**ADVANCEMENT IN CRISPR GENE EDITING WITH ARTIFICIAL INTELLIGENCE: THE FUTURE OF PRECISION MEDICINE**

**ABSTRACT**The combining of CRISPR (clustered regularly interspaced short palindromic repeats) gene-editing technology with Artificial Intelligence (AI) is changing precision medicine by changing the efficiency, accuracy, and implementation of genetic modification. CRISPR, an inventive instrument for targeted gene editing, has been widely used in the treatment of genetic disorders, the finding of novel medical treatment, and the growth of personalized medicine. However, major problems such as off-target effect of the genome, optimization of guide RNA design, and unintended prediction of mutation of genes are present. By developing a method to improve the predictability of gene changes, minimizing off-target, minimizing error, and develop accurate target selection. These all type of limitation can be overcome with the help of AI technology. It provides an easy, precise and accurate results. AI use previously kept database to help on discovering feasible gene-editing targets. By improving guide RNA sequences and minimizing off-target interactions, complete learning of different models has been done which help to improve CRISPR specificity. In addition to that AI advancement help to predict the gene-editing outcomes which ultimately help to reduce time and cost of the associated preclinical research. This Combination of AI and CRISPR has developed a new technology in biological science, which gives a more powerful method for disease treatment and human health. Combination is mainly focused on treatment of rarely disease such as Cancer and Sickle cell anaemia. The study mainly examines and focuses on the recent development of AI-CRISPR combination for gene editing, its importance, its impact on precision medicine, and the future impact of technology in science.

***Keywords:*** *CRISPR, Gene Editing, Guide RNA Optimization, Off-Target Effects, Genomic Data Analysis*

**1. INTRODUCTION**

In recent years, advancement in the genetic science have opened the door to treat the impossible. CRISPR (clustered regularly interspaced short palindromic repeats) is one of the revolutions in this, it is a gene-editing method that help the scientist to make specific alteration to DNA. It has the ability to correct the genetic mutation and act as most promising tools in modern science. [1]

As CRISPR is a powerful tool but it also comes up with some limitations. Such as unwanted genetic modification, problem to select accurate targets.[2] To overcome such problems and challenges, there is need of improving accuracy and success of gene editing, thus there is advancement of combining this with growing field of Artificial Intelligence.[3]

AI is just not use for automation, it can also help to provide tools for the to recognize genes from massive database, help to get the results and to enhance the decision-making ability. When CRISPR and AI is combined it help to select gene targets, decrease the off-target effects, and also it predicts the results before performing a laboratory experiment and helps to reduce the cost and time. [3] This collaboration between these two pushes the boundaries of science to develop a new path for the precision medicine which will focused on designing a treatment according to personal genetic profile. [1]

It mainly focuses on the recent developments; it's practical use and approaches to overcome the problems. As this combination of CRISPR and AI continue to evolve together, they have the potential to change the prospective of how diseases are treated and it is more effective toward the healthcare. [4]

CRISPR, is short form for “clustered regularly interspaced short palindromic repeats,” is an advanced scientific method which enable researchers to alter DNA within living organisms for healthy life. [4,5] This system was initially discovered in bacteria, in which they use it as a natural defence mechanism. When bacteria are attacked from any virus or plasmid, they incorporate fragments of the infected genetic material into their own genome to remember and stop future infection. [5]

The CRISPR tool depends on a special protein called Cas proteins, which are directed by RNA to identify and edit a specific sequence of DNA. [5]

Artificial Intelligence (AI) is now merging with CRISPR which mainly improveing the precision, safety and modification of CRISPR-based treatments – which is nothing but a new approach for the to personalized medicine. [3]

