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Review Article

CRISPR-Cas9 gene editing technology in human gene therapy: the new realm of medicine

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ABSTRACT

Gene therapy has a huge clinical relevance in the present therapeutic world and is one of the many research fields of biology which received many benefits from the recent advancements of modern clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing technology. Researchers are on the way to make significant changes in the ways of treating genetic abnormalities. An increase in the number of approved clinical trials of CRISPR based gene therapy shows we are not too far from eliminating deadly diseases such as acquired immunodeficiency syndrome (AIDS), cancer and many inherited genetic conditions from the society. However, there are some challenges associated with the development of CRISPR technology in medical field most of which revolves around its safety, efficiency and ethics. Lack of an optimized method by which the CRISPR-Cas9 expression cassette can be delivered to cells is one of the main challenges when it comes to its application in human gene therapy. Although viral vectors are the most common delivery systems used in gene therapy, recent researches show promising results on using lipid- based l delivery systems such as liposome-templated hydrogel nanoparticles (LHNPs). As these could eliminate the safety concerns of using viral vectors, it is expected to have potential therapeutic applications in future. Nevertheless, the efficiency of non-viral systems is still not fully comparable with that of viral vectors. Hence, CRISPR based therapies might take longer than expected to be prevalent in the medical field. In this short review, the recent advances of CRISPR technology in gene therapy is discussed along with its challenges and limitations.

Keywords: CRISPR-Cas9, Gene therapy, Gene editing, LHNPs

INTRODUCTION

Gene editing is an emerging molecular biology technique which allows scientists to alter the genetic material of organisms by adding, deleting, modifying or replacing genes within their deoxyribonucleic acid/ribonucleic acid (DNA/RNA). Although there are now a variety of gene editing approaches such as zinc-finger nucleases (ZFNs), homing endonucleases, and transcription activator-like effector nucleases (TALENs) available, Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology is said to be the one that revolutionizes the medical field.[1] CRISPR technology has already been successfully used to make changes in the genetic makeup of a wide range of cells and organisms ranging from virus to human embryos. Applications of CRISPR-Cas9 in various medical researches including that of gene studies, disease modelling, therapeutics and diagnosis is being investigated by researchers. Apart from medical research, CRISPR also dominates in other industries as well. In agriculture sector, CRISPR has found to give promising results in the development of genetically modified crops with more improved beneficial characteristics that had never been achieved before with other gene editing tools. Plants with good quality and high yield, adapted to climate changes, and disease resistance is going to help humanity in food production crisis that could occur in future as the world's population is estimated to reach 9.6 billion by

2050.[2] In synthetic biology, CRISPR-Cas system has potential applications as genetic recorders which helps in lineage tracing, molecular barcoding, and in intracellular biological activity recording.[3] Recent researches also outline the advancement of synthetic biology in conjugation with CRISPR technology for developing novel therapeutics and treatments by identifying new drug targets.[4]

Gene therapy is one of the most promising and emerging applications of CRISPR technology in modern medicine. As a therapeutic type which focuses on treating diseases by modifying faulty genes of cells, gene therapy has had great benefits from this recently discovered gene editing tool. With the development of gene editing technologies, medical field has been witnessing the evolution of gene therapy strategies since 1900 when the first gene therapy clinical trial was approved for treating patients with adenosine deaminase-severe combined immunodeficiency (ADA-SCID).[1] In this short article, the most recent advancements of CRISPR-Cas 9 technology in human gene therapy are highlighted along with a little background on this new gene editing technology and gene therapy.

