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# ADVANCEMENT IN GENE EDITING TECHNOLOGIES: IMPLICATION FOR DRUG DEVELOPMENT IN PERSONALISED MEDICINE

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## ABSTRACT

The development of genome editing technologies has opened up the possibility of directly targeting and modifying genomic sequences in almost all eukaryotic cells. Genome editing has extended our ability to elucidate the contribution of genetics to disease by promoting the creation of more accurate cellular and animal models of pathological processes and has begun to show extraordinary potential in a variety of fields, ranging from basic research to applied biotechnology and biomedical research. Recent progress in developing programmable nucleases, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases, has greatly expedited the progress of gene editing from concept to clinical practice. Here, we review recent advances of the three major genome editing technologies (ZFNs, TALENs, and CRISPR/Cas9) and discuss the applications of their derivative reagents as gene editing tools in various human diseases and potential future therapies, focusing on eukaryotic cells and animal models.

**Keywords: Gene editing, CRISPR, Drug development, Prime editing, TALEN, ZFN**

## INTRODUCTION:

The development of gene editing technologies has been the fastest ever in biomedicine, offering to revolutionize many aspects and niches. Of these technologies, CRISPR-Cas9 has been the most transformative providing unprecedented precision, efficiency and versatility. The implications are profound for drug development, particularly personalised medicine which aims to treat a patient based on their individual genetics. The goal of personalised medicine is to enhance therapeutic efficacy and reduce risk, by accounting for individual genetic variability, lifestyle and environmental exposure. In this edition, we look at how gene editing technologies are driving new frontiers in drug development and personalized medicine **[1]**.

The availability of the human genome sequence along with progress in the field of bioinformatics has triggered an inexplicable pace of upsurge in the post-genomic drug discovery process. Currently small molecules as well as macromolecules have embraced gene targets as their major focuses. At the same time, a vast amount of species-specific information is encoded in the nucleotide sequence. Molecular drugs initially designed based on a wild-type phenotype may cause such side effects and at times, loss of function mutations in targets are inherited making such drugs ineffective **[2]**. The Cre/LoxP system for deletion of any fragment of DNA by using the LoxP sites is a genetic tool for further development of model systems. Spontaneous insertions of the LoxP/Cre pair result into knockout alleles. Most of the animals generated by random deletions will be heterozygous for the knocked-out alleles. The offspring generated through such a cross with Cre transgenic animals will harbour their individual genetic manipulation. A LoxP site can be inserted at some desirable point, thus the wild type alleles will not be deleted, than a that is homozygous knockout. Transgenic means the ability to transfer one or more genes from one organism to another entirely different from it.

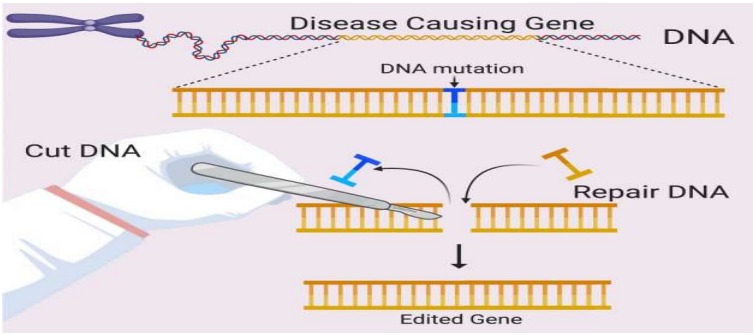


**Figure 1: Concept of Gene editing [65]**

The most typical transgenesis technique is an expression cassette containing a transgene and regulatory sequences (promotor/enhancer and polyA tract). Such random integration will give a polyclonal line with different genetic changes. If the genetic modification can be put into practice in germline, the modified offspring will be bred in the population. Effective mutations by means of random insertions of foreign genes in animals transmitted by germline tend to occur in ectopic manner. Abnormal phenotypes may be occasioned by improper expression. Site-specific integration may be affected through Cre/loxP. 1st an expression vector with a LoxP site and a selectable marker gene having two FRT sites on either side is transported into cells. After screening, a single-genic FRT-excised clone is grown. This vector will be then harvested to co-transfect a Cre-expressing vector in those cells **[3, 4]**.

**Historical Milestones in Gene editing:**

**1970s:** Uncovering Restriction Enzymes: Scientists discovered some enzymes that can cut DNA at specific sequences hence serving as the basis for gene editing **[5]**.

**1980s:** Development of Gene Targeting: There has been limited success, but there are established methods that can be used to introduce exact changes into genes **[6]**.

**1990s:** Invention of Homologous recombination: Researchers now understand how cells normally repair breaks in DNA, making gene editing possible **[7]**.

**2000s:** Development of Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs): In order to make gene editing more effective researchers develop enzymes with predetermined DNA sequences as their targets **[8]**.

**2010:** First Discovery of the CRISPR/Cas9 System: Scientists discover the CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats) system present in bacteria and subsequently revolutionize the field of gene editing **[9]**.

**2012:** The First Gene Editing Using CRISPR/Cas9 in Human Cells: Researchers successfully use CRISPR/Cas9 to modify human cells **[10]**.

**2013:** CRISPR/Cas9 Gene Editing in Animals: The scientists carried out gene editing on animals, and this was to show the way for treating genetic disorders **[11]**.

**2014:** CRISPR/Cas9 Gene Editing in Plants: Through researchers edited genes in plants they were able to indicate that agriculture is where it can be applied **[12]**.

**2015:** First Clinical Trials Using CRISPR/Cas9: The journey of trying CRISPR/Cas9 as a drug to treat people began here **[13]**.

**2017:** CRISPR/Cas9 Used to Treat Genetic Disease in Humans: In yet another case, human trials propagate the researchers'

success in the treatment of an inherited metabolic disorder through the use of CRISPR/Cas9 **[14]**.

**2018:** CRISPR/Cas9 Used to Edit Human Embryos: This caused an ethical debate after scientists used gene editing tool to correct disease-causing mutations in human embryos **[15]**.