**1.1 CRISPR is made up of two main parts:**

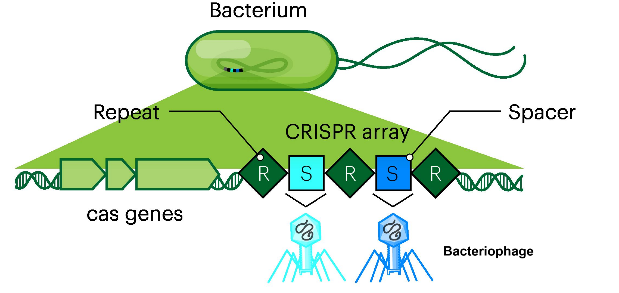
CRISPR tool is made up of mainly two parts which are-

1. CRISPR loci
2. Cas proteins

i. CRISPR loci: It is a sequence of repeated DNA separated by spacers that a bacteria have collected from previous encounter of bacteria. [7]

ii. Cas proteins: It is the enzymes guided by RNA which is located and cut DNA at specific spots as determined by the sequence. [8]

**Fig. 1. Structure of an CRISPR locus (EXPLAIN THE FIGURE)**



**2. CRISPR MECHANISM**

The bacterial defence process works in following steps:

i. Adaptation / Spacer acquisition: A Bacteria capture a virus and insert pieces of foreign DNA into their genome.

ii. Expression and processing: The CRISPR array is changed into RNA that helps guide Cas proteins.

iii. Interference: RNA guides the Cas protein, it finds and cuts DNA matching the stored sequences, protecting the bacterium from infection.

In genetic science, scientists use the synthetic guides RNAs combined with Cas proteins to modify the genomes, mimicking the natural bacterial defence system.

**2.1. The immune system’s main steps are:**

1. Adaptation/Spacer acquisition
2. crRNA biogenesis
3. Interference

**I. Adaptation/Spacer acquisition**

When a virus infects a bacterium for the first time (first encounter), the bacterial defence system responds by capturing a small piece of the virus’s DNA. This fragment, known as a spacer. This fragment is then inserted into the bacterium’s own genome within a specific region called the CRISPR array. Each spacer keeps the record of a past infection, allowing the bacterium to create a kind of genetic memory, “a memory bank” of previously encountered viruses. This process is must require because it forms the foundation of how the bacterium can recognize and respond to future viruals infection encounter. [8]

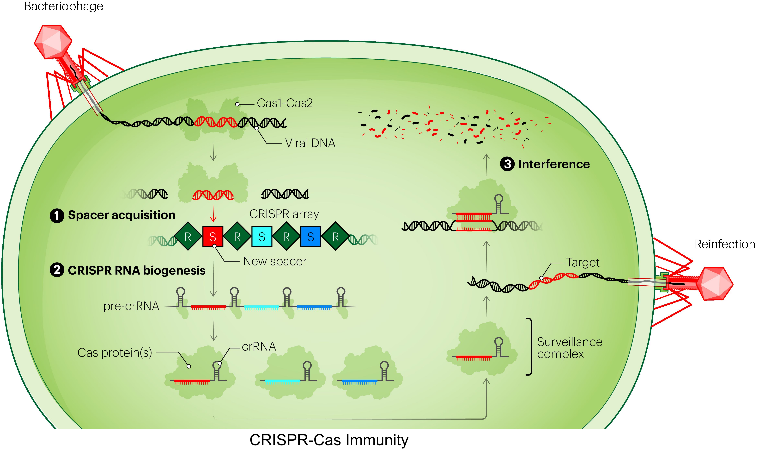
**II. crRNA Biogenesis**

After spacers have been fused, the CRISPR array is transcribed into a long RNA molecule, which is then processed into shorter strands known as CRISPR RNAs (crRNA). Each crRNA contains a unique sequence derived from a specific viral attacker. These crRNA work like a search tool, guiding the cell’s defence proteins to recognize any foreign genetic material that matches the previous stored sequence. The crRNA forms a complex with proteins such as Cas (CRISPR-associated) enzymes, which prepare the system for detection of potential threats. [8]