Evolution of CRISPR-Cas9 technology

CRISPR-Cas 9, the powerful tool of modern gene editing era has been first described as a DNA repair mechanism of bacteria following the discovery of a repeating DNA sequence in *E. coli* in 1987 by a Japanese scientist, Yoshizumi Ishino. This repeating DNA sequence with different spacer sequences in between them is named as CRISPRs by researchers Francisco Mojica and Ruud Janse. Later researches conducted by the same researchers revealed that the spacer sequence in CRISPR is identical to the sequences present in viruses those infect bacteria (called bacteriophages) and it acts as defensive mechanism against bacteriophages.[5] Spacers which carry the sequence of previously infected virus gets transcribed to form short CRISPR RNAs (crRNAs) upon the next invasion of same virus. This crRNAs function as a guide for CRISPR-associated sequence (Cas) protein to target and cleave the complimentary sequence found in infected virus. In this way, the role of CRISPR-Cas system in bacteria has been characterized as an important adaptive immune response against bacteriophages.[5] Further researches were done on the components involved in the CRISPR-Cas system to understand this amazing defensive mechanism in depth. As a result, the site-specific nuclease activity of Cas was identified, whereas crRNA found to form duplex with trans-activating CRISPR RNA (tracrRNA) to accomplish its function as a guide for Cas to target DNA. The association crRNA which contains complementary sequence of target DNA and tracrRNA which act as a binding space for the Cas nuclease were later termed as a guide RNA (gRNA).[6] In addition to Cas protein and gRNA, in 2012, researches led by Charpentier and Doudna reported an another essential component of CRISPR-Cas system called protospacer adjacent motifs (PAMs) which is found upstream of target sequence. This short base-pair sequence makes sure only the specific nucleic acids of interest is cleaved by Cas 9 as the nuclease doesn't start cleavage until it recognizes PAMs.[7] In the same year of 2012, a group of researchers led by Jennifer Doudna and Emmanuelle Charpentier designed the first guide RNA to cut DNA sequences of a predetermined site. They proved that the CRISPR-Cas system could be controlled so that they can edit any DNA molecules of interest at a specific site.[8] This breakthrough research in genetic engineering won them the Nobel prize in 2020 in Chemistry.[9] Overall, the most powerful gene editing technology, CRISPR is a result of efforts done by multiple researchers and has an unexpected starting point.

CRISPR is now being used to manipulate DNA for a wide range of applications. As CRISPR could overcome most of the limitations of other gene editing tools, it took no time for CRISPR to become popular in life science researches. One of the main advantages of CRISPR-Cas system is that it doesn't need a separate protein to recognize the target site as it has gRNA to mediate the nuclease activity and these gRNAs can be designed readily. This makes the designing of CRISPR-Cas system easy and cost effective by eliminating the need of protein engineering unlike ZFNs and TALENs. Moreover, the ability of CRISPR to multiplex enable researchers to introduces changes in more than one target sites simultaneously in the same cell by using multiple guide RNAs in parallel. Another potential advantage of CRISPR is that it saves time and effort of going through long lab procedures such as protein design, transfection, selection of required cells and optimization. These striking characteristics makes CRISPR-Cas system highly efficient, easy to use and cost-effective.[10] However, there are some concerns about the specificity of CRISPR because the nuclease is found to target sequences which are not an exact match of gRNA. Numerous researches have been going on to eliminate the off-target issue of CRISPR technology and thus increase its specificity in gene editing.[11] One of the other popular researches in CRISPR is the study to figure out the most efficient mode of CRISPR-Cas9 delivery to mammalian cells as the size of cDNA coding Cas9 is large.[12] Resolving these two major disadvantages of CRISPR-Cas system could unravel many of its potential applications in therapeutics.

Human gene therapy

Gene therapy is a gene modification technique which is intend to produce therapeutic effects in abnormal cells. It involves inactivation or replacement of disease- causing gene to prevent or treat disease. Development of gene therapy has gone through many challenges and failures but it has always been considered as one of the biggest achievements of medical field as it could bring cure to many diseases which were once thought as incurable. After the success of first gene therapy trial in 1990, it met a tragic situation in 1999 when an 18-year-old boy who signed up for gene therapy trial died due to immune reaction to the treatment.[13] As a result, further

development of gene therapy was in a slow pace with more precautions and safety measures. However, it bounced back to therapeutic field in 2003 when the first gene therapy called gendicine has been approved in China for head and neck cancer. Followed by China, Russia and European Commission approved neovasculgen for peripheral artery disease in 2011 and Glybera for lipoprotein lipase deficiency respectively.[13] Unfortunately, Glybera had to leave the medical field in 2017 because of its high cost of €1M for a single dose.[14] Several advancements in vector engineering, gene editing and gene delivery techniques helped gene therapy to pick up a rapid pace of development in such a way that by the year of 2025, Food and Drug Administration (FDA) expects to grant approval for approximately 10 to 20 cell and gene therapies every year.[15]