**2019:** First CRISPR/Cas9 Gene Editing Treatment Approved: CRISPER/case will soon make history with FDA clearance making it possible for doctors to begin using a technique for fixing DNA called Crispr-cassette **[16]**.

**Overview of gene editing technology:**

- **CRISPR/Cas9:** Genetic editing has come a long way using the CRISPR/Cas9 system, among other things in oh, Gene edited sponsored scientific development. This capacity to create shift in DNA directionally, can cause learning of quality capacities and entitlements for treatment thereof **[1, 2]**. TALENs (Transcription Activator-Like Effector Nucleases) Uses a protein RNA complex to bind and cleave a given DNA sequence **[17]**. The ZFNs (Zinc Finger Nucleases) Uses a protein-DNA complex to bind and cleave a target DNA sequence **[18]**.
- **Base Editing:** To put it simply, base editing was CRISPR/Cas technology-base without the DSB cutting capability. Additionally, it can be used to change point mutations associated with a disease **[19]**.
- **Prime Editing:** Prime editing is more precise than the gene-editing tool CRISPR/Cas9 it can "search for, and modify" specific parts of a target DNA sequence by inserting new genetic information or deleting existing sequences. It is supposed to fix more of these mutations at each time and thus helps by correcting those errors that happen most commonly **[20]**.

**1. CRISPR/Cas9**

CRISPR adds yet another important feature to the existing concept of gene-editing based medicine as an RNA based natural system of sequence specific immunity as found by researchers in prokaryotes **[2]**.

The discovered prokaryotic immune systems have pointed out possibilities of RNA based natural systems for sequence specific recognition of homologous foreign DNA and RNA in Metazoan. Onca-CRISPR, the first CRISPR based gene editing therapeutic was concurrently filed for trial on patients suffering from diseases **[21]**. New advanced technologies include single adeno-associated virus (AAV) containing multiple Cas9 and gRNAs dual RNA viruses that continue Cas9, as well as gRNA vectors, and integrating viral vectors containing gRNA and fluorescent markers

have expanded the use of the CRISPR systems to the therapeutic level **[22]**. This year, the discovery of bacteriophages that inactivate the CRISPR/Cas bacterial immune system published. Later, more research works that looked at the discovery of anti- CRISPRs, how they worked and their impact on evolution shape a new wing of evolutionary biology. After that the definition of small CRISPR RNAs provided manners for antiviral defense in prokaryotes. In the course of one night, the small RNAs reversed the thinking about how RNAs could function at the DNA level. This discovery of this small RNA mediated prokaryotic version of RNAi system was another year of Oscar for its discoverers, one of whom (Rodriguez) was an ONR funded academic in the glorious days of computational biology, bioinformatics and genomic sequencing. By comparative genomics, tens of thousands of metagenomic CRISPR sequences have been collected to explore the CRISPR world **[23]**. Analyses of phylogenomic indicated that Type I systems were older and shared information about CRISPRs in bacteria which in its own right deserved a Nobel Prize. On the basis of computational analyses, the emergence, development and possible directions of the future anti-CRISPRs, sCRISPR-RNAs, Type III systems, CRISPR immunity in Archaea and eukaryotes were also argued and explored **[24]**.

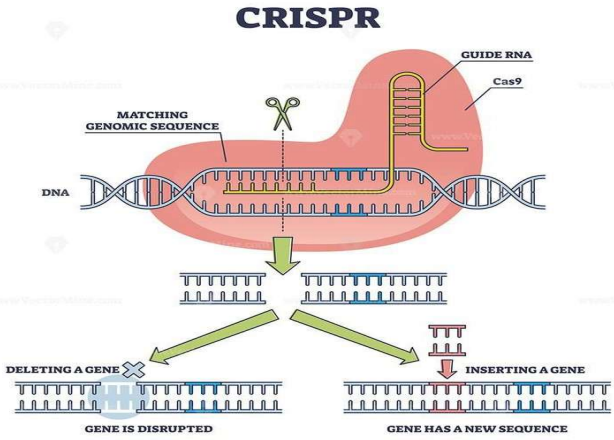


**Figure 2: CRISPR [66]**

**Mechanism of CRISPR system:**

CRISPR is changing how we modify genes at a rapid pace by introducing pinpoint adjustments to an organism's DNA. Its operation can be summarized as:

**Guide RNA (gRNA) Design:** A genomic sequence has been designed for a specific but unknown DNA strand that is subsequently used by the CRISPR-associated protein (for instance Cas9) as its guide.

**Binding of Cas9 Protein:** An endonuclease

known as Cas9 aligns itself with the guide rRNA it is part of. The combination then identifies and achieves the complementary base pairs with reference to any particular location in a genome.

**DNA Cleavage:** The moment gRNA-Cas9 hybrid attaches itself onto the anchor strands in one strand of double-helical structure, however, lysating it through amino acid units on the other strand, opens up at whereby such double strandedness was dictated by the sequence itself **[22]**.

**DNA Repair:** Lastly, non-homologous end joining repairs this damaged segment through two ways: Non-Homologous End Joining (NHEJ): Which sometimes produces frame shifts or random nucleotide sequences which might lead into gene disruption or cause mutation to happen. Homology-Directed **Repair (HDR):** In case a repair protocol provides an alternative template there will be exactly no change until repaired by cell itself at this broken point **[25, 26]**.

**CRISPR-transposases for large genome insertions**

Many strategies are enabling targeted, precise, and precise insertion due to transposon-linked CRISPR-Cas systems. Two groups have shown that the type I-B, I-F, and V-K CRISPR-Cas systems can prompt a Tn7-like transposase to place donor DNA at target sites that are subsequent to targeted sequences in E. coil expanding upon earlier computational analysis **[27]**.

CAST or type V-K transposon-associated CRISPR system consist of Tn7-like transposase and Cas12k and might mediate DNA integration in bacterial cells at a frequency up to 80% without the necessity of selection. Here, the INTEGRATE system containing the type I-F transposon-associated CRISPR is utilized the TniQ-Cascade complex for DNA integration. To the extent it has an off-target effect, it is less than 5% as a percentage of control MEFs, yet it demonstrates very high on-target non-directional integration activity. A single-plasmid INTEGRATE system has since simultaneously integrated bacterial DNA and exhibited fairly high efficiency. This has also allowed designing insertions within independent natural communities that evolved to be specific to organisms and locales using a single-RNA guided DNA editing and transposition CRISPR Cas (DART) method **[28]**.