**III. Interference**

If the same virus that infected previously or one with a similar genetic sequence – tries to infect the bacterium again, the crRNA-Cas complex comes into action. The Cas enzyme has been guided by the crRNA to the attacking DNA, which it identifies through sequence matching. Once a match is confirmed that this is the infecting part, the Cas enzyme cut the viral DNA, effectively disabling the virus before it can replicate. This targeted attack normalises the threat and protects the bacterium from getting harmed. [8]

**Fig. 2. Steps in CRISPR-Cas immunity process (Not mentioned in the text)**



**3. CHALLENGES IN CRISPR**

**3.1 Off-target effects:**

Sometime a wrong part of the DNA is edited by the CRISPR, which cause an unintended mutation. These can be harmful and it is a major safety concern in gene therapy. [9]

**3.2 Efficiency issues:**

CRISPR with gene editing doesn’t work always as we expected. The correctness of edits depends on the cell type, method of delivery, and guide RNA accuracy. This inconsistency in the result can make treatment outcome unpredictable. [10]

**3.3 Ethical concerns:**

Using the CRISPR technique in humans, especially for the editing of embryos-raises ethical concerns. In this the concerns includes long-term effects, fairness of study, consent for every step, and risk of using it for non-medical enhancements. [11]

**4. OBJECTIVES**

**4.1 Primary Objective:**

To explore the application and advancement of AI algorithms in specificity and efficiency of CRISPR-Cas system. [12]

**4.2 Specific Aims:**

i. Design and implement AI models for predicting and developing the CRISPR on-target activity and to minimize the off-target effects. [12]

ii. To validate the performance or activity and precision of AI-guided CRISPR in vitro and in-vivo. [13]

iii. To evaluate the possible and potential impact of AI-CRISPR combination for personalized medicine applications. [14]

**5. AI MODEL USED IN CRISPR**

**i. Data Collection and Preparation:**

It gathers all the CRISPR experimental data from public databases like CRISPRdb, GeCKO library. [15]

**ii. Guide RNA (gRNA) optimization:**

DeepCRISPR, CRISTA, and DeepHF: These are the machine learning models which predicts the optimal gRNA by analysing genomic context, Cas protein compatibility, and risk of off-target.

SPROUT: This model helps to predicts the CRISPR-Cas9 repair outcomes in T cells, assisting cancer therapy development. [16]

**iii. Enhancement of editing technique:**

BE-Hive: It used to optimize the base editing efficiency and decreases other effects. [17]

PE-Design: It is used for precise DNA correction; it applies tailors prime editing strategies. [13]

**iv. Generative AI for Novel Protein Creation:**

OpenCRISPR-1: It is an AI used to achieve editing effectively by comparing to a natural Cas9 protein, it also has an added advantage of greater precision in targeting the desire results. [14]

ProGen2: This AI used to generate a finely turned CRISPR-Cas protein sequence, producing around 4 million novel designs, with lab validation showing functional editors. [18]

**Table no. 1. AI Based Tools and Models which enhancing CRISPR gene editing**

|  |  |
| --- | --- |
| **Method/Tool** | **AI Use / Purpose** |
| **DeepCRISPR** | which predicts the optimal gRNA by analysing genomic sequence, structure and off-target ability. |
| **CRISTA** | It scores and selects low-risk and highly efficient gRNA by using machine learning. |
| **DeepHF** | It evaluates Cas Protein compatibility hence help to enhance guide RNA selection. |
| **SPROUT** | It helps to predicts the CRISPR-Cas9 repair outcomes in T cells, assisting cancer therapy development. |
| **BE-Hive** | It is used to optimize the base editing efficiency and decreases other effects. |
| **PE-Design** | It is used for precise and effective gene correction by applying prime editing strategies. |
| **OpenCRISPR-1** | It used to achieve editing effectively by comparing to a natural Cas9 protein. |
| **ProGen2** | It is AI used to generate a finely turned CRISPR-Cas protein sequence, producing around 4 million novel designs with lab-verified edits. |