Up to date, there are about 2600 gene therapy trials going on for curing various conditions including genetic disorders such as hemophilia, cystic fibrosis, and cancer and cardiovascular diseases, rare diseases and neurodegenerative diseases. Cancer is on the top of list of diseases treated by gene therapy followed by monogenic disorders.[16] Until November 2017, more than 65% of greater than 2597 clinical trials conducted on gene therapy was for cancer. But, for some reasons, only 22 gene therapy products got approved by August 2019 for the management of different diseases. Immunotherapy including the popular one using engineered CAR-T cells are found to have promising results in cancer treatment.[17] Some of the most recent gene therapy medications approved by regulatory bodies includes zolgensma, zynteglo, and abecma. Zolgensma was approved by FDA in May 2019 for spinal muscular atrophy. Affected children under the age of 2 is administrated with zolgensma which delivers a healthy copy of human SMN gene to restore the normal function of motor neurons. Zynteglo which contains healthy copy of gene responsible for making hemoglobin has gained approval from E.U. in May 2019 to treat individuals of 12 years and older with beta thalassemia.[18] Abecma, approved in March 2021 by FDA is the first CAR-T cells-based gene therapy for the treatment of adult patients with multiple myeloma.[19]

Even though gene therapy is expected to become one of the standard treatments for many diseases in future, it is still going through many challenges with regards of safety, quality, stability, cost and long-term risks. Currently, gene therapy is allowed in only somatic cells considering its possible potential effects that could occur on offspring.[20] The use of viral vectors such as retrovirus and adenoviruses to deliver the therapeutic gene in cells during gene therapy has always attracted serious safety concerns.[21] Follow up studies conducted in patients who received viral vector mediated gene therapies reported that the patients developed cancer due to insertional mutagenesis.[15] Therefore, questions have risen about the efficiency of gene therapy to deliver the gene at a pre-determined site. Moreover, the production of high-quality products, storage, distribution and transportation of gene therapy are also challenging in prospective of manufacturing companies.[22] Furthermore, ethical issues surrounding the mode of action and cost of gene therapy products are also discussed in medical field. The most expensive drug in the world is a gene therapy called zolgensma which costs £1.795 million for a single dose.[23] Hence, not everyone can afford the price of gene therapy which restricts its use to only wealthy people around the world. Difficult questions such as: Are humans allowed to change their god given traits by gene therapy? Is it fair to put such a high price tag for human life? Is it another representation of human discrimination between rich and poor? Will human gene therapy able to be controlled in future? -remains unanswered by regulatory bodies.

DISCUSSION

Gene editing using CRISPR-Cas9 could make significant changes in the realm of gene therapy. The potential use of this modern gene editing technology in gene therapy has revolutionized the filed by overcoming many limitations of conventional gene therapy approaches. Easy to use, efficiency, versatility, and simplicity is the main advantages of CRISPR. It has helped researchers to precisely edit the disease-causing gene of interest located in the specific locations.[1] However, CRISPR technology also have its own limitations and challenges. One of the main hurdles of CRISPR technology that needs to overcome is the lack of a perfect delivery system as the direct delivery of CRISPR/Cas9 is not possible because of its instability and susceptibility to digestion by endonuclease.[24] Extensive researches have been going on to develop an efficient method for delivering gDNA to cells so that maximum number of required cells could receive it as well as off target effects can be avoided. AAV, the most commonly used viral vector in gene therapy shows high efficiency in terms of delivering the therapeutic gene as compared to non-viral vectors. Because of that, AAV vector mediated delivery of Cas9 and gRNA remains as a key mode of delivery procedure in CRISPR gene therapy too.[1] If researchers could optimize non-viral systems such as plasmids, nanoparticles, electroporation, microinjection and liposomes for the efficient delivery of CRISPR/Cas9 in cells, it could eliminate several issues associated with viral vectors including insertional mutagenesis, less insertional size, off target effects, immunogenicity, cytotoxicity, and the expensive large-scale production.[25] Generally, the use of electroporation