It is the direct DNA insertion that is accomplished by TA- CRISPR with the help of its associated transposases and was one of the biggest advantages of this system. This has a huge potential of application to organisms with low HDR repair efficiencies especially poikilothermic organisms such as plants. At the same time, no information has been identified on the use of transposon-associated CRISPR systems in eukaryotic

organisms yet. This may be as a result of differences in the chromatin structures, the suppression of transposases or differences in post transcription modifications **[29]**.

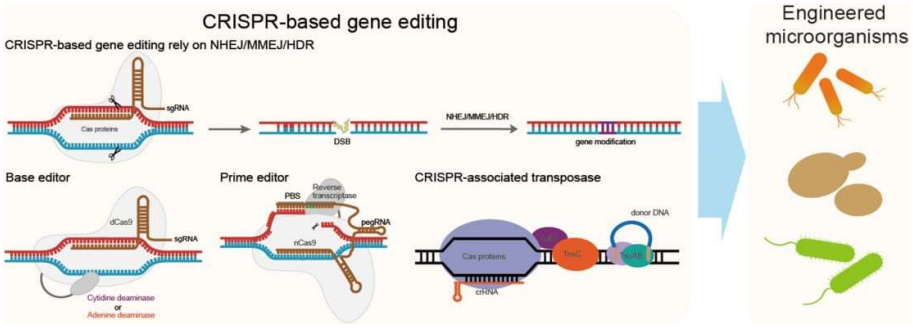


**Figure 3: CRISPR- based gene editing [67]**

**CRISPR Applications:**

**CRISPR in medical research**: To understand gene function and the genetic basis of diseases. Researches can model diseases to better understand their development and treatment alternatives. Gene Therapy CRISPR for editing genetic mutations causing hereditary diseases It is currently under investigation for the treatment of diseases such as cystic fibrosis, muscular dystrophy or sickle cell anemia.

**Cancer treatment:** Armed with CRISPR, immune cells can be tweaked to more readily recognise and kill cancerous invaders. Investigators are also testing it to try and sensitize cancer cells themselves to treatment.

**Agriculture:** In agriculture, CRISPR has been used to create genetically modified crops of desired traits such as increased resistance against pests and diseases or abiotic stress, improved yield, removing deleter.

**Biotechnology:** The technology can be used to develop new synthetic biology tools, engineer microorganisms for industrial processes and produce novel biomaterials.

Instruments that allow genes to be specifically deleted or inserted are revolutionary, but the use of these is continuously being studied and regulated under controversy related to ethics and safety **[1, 2]**.

**Future directions for CRISPR:**

Enhanced specificity: Decreasing off-target effects.

Productivity Improvement: This is essentially improved editing productivity.

Novel Uses: Innovating examples of what can be done with CRISPR tech **[30]**.

**2. TALENs:**

The TALE proteins are multimeric and consist of DNA binding core, nuclear localization signal and the transcription activating domain of the target gene. These

proteins were found to possess the capability to bind to DNA in 2007 but it was only in 2008 that two groups of researchers deciphered the code for wielding TALE proteins to identify the target DNA. For example, the conformation of DNA-binding domain was determined and each of them binds to only one nucleotide in target nucleotide sequence. These are called monomers and there are 34 amino acid residues; however, there are 2 which are in positions 12 and 13 and are RVD because they are variable and in fact it is these two that differentiate a particular nucleotide. This code is degenerate; some RVDs can interact with several nucleotides and do this with different affinity. When 5'-end of a sequence bound by a TALE monomer, the target DNA molecule always has one specific nucleotide, thymidine, affecting the efficacy of binding. The final tandem repeat that connects to a nucleotide at the 3'-end of the target sequence is however formed from merely 20 amino acid residues and is as such referred to as the half-repeat **[17]**.

**TALEN gene editing applications:**

The opportunity of using TALEN technology can be useful in various fields including:

**Disease correction:** The TALENs can also provide the role of gene editing for many of the genetic diseases such as cystic fibrosis, sickle cell anemia, Huntington disease and many others.

**Crop enhancement:** They said that this technique has the possibility of creating crops that come with characteristics such as high yield, pest and disease tolerance, improved nutritional value among others **[31, 32]**.

**Biotechnology:** TALENs are used in designing organisms through genetic engineering for diverse purposes such as in production of bio fuels, enzymes and the likes.

**Research:** TALENs- A Versatile Gene Editing Tool to Investigate Genes Function and Biological Processes **[33, 34]**.

**Mechanism:**

**Design:** Since TALENs can be designed to contain specific cutting DNA sequence, scientists design TALENs with patterns that are complementary to the desired DNA sequences **[35]**.

**Target:** The TALENs go and then attach themselves to the correct area of DNA then stick.

**Cut:** The TALENs made the cut in two DNA.

**Repair:** Then, the body attempts to heal this cut, and that in turn, has the possibility to cause adjustments to the DNA **[36]**.

**Applications:**

**Fixing broken genes:** TALENs can be used widely for correcting mutated genes that cause diseases **[37]**.

**Improving crops:** It can also be used in order to create plants that can grow more

efficiently or plants that are immune to pests **[38]**.

**Creating new products:** It is possible to create organisms that can produce substances which are of value such as fuels or drugs **[39]**.

**Studying genes:** TALENs can be applied in order to gain a greater understanding of how genes function. Despite this, TALENs are very strong, yet they are not perfect and they do have some drawbacks. They are even more complex to design and to employ in comparison to CRISPR and are likely to act as scissors in the wrong locations of DNA **[40]**.

**3. ZFNs (Zinc Finger Nucleases):**

ZFNs: Jewish geneticists taken the first step toward gene editing. Think of gene editing as a fine process of eradicating minuscule errors in a book. Zinc Finger Nucleases or ZFNs were one of the first that were designed for the purpose. They look like scissors with two sharp blades which can manage to clip the DNA at particular sites **[17]**. Zinc finger gene editing is a technology in gene modification involving changes in specific genes within the DNA of an organism. This method involves the use of artificially engineered zinc finger proteins that are small protein domains capable of recognition and binding to particular DNA sequences. Designing such proteins targets those genes in which they can cause double-strand breaks in DNA **[18]**.