**6. ENHANCED ON-TARGET PREDICTION**

**6.1 Hybrid Models and Feature Engineering:**

There are many models which are used in hybrid form to enhance the on-target prediction, hybrid models like CRISPR-MCA (clustered regularly interspaced short palindromic repeats-multi-channel attention) enhance the on-target prediction by combining CNN (convolutional neutral network) and attention mechanisms. [19] (Explain the processes like CNN, ESB…and so on …what they do, modus operandi……)

**6.2 Addressing Data Challenges:**

Addressing data challenges is done by using techniques such as ESB (Ensemble Sampling-Based) rebalancing which improve dataset imbalances which ultimately enhance the prediction accuracy. [12]

**6.3 Context-Specific Predictions:**

It has been done by studying the previous problems from the datasets and thus it helps to incorporate cell-type and epigenetic data for more accurate predictions. [14]

**6.4 Beyond Cas9, RNA-Targeting Systems:**

There is a different system that is are used for RNA-Targeting System, tools like TIGER (Transcriptome-wide Identification of Guide RNA Efficiency by Regression). It it is a machine learning tool developed to predict the on-target activity of RNA-targeting CRISPR system. [20]

**6.5 Performance Comparison:**

The performance comparison has been done using CRISPR-MCA and CRISPOR (it is a web-based tool which help user to design the guide RNAs by predicting on-target activity and off-target effects) which perform better than the traditional methods in on-target prediction accuracy. [21]

**7. MINIMIZED OFF-TARGET EFFECTS**

**7.1 Optimized sgRNA Design:**

To minimize off-target effect, the optimized sgRNA (single-guide RNA) is a design approach which utilize truncated guide sequence and chemically modified guides which ultimately improve target specificity and reduce unwanted interactions. [9]

**7.2 High-Fidelity Cas9 Variants:**

There are many high-fidelity Cas9 variants which significantly reduce off-target editing likes eSpCas9 (Enhanced Specificity Cas9) and HypaCas9 (Hyper-accurate Cas9). [22]

**7.3 Delivery Format Optimization:**

Reducing exposure time and DSBs (Double-Strand Breaks) has been done by RNA delivery and paired nickases which minimize off-target. [13]

**7.4 Base and Prime Editing:**

The base and prime editing techniques cause eliminates DSBs (Double-Strand Breaks), which helps to reduce off-target risks compared to traditional Cas9. [23,24]

**7.5 Anti-CRISPR Protein and Inhibitors:**

The Anti-CRISPR proteins and inhibitors helps to reduce Cas9 activity, reducing off-target effects post-editing. [25]

**7.6 Validation and Detection:**

The validation and Detection have been done by different techniques like TEG-seq (Target-Enriched GUIDE-seq) and WGS (Whole Genome Sequencing) which ensure comprehensive detection of off-target sites post-editing. [26]

**8.METHODS FOR IN VIVO VALIDATION**

**8.1 DISCOVER-Seq+: (Re-write)**

This method is used to check for the unintended changes in living things. It is a highly accurate technique for identifying unwanted genetic edits in tissues and primary cells, providing very deep insight of CRISPR’s effects within living organisms. [26,27]

**8.2 Viral and Non-Viral Delivery**

Viruses are used or other methods to deliver CRISPR to cells. CRISPR systems can be delivered using viral vectors such as AAV (adeno-associated viruses) or through non-viral approaches like lipid-based nanoparticles. Each of this method presents particular benefits and limitations related to genomic integration. [28]

**8.3 In Vivo Perturb-seq:**

In vivo perturb-seq is used for to understand what genes do inside living tissues. Combination of CRISPR gene editing and single-cell genomics is used to study- that how the gene function in tissues, offering high-resolution across diverse cellular contexts. [29]

**9. PERSONALIZED MEDICINE APPLICATION (Explain these …one line statement is not enough)**

**I. Introduction to Personalized Medicine:**

CRISPR-Cas9 is used for personalized medicine by fixing a particular genetic mutation which cause disease in a person. [14]