**Mechanism:**

**Design:** The scientists create ZFNs to have one or more domains that are specific to different DNA target sequences.

**Target-** The ZFNs identify the place on the DNA to which they have to bind themselves.

**Cut-** The DNA gets cut at that position in the target sequence by the ZFNs making it two.

**Repair –** The cell tries to stick the cut and, in that process, more change the DNA sequence **[39]**.

**ZFNs application:**

**Gene correction –** It is possible to try and fix genes that contain a disease.

**Improvement of crops-** It can be used to make the plants insect-resistant **[41]**.

**Research:** It would help in expounding more about the genes operation.

However, despite such a huge improvement, ZFNs had been done away with by better tools that are even better such as CRISPR and TALENs. The use of these newer tools is easier and less error prone compared with older tools that once were used for data analysis. However, what was introduced by the ZFNs was still in its infancy now that a new developed technology in the area was available **[39]**.

**4. Base editing:**

Base editing is a new genetics engineering tool that allows very precise changes to DNA sequences in living cells. While regular CRISPR/Cas9 makes double-

stranded breaks and depends on the cell's repair mechanisms to make the change, base editing actually converts one base in the DNA to another without making a double-stranded break. That allows changes in selected point mutations, some of which are known to cause genetic diseases; therefore, this technology could be used for the treatment of a variety of illnesses. This works by fusing catalytically inactivated CRISPR/Cas9 proteins to DNA-modifying enzymes so that it can make a targeted and efficient base **[19]**

**Mechanism:**

**Fusion protein:** Base editing uses a fusion protein of a catalytically dead CRISPR-Cas9 protein (dCas9) and a base editor enzyme. The dCas9 part directs the editing machinery to the target DNA sequence.

**Base editor enzymes**: Base editor enzymes typically contain cytidine deaminase (which converts cytosine to uracil) or adenosine deaminase (which converts adenosine to inosine). These enzymes act on the DNA base at the target location.

**DNA repair:** After base editing enzymes edit the bases, the cell's natural repair machinery replaces the edited bases with the new bases and you get the correct nucleotide substitution **[42]**.

**Applications:**

**Correction of genetic diseases:** Base editing is possible in the event of point mutations causing genetic diseases, such as sickle cell disease, cystic fibrosis, and muscular dystrophy.

**Functional genomics:** There are studies using base editing to introduce specific mutations to understand how a mutation affects gene expression and cellular processes.

**Agricultural Biotechnology:** Application of base editing in introducing desirable traits in crops also involves resistance to specific diseases, nutritional improvement.

**Cell and genetic therapy:** There is possibility of treatment in cells specific to the patient for transplantation or correct genetic defects.

Basic editing will become a powerful tool for basic research and clinical applications. It reduces the off-target effect of the targeted genetic change compared to the traditional method of genome editing **[43]**.

**5. Prime editing:**

Prime editing is a fresh method for genetic alteration that enables us to the alteration of DNA to be more precise. The 2019 innovation of prime editing by researchers is much better compared to conventional CRISPR-based techniques. It permits precise genome modifications with fewer unintended consequences **[20]**.

**Mechanism:** Components of Prime Editor:

The system is made out of two main parts:

A) **Prime Editor Protein:** It is made of a catalytically inactive CRISPR/Cas9

protein that is complexed with a reverse transcriptase enzyme.

B) **Prime Editing Guide RNA (pegRNA):** Not only does it have the usual sequence corresponding to the upset DNA base in the DNA but also it is accompanied by a model to replicate the preferred DNA chain.

**Process: -** The peg RNA carries the prime editor to the DNA-the strand of DNA then serves as an approach route for the prime editor down to its target in the DNA. The prime editor protein nicking made a single-strand break thereby the location will be that in the DNA at which the DNA ends the required change. The reverse transcriptase is taking the RNA template given by pegRNA and it will maliciously insert the new sequence into the DNA resulting in the desired change **[44]**.

**Application**

**Disease treatment:** Prime editing can correct the causal genetic error of a variety of hereditary diseases, including sickle cell anemia, muscular dystrophy and cystic fibrosis.

**Genetic study:** It makes the deletion of genes in research models more accurate, which improves studies on gene functioning and enables a higher accuracy on how these works together.

**Farming:** Prime editing can help geneticists to enhance disease resistance and yield in cattle, the other one local animals animal or agrarian item without adding new DNA sequences **[45]**.

**Biotechnology-** This helps to manufacture the product as per our need by changing genes of some organisms. Since it is a more targeted and less error-prone method for genome editing as compared to other similar methods, prime- editing has powerful potential in genetic engineering **[20]**.

**Multiplex gene editing technology**

About multiplex genetic modification TALENs and ZFNs are less efficient than the CRISPR-Cas systems. For multiple gRNAs to be used to express within the same cell, many strategies have been adopted. First, multiple gRNAs can be obtained because of pol III promoter-driven expression cassettes, and then the user can choose the appropriate gRNA for knocking out the corresponding gene **[46]**. Secondly, mature gRNAs can be for example released by tRNA processing, ribozyme auto-cleavage or Csy4 ribonuclease cleavage if several gRNAs are transcribed from one Pol II or Pol III promoter and are together in one transcript. Lastly, we believe the inherent ability of CRISPR systems to self- process can be utilized to create many gRNAs. Among the type II CRISPR arrays, Tracer RNA expression is also necessary. The compact size of the crRNA sequences and the existence of different Cas12 proteins make multiplexed genome editing possible or easy **[47]**.

Other researchers have also been used in delivering multiplexed orthogonal editing with many Engraleted sgRNAs for creating DSBs. The first documented usage is in the regulation of gene editing and at the same time regulating the Geneon within the same cell using CAS-Orthogonal fusing to the effectors with unique functions. Furthermore, one could obtain multiplexed orthogonal editing by knocking in the gRNAs and simultaneously, one gRNA for the other use. For instance, it is disadvantageous as gene regulation and base editing can be carried out at the same time while one is using aptamer-modified gRNAs as it is possible to recruit different effectors **[48]**.

**Discovery, Research and Medicine Applications**

The system can also use the CRISPR-Cas9 to create loss-of-function mutations of said gene to study its function in cell physiology in addition the role it plays in a disease's pathogenesis. Gene therapy uses a technique to correct genetic mutations linked with hereditary diseases. CRISPR has been used by researchers to customize the genes that cause diseases including Duchenne muscular dystrophy and cystic fibrosis **[49]**. Functional Genomics: Deleting these genes or modifying them can give some clues about their roles in such complex physiological processes and conditions.

**Implication for drug development**

**Drug target detection and validation grew rapidly**

Gene editing: It makes possible the controlled manipulation of genes, which can shed light on gene function and validate novel drug targets. Of course, knocking-out, or later changing genes themselves within an organism have allowed those working with different models (including plants) see how traits linked to potential drug targets change. Enhances drug target validation success that fast-tracks the progress of a drug to market **[22, 50]**.

**Rapid, scalable disease modeling and drug screening**

The precision of disease models, in particular for genetically complex diseases like cancer and neurological disorders, has dramatically grown since the arrival of CRISPR-Cas9. This way, the researchers can edit genes in human cell lines and animal models to look like the disease state on top of which they hope to rebuild complex traits. These models are very important for discovery stage biology work, to understand disease pathology and drug efficacy early in preclinical stages so as to accelerate more successful treatments **[2, 51]**.

**Therapies Tailored to Genetic Disorders Unique**

A deeper layer of implication with gene editing is the ability to develop personalized therapies for genetic disorders. For example,

treatments based on CRISPR are in the works for diseases like cystic fibrosis, muscular dystrophy, and sickle cell anemia. In the case of these, the defective gene responsible for the disorder can be precisely corrected or replaced. This could also present an application of personalized gene therapy-engineered from a person's highly specific genetic signature-where curative options might exist beyond traditional drugs **[52]**.

**Pharmacogenomics**

Pharmacogenomics is the study of how genes influence one's response to drugs. Gene editing takes this capability to a whole new level, as in the study of gene-drug interaction, predicted adverse drug responses are done. In pharmacogenomics, discovery for genetic variation is allowed, which may act like markers for modified drug metabolism or variable efficacies. These genetic variants will aid pharmaceutical firms in devising targeted therapies for particular classes of patients. This will lead to the development of more appropriate, safer, and effective drugs for specific classes of patients. Drugs would not be put through that ritualistic trial-and-error phase just to get prescribed to the patients **[53]**.

**Drug Repurposing via Gene-Driven Editing**

With gene-editing technology, drugs can be repurposed-meaning, drugs previously approved can be evaluated for new disease indications. Modeling a nonhuman disease model by gene editing allows one to make inferences about whether an available drug has unexpected effects, probably in different pathways **[39]**. This approach not only fosters and accelerates drug discovery but also reduces the development costs greatly since available drugs are being evaluated for new indications **[54]**. **Accelerated Drug Screening and Development**

Traditional drug development is long and expensive; it usually takes a number of years before a new drug hits the market. Gene-editing technologies will enable such processes to be accelerated via high-throughput screening of drug candidates. Large libraries of genetic alterations, through construction, will enable researchers to identify rapidly what kind of mutations respond best to a particular drug. The output of this will be a fast-track approach toward identifying promising drug candidates, which especially would be important in personalized medicine **[55]**.

Moreover, gene editing enables the establishment of patient-derived cell lines that might serve for the examination of efficacy and drug safety. This strategy can accelerate drug development while reducing the risk of drug reactions, as treatments can be examined on cells similar to the patient's cells **[56]**.

**Enhanced Disease Modeling for Drug**

**Discovery**

These gene-editing technologies further revolutionized the making of disease models crucial for understanding the mechanisms behind diseases and testing potential therapies. Scientists are able to introduce certain mutations into cells or organisms' DNA in order to create disease models mimicking exactly those taking place in a human organism. Further on, such models can be used in the study of the process of disease development and for screening drugs which can be effective against conditions of interest.

This capability is especially important for personalized medicine, in that it allows researchers to test how a certain drug will interact with the genetic makeup of an individual patient. This, in turn, allows developers of drugs to identify therapies that would more likely be effective, reducing trial-and-error approaches to drug development **[57]**.

**Challenges**

There are a range of challenges associated with gene editing which has two specific ethical concerns off-target effects changes may occur in unintended genes or genomic regions away from the target Mosaicism a mix of brood, edited cells, and unedited cells, which may lead to inconsistent outcomes. Finally, there are a always the concern of efficiency, success rate, because as long as reasonable use of the technology is available, logistics and cost to be determined when as the end user, there is always efficiency to be determined. delivery difficulty in delivery of gene editing and zygotic editing tools to target cell delivery facilities within the organism. Scalability creating a logistical plan for gene editing at a larger scale action to become reasonable return of efficiency evidence **[58]**.

**Ethical and Regulatory Consideration**

With all of the excitement around a new technology, advances in gene editing definitely present ethical and regulatory challenges, as with all disruptive technologies. One of the major concerns for personalized medicine is the idea of a germline edit-an inheritable change (a modification passed down to future generations). Change to somatic cells will only affect the individual treated, but germline editing or changes to existing human germline development has caused more fear and controversy because of the possibility of unforeseen changes and patterns-moral dilemmas created in part because of the willingness to modify human genetic material. Regulators across the globe are still challenged in figuring out how to regulate the incorporation of gene editing into drug development efforts knowing that the application has the potential benefit of speeding up new medicines, but are also tasked with protecting human subjects from

harm. In personalized medicine, the plot thickens-individualized therapies, which may be considered individualized based on a certain genetic profile of individuals may consider interventions on a case by case basis **[59]**.

Aside from these practical concerns, modifications of the human germline (heritable changes) could pose serious risks and ethical challenges in terms of their potential for abuse and long-term effects on human genetics.

Off-Target Effects: Ensuring CRISPR accuracy is critical to avoid unintended genetic alterations. It is these off-target effects that scientist work hard to reduce as much a possible.

Regulatory Issues: The creation of research and clinical opportunities for CRISPR applications will be affected by the varying legal standards between countries, such as those regarding human germ-line editing.

With potential for use across many areas, CRISPR technology is an exciting and significant development in the realm of gene editing. It can be a double-edged sword, and researchers as well as legislators must handle both the responsibilities that come with using it challenges aside **[60]**.

**Future direction in gene editing**

Clustered regularly interspaced short palindromic repeat (CRISPR)-based genome editing technologies have opened completely new and exciting perspectives both for basic research in genes and for the improvement of human medical conditions. On the other hand, artificial intelligence allows for genome editing to be more precise, efficient, and low in cost in targeting various diseases, such as Sickle cell anemia or Thalassemia **[61]**. Various AI models have recently been utilized for the design of guide RNAs for CRISPR-Cas systems. Some of the tools that carry out such predictions of optimal gRNAs for a given target sequence include DeepCRISPR, CRISTA, and DeepHF. In these predictions, multi- dimensional factors are considered that include genomic context, Cas protein type, type of desired mutation, on-target/ off-target scores, potential off-target sites, and potential impact of genome editing on gene function and cell phenotype. The models allow for the optimization of other genome editing technologies, including advanced base, prime, and epigenome editing to achieve precise and programmable alterations in DNA without relying on mechanisms such as homology-directed repair or donor DNA templates. On top of this, AI helps in personalized treatments with genome editing and precision medicine based on genetic profiles **[62]**. AI analyzed genomic data of patients for the identification of mutations, variations, and biomarkers attributed to various diseases: Cancer, Diabetes, and Alzheimer's. Whereas many

challenges remain on costs, off-target editing, suitable delivery methods for CRISPR cargoes, enhancing editing efficiency, and ensuring safety in clinical applications, this review discusses the contribution of AI in the advancement of CRISPR-based genome editing technologies and addresses the different existing challenges. It also discusses possible areas of future research in AI-driven CRISPR-based genome editing technologies. Merging AI and genome editing opens new horizons for genetics, biomedicine, and healthcare, having significant consequences for human health **[63]**.

Novel techniques using this basic genomic site identification provided by the canonical CRISPR-Cas9 system are beyond the technologies discussed above. Most relevant to the situation at hand are those mechanisms which abort the generation of DSBs, thereby reducing the risk of on- and off-target genotoxicity and uncontrollably random repair **[62]**.

**Limitation and risk of gene editing**

As stated above there are disadvantages and also risky consequences of gene editing.

Both somatic or germline cell can be targeted for gene editing process. Now, most nations' laws permit germline genome editing only in a Petri dish since this alteration can be transmitted to subsequent generations. However, one cannot ignore the numerous moral and legal issues together with evidence that indicates that blastocysts are more vulnerable to genomic damage than somatic cells. Several off-target effects have been reported in the experiment concerning germline gene editing in embryos. Among them there may be quite a severe chromosomal effect **[64]**.

**CONCLUSION**

Gene-editing technologies are new drug development power drivers, especially in personalized medicine. Health care will be revolutionized by these technologies through the precision targeting of genetic mutations, better modeling of diseases, accelerated discovery of drugs. It is prudent, however, that ethical and regulatory challenges be cautiously worked through so that benefits accruing from these advances are captured while risks are minimized. In conclusion Gene editing technologies such as CRISPR, TALEN, base editing, prime editing, zinc finger nucleases, and multiplex gene editing are explained in the above review along with how they can be used to develop new drugs, improve drug discovery models, speed up drug development and screening, overcome obstacles, take ethical and legal issues into account, and shape gene editing in the future. In the future, gene editing will help shape the future of personalized medicine as it gets more fully developed, taking us even closer to a day when treatments will be tailored to the specific genetic makeup of the individual.

**LIST OF ABBREVIATIONS**

| Abbreviation | Full form |
|---|---|
| AAV | Adeno-associated virus |
| AI | Artificial Intelligence |
| AID | Activation-Induced Cytidine Deaminase |
| BFP | Blue Fluorescent Protein |
| CAS | CRISPR-Associated System |
| Cre | Cyclization recombination |
| CAR-T | Chimeric Antigen Receptor T Cells |
| CAST | CRISPR-Associated Transposase |
| CRISPR | Clustered regularly interspaced short palindromic repeat |
| CRISPR-Cas12k | CRISPR-Associated Protein 12k |
| CRISPR/Cas-MGE | CRISPR/Cas Mobile Genetic Elements |
| CRISPR-Cas Type I- B | CRISPR-Associated Type I-B |
| CRISPR-Cas Type I- F | CRISPR-Associated Type I-F |
| CRISPR-Cas Type V-K | CRISPR-Associated Type V-K |
| CRISPRi | CRISPR Interference |
| CRISTA | CRISPR Target Assessment |
| Csy4 | CRISPR System 4 Endoribonuclease |
| DART | DNA and RNA Targeting |
| dCas9 | Catalytically Dead CRISPR-Cas9 |
| DeepCRISPR | Deep Learning-Based CRISPR Prediction Tool |
| DeepHF | Deep Learning-Based High Fidelity |
| DNA | Deoxyribonucleic acid |
| DSB | Double-Strand Break |
| FRT | Filppase recognition target |
| FDA | Food and Drug Administration |
| GFP | Green Fluorescent Protein |
| gRNA | Guide ribonucleic acid |
| HDR | Homology-directed repair |
| HLA | Human Leukocyte Antigen |
| INTEGRATE | Integrative Genome Engineering Platform |
| LoxP | Locus of X-over P1 |
| LTR | Long Terminal Repeat |
| MEFs | Mouse Embryonic Fibroblasts |
| MGE | Mobile Genetic Elements |
| mRNA | Messenger RNA |
| NGS | Next-Generation Sequencing |
| NHEJ | Non-homologous end joining |
| NMD | Nonsense-Mediated Decay |
| ONR | Office of Naval Research |
| PAM | Protospacer Adjacent Motif |
| PCSK9 | Proprotein Convertase Subtilisin/Kexin Type 9 |
| PDB | Protein Data Bank |
| pegRNA | Prime Editing Guide RNA |
| RAG1/2 | Recombination Activating Gene 1/2 |
| RISC | RNA-Induced Silencing Complex |
| RNA | Ribonucleic acid |
| RNAi | RNA Interference |
| rRNA | Ribosomal ribonucleic acid |
| RVD | Repeat Variable Diresidues |
| RNP | Ribonucleoprotein |
| RT | Reverse Transcriptase |
| sCRISPR-RNAs | Small CRISPR RNAs |
| sgRNA | Single Guide RNA |
| TALEN | Transcription Activator-like effector nucleases |
| TCR | T Cell Receptor |
| tRNA | Transfer RNA |
| Tn7 | Transposon 7 |
| TniQ | Transposition Insertion Protein Q |
| TracrRNA | Trans-Activating CRISPR RNA |
| TSD | Target Site Duplication |
| ZFN | Zinc Finger Nucleases |
| ZFN-FokI | Zinc Finger Nuclease-FokI (Restriction Enzyme) |

**REFERENCES:**


**[1]** Doudna, J. A., & Charpentier, E., The CRISPR-Cas9 system for genome editing. Science. 2014; 346(6213): 1258096.

**[2]** Hsu, P. D., Lander, E. S., & Zhang, F., Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014; 157(6): 1262-1278.

**[3]** Sauer, B., & Henderson, N., Site-specific recombination of genes: a tool for functional genomics. Current Opinion in Biotechnology. 1988; 9(5): 519-525.

**[4]** Zhang, L., & Liu, X., Human germline genome editing: Scientific and ethical issues. Journal of Medical Ethics. 2019; 45(7): 475-478.

**[5]** Smith, H. O., & Nathans, D. A suggested nomenclature for bacterial restriction endonucleases. Journal of Molecular Biology. 1973; 79(2): 201-203.

**[6]** Thomas, K. R., & Capecchi, M. R. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell,1987; 51(3): 503-512.

**[7]** Zhang, Y., & Choi, K. Gene targeting and homologous recombination in embryonic stem cells. Current Opinion in Biotechnology. 1995; 6(5): 597-603.

**[8]** Kim, H. S., & Kim, J. S., A guide to genome engineering with programmable nucleases. Nature Reviews Genetics. 2014;15(5):321-334.

**[9]** Jinek, M., & Doudna, J. A., A three-dimensional view of the RNA-interference machinery. Nature. 2012; 482 (7385): 331-338.

**[10]** Mali, P., & Yang, L., RNA-guided human genome engineering via Cas9. Science. 2013; 339(6121):823-826.

**[11]** Cong, L., & Zhang, F. Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Science. 2013; 339(6121): 612-617.

**[12]** Feng, Z., & Zhang, B., Efficient genome editing in plants using a CRISPR/Cas system. Science. 2014;346(6213): 769-774.

**[13]** Doudna, J. A., & Charpentier, E., The CRISPR-Cas9 system for genome editing: A history of its development and its applications in clinical research. Molecular Therapy. 2015; 23(5): 1037-1042.

**[14]** Ma, H., & Zhang, L., Correction of a pathogenic gene mutation in human embryos. Nature. 2017. 548(7668): 413-419.

**[15]** He, J., & Wu, M., CRISPR/Cas9-mediated genome editing in human embryos. Molecular Genetics & Genomic Medicine. 2018; 6(1): 27-36.

**[16]** Ma, H., & Liu, Y., First CRISPR-Cas9 based therapeutic for genetic diseases: The case of Sickle Cell Disease. New England Journal of Medicine. 2019; 381(8): 764-770.

**[17]** Gaj T, Gersbach CA, Barbas CF III, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013; 31(7):397–405.

**[18]** Carroll D, Genome engineering with zinc-finger nucleases. Genetics. 2011, 188(4):773–782.

**[19]** Mali, P., & Esvelt, K. M., Base editing: The next generation of CRISPR/Cas9. Cell, 2018;173(1): 24-27.

**[20]** Liu, Y., & Zhang, T., Prime editing: An emerging tool for precise genome engineering. Nature Reviews Genetics. 2021;22(12): 827-842.

**[21]** Barrangou, R., & Marraffini, L. A., CRISPR-Cas systems: Prokaryotes upgrade to adaptive immunity. Molecular Cell. 2014; 54(2): 234-244.

**[22]** Sander, J. D., & Joung, J. K., CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotechnology. 2014; 32(4): 347-355.

**[23]** Seed, K. D., & Lazinski, D. W., A bacteriophage that targets CRISPR/Cas bacterial immune systems. Nature. 2013; 496(7446): 235-238.

**[24]** Makarova, K. S., & Koonin, E. V., Classification and nomenclature of CRISPR-Cas systems: A revised proposal. Philosophical Transactions of the Royal Society B: Biological Sciences.2015; 370(1672): 20140260.

**[25]** Cong, L., & Zhang, F., Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Science. 2015;339(6121): 619-623.

**[26]** Shen, B., & Zhang, Y., Regulation of DNA double-strand break repair by DNA damage response signaling. Current Opinion in Genetics & Development.2014; 28:10-17.

**[27]** Sikora, M., & Liang, Q., Challenges and advances in applying CRISPR-transposon systems to eukaryotic organisms. Nature Reviews Molecular Cell Biology. 2020; 21(11): 719-731.

**[28]** Geng, C., & Kim, H., CAST: A type V-K CRISPR-transposon system

for efficient large-scale genome editing in bacteria. Journal of Biological Chemistry. 2021; 296:100268.

**[29]** Sakai, T., & Fuchino, Tn7-like transposase-mediated integration of donor DNA in bacterial genomes. Nature Communications. 2020;11(1):1834.

**[30]** Zhang, Y., & Zhang, Y., Advances in CRISPR/Cas9 technology for improving genome editing efficiency and accuracy. Journal of Genetics and Genomics. 2021;48(2): 91-102.