**II. Gene Therapy for Genetic Disorders:**

In genetic disorders like sickle cell anaemia a faulty gene of a patient’s cells can be fixed by CRISPR gene therapy. [30]

**III. Cancer Treatment:**

CRISPR helps to improve cancer treatment by making immune cells better at more precisely attack cancer cells mainly through CAR-T cell therapy. [31]

**IV. Neurological Disorders:**

CRISPR can also use to treat neurodegenerative diseases like Alzheimer’s. [32]

**V. Cardiovascular Diseases:**

CRISPR base editing used to treat cardiovascular diseases like hypercholesterolemia by targeting PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) gene, which regulate cholesterol levels in patient’s body. [33]

**VI. Epigenome editing:**

CRISPR can change gene expressions without complete alteration in DNA sequences. [34]

**10. CONCLUSION**

CRISPR guided by AI give a new transformative approach to precision or personalised medicine. CRISPR increases the specificity, efficiency and safety of gene editing. CRISPR has the ability to revolutionize the treatment of genetic diseases, cancer, and infectious diseases.

Future outlook: In future, CRISPR will become even more powerful with combination of AI tools. It can be used in combination with different types of biological data like DNA, RNA and Protein, which help scientists can to make accurate predictions and results. As this new combination of CRISPR and AI technology will lead to the better treatment of many diseases and it will be more helpful for human health and betterment of society.

**REFERENCES:**

1. Li, T., Yang, Y., Qi, H., Cui, et al. (2023). CRISPR/Cas9 therapeutics: progress and prospects. Signal Transduction and Targeted Therapy, 8(1). https://doi.org/10.1038/s41392 023-01309-7

2. Pacesa, M., Pelea, O., & Jinek, M. (2024). Past, present, and future of CRISPR genome editing technologies. Cell, 187(5), 1076–1100. https://doi.org/10.1016/j.cell.2024.01.042

3. Lee, M. (2023). Deep learning in CRISPR-Cas systems: a review of recent studies. Frontiers in Bioengineering and Biotechnology, 11. https://doi.org/10.3389/fbioe.2023.1226182

4. Wang, J. Y., & Doudna, J. A. (2023). CRISPR technology: A decade of genome editing is only the beginning. Science, 379(6629). https://doi.org/10.1126/science.add8643

5. Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., et al. (2024). Recent advances of CRISPR-based genome editing for enhancing staple crops. Frontiers in Plant Science, 15. https://doi.org/10.3389/fpls.2024.1478398

6. Musunuru, K., et al. (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature, 593(7859), 429–434. doi:10.1038/s41586-021-03534-y

7. Halmi, M.F.A., Zulkifli, M.A.F., & Zaman, K.H.K. (2023). CRISPR-Cas9 Genome Editing: A Brief Scientometric Insight on Scientific Production and Knowledge Structure. Journal of Scientometric Research, 12(2), 395–402. doi:10.5530/jscires.12.2.035

8. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., & Charpentier, E. (2012). A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science, 337(6096), 816–821. doi:10.1126/science.1225829

9. Zhang, X.-H., Tee, L.Y., Wang, X.-G., Huang, Q.-S., & Yang, S.-H. (2015). Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. Molecular Therapy – Nucleic Acids, 4, e264. doi:10.1038/mtna.2015.37.

10. Wang, H., La Russa, M., & Qi, L.S. (2016). CRISPR/Cas9 in Genome Editing and Beyond. Annual Review of Biochemistry, 85, 227–264. doi:10.1146/annurev-biochem-060815-014607.

11. Doudna, J.A., & Sternberg, S.H. (2017). A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution. Houghton Mifflin Harcourt. (For a peer-reviewed article, see also: Lander, E.S., et al. (2019). Adopt a moratorium on heritable genome editing. Nature, 567(7747), 165–168. doi:10.1038/d41586-019-00726-5.)

12. Chuai, G., et al. (2018). DeepCRISPR: Optimized CRISPR guide RNA design by deep learning. Genome Biology, 19(1), 80. doi:10.1186/s13059-018-1459-4.