**[31]** Doyon, Y., et al., "Transcription activator-like effector nucleases (TALENs) for gene modification. Nature. 2008; 459(7245): 421-425.

**[32]** Gao, C., The future of CRISPR and other genome-editing technologies for plant improvement. Nature Reviews Molecular Cell Biology. 2018; 19(5): 289-291.

**[33]** Kwon, J. H., & Lee, M., TALEN-based approaches for the improvement of biotechnology applications. Biotechnology Advances. 2014;32(5: 648-658.

**[34]** Maeder, M. L., & Gersbach, C. A., Genome-editing technologies for gene and cell therapy. Molecular Therapy. 2016; 24(3): 430-446.

**[35]** Miller, J. C., & Holmes, M. C., An improved TALEN nuclease architecture for highly specific genome editing. Nature Biotechnology.2011; 29(2):143-148.

**[36]** Bogdanove, A. J., & Voytas, D. F., TAL effectors: Customizable proteins for DNA targeting. Science. 2011; 333(6051): 1843-1846.

**[37]** Kang, J., & Kim, J. S. Genome editing with TALENs and CRISPR/Cas9: A comparative review. Current Opinion in Biotechnology. 2015; 32:1-9.

**[38]** Li, J., & Wang, L., TALENs: A powerful tool for plant genome editing. Journal of Biotechnology. 2014; 185: 10-17.

**[39]** Gaj, T., & Gersbach, C. A., ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Molecular Therapy. 2013; 21(5):717-725.

**[40]** Miller, J. C., & Zhang, L., TALENs and other tools for genome editing. Current Opinion in Biotechnology. 2016; 37:10-17.

**[41]** Shukla, V. K., & Doyon, Y., Precise genome modification using zinc-finger nucleases. Nature Biotechnology.2009; 27(9): 881-891.

**[42]** Komor, A. C., & Kim, Y. B.

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016; 533(7603): 420-424.

**[43]** Frangoul, H., & Altshuler, D. CRISPR-based therapies: The new era of personalized medicine. Science Translational Medicine. 2020;12(564): 7326.

**[44]** Anzalone, A. V., & Zhang, F. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785): 149-157.

**[45]** Mendelian, L., & Kintisch, E. Prime editing and its potential in treating genetic disorders. Science Translational Medicine. 2021; 13(587): 4302.

**[46]** Kang, J., & Kim, J. S. Genome editing with TALENs and CRISPR/Cas9: A comparative review. Current Opinion in Biotechnology. 2015; 32: 1-9.

**[47]** Xie, K., & Yang, Y., RNA-guided genome editing for plant biotechnology. Current Opinion in Biotechnology. 2013; 24(2): 229-236.

**[48]** Doudna, J. A., & Charpentier, E., The new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346(6213): 1258096.

**[49]** T. Gaj, S.J. Sirk, S.L. Shui, J. Liu. Genome-editing technologies: principles and applications Cold Spring Harbor Perspect. Biol.,2016; 8(12): 540 -545.

**[50]** Kiran, M., & Smith, L., Gene editing for drug discovery and development: Recent advances and future perspectives. Pharmacology & Therapeutics. 2020; 212: 107562.

**[51]** Shen, B., & Zhang, X., CRISPR/Cas9-mediated gene editing in human disease modeling and drug screening. Molecular Therapy. 2021;29(6): 1612-1626.

**[52]** Jinek, M., & Doudna, J. A., A three-dimensional view of the CRISPR-Cas9 RNA-guided surveillance complex. Science. 2012; 337(6096): 816-821.

**[53]** Ingelman-Sundberg, M., Pharmacogenetics and pharmacogenomics of cytochrome P450 2D6: Clinical implications. *Current Drug Metabolism.* 2017;*18*(3): 223-229.

**[54]** Liu, Y., & Zheng, Z., The role of CRISPR in drug repurposing. *Frontiers in Pharmacology.*2019; *10*: 504.

**[55]** Firth, A. L., & Doudna, J. A., "CRISPR and the Patient-Derived Cell Revolution. *Nature Reviews*

*Drug Discovery.* 2017; *16*(8): 525-526.

**[56]** Pomerantz, J. L., & Auerbach, S. J., Patient-Derived Models in Drug Development. *Journal of Medicinal Chemistry.*2018; *61*(4): 1223-1234.

**[57]** Koblan, L. W., & Miller, J. C. Targeted Gene Editing for Disease Modeling and Therapeutic Development. *Nature Reviews Drug Discovery.* 2021; *20*(3): 222-234.

**[58]** Rohan Ameratunga, Euphemia Leung, See-Tarn Woon, Edward Lea, Caroline Allan, Lydia Chan, Hilary Longhurst, Richard Steele, Russell Snell, Klaus Lehnert. Challenges for gene editing in common variable immunodeficiency disorders: Current and future prospects. Clinical Immunology. 2024; 258:109854

**[59]** Krisztina, B., et al., CRISPR/Cas9 and Off-Target Effects: A Review. *International Journal of Molecular Sciences.*2017; *18*(9):1953.

**[60]** Schaefer, K. A., et al. Unexpected mutations after CRISPR-Cas9 editing in vivo. *Nature Communications.*2017; *8*:143.

**[61]** Chen, J. S., et al. CRISPR-Cas9 genome editing: a review of the current state of the art." *Journal of Gene Medicine.* 2018; *20*(1): e3000.

**[62]** Garber, K., Toward a new era of personalised medicine. Science. 2015; 350(6264): 650-652.

**[63]** I. Hatada, T Horii, Genome editing comes of age, Nat. Protoc., 2016;11:1573-1578.

**[64]** Clement, K., et al., CRISPResso2 provides accurate and robust analysis of CRISPR genome-editing experiments. *Nature Biotechnology.,*2019; *37*(5): 455-458.

**[65]** https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcSIphXrCqEHalDZChTzt0vukLGCtZEqFFTguA&usqp=CAU

**[66]** https://labassociates.com/wp-content/uploads/2022/10/crispr-720x720.jpg

**[67]** https://ars.els-cdn.com/content/image/1-s2.0-S2667370323000334-ga1_lrg.jpg