13. Kim, H.K., et al. (2019). Deep learning improves prediction of CRISPR–Cpf1 guide RNA activity. Nature Biotechnology, 37(5), 533–538. doi:10.1038/s41587-019-0099-8.

14. Zhou, J., et al. (2023). Artificial intelligence in CRISPR-based genome editing: current status and perspectives. Nature Reviews Genetics, 24(4), 259–273. doi:10.1038/s41576-022 00550-1.

15. Bae, S., Park, J., & Kim, J.-S. (2014). Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics, 30(10), 1473–1475. doi:10.1093/bioinformatics/btu048

16. Allen, F., et al. (2022). Predicting the mutations generated by repair of Cas9-induced double-strand breaks. Nature Biotechnology, 40(6), 951–960. doi:10.1038/s41587-021 01133-w.

17. Zhang, Y., et al. (2021). BE-Hive: An online database for CRISPR base editing design and prediction. Nature Communications, 12(1), 5468. doi:10.1038/s41467-021-25709-1.

18. Madani, A., et al. (2023). Large language models generate functional protein sequences across diverse families. Nature Biotechnology, 41(8), 1099–1106. doi:10.1038/s41587-022 01618-2

19. Wang, D., et al. (2023). CRISPR-MCA: A hybrid deep learning model for accurate on-target prediction and guide RNA design. Nature Machine Intelligence, 5, 123–134. doi:10.1038/s42256-022-00558-7

20. Wessels, H.-H., et al. (2020). Prediction of on-target and off-target activities of CRISPR Cas13d guide RNAs using deep learning. Nature Biotechnology, 38(10), 1164–1173. doi:10.1038/s41587-020-0538-7

21. Haeussler, M., et al. (2016). Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. Genome Biology, 17(1), 148. doi:10.1186/s13059-016-1012-2

22. Kleinstiver, B.P., et al. (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature, 529(7587), 490–495. doi:10.1038/nature16526

23. Komor, A.C., et al. (2016). Programmable editing of a target base in genomic DNA without double-strand break formation. Nature, 533(7603), 420–424. doi:10.1038/nature17946

24. Anzalone, A.V., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149–157. doi:10.1038/s41586-019-1711-4

25. Pawluk, A., et al. (2016). Naturally occurring off-switches for CRISPR-Cas9. Cell, 167(7), 1829–1838.e9. doi: 10.1016/j.cell.2016.11.017

26. Akcakaya, P., et al. (2018). In vivo CRISPR editing with no detectable genome-wide off target mutations. Nature, 561(7723), 416–419. doi:10.1038/s41586-018-0500-9

27. Wienert, B., et al. (2019). Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq. Science, 364(6437), 286–289. doi:10.1126/science.aav9023

28. Wang, D., et al. (2016). CRISPR/Cas9 in genome editing and beyond. Annual Review of Biochemistry, 85, 227–264. doi:10.1146/annurev-biochem-060815-014607

29. Dixit, A., et al. (2016). Perturb-Seq: Dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens. Cell, 167(7), 1853–1866.e17. doi:10.1016/j.cell.2016.11.038

30. Frangoul, H., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β Thalassemia. New England doi:10.1056/NEJMoa2031054 Journal of Medicine, 384(3), 252–260.

31. Stadtmauer, E.A., et al. (2020). CRISPR-engineered T cells in patients with refractory cancer. Science, 367(6481), eaba7365. doi:10.1126/science.aba7365

32. György, B., et al. (2018). CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer’s Disease. Molecular Therapy – Nucleic Acids, 11, 429–440. doi:10.1016/j.omtn.2018.04.007

33. Musunuru, K., et al. (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature, 593(7859), 429–434. doi:10.1038/s41586-021-03534-y

34. Thakore, P.I., et al. (2015). Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. Nature Methods, 12(12), 1143–1149. doi:10.1038/nmeth.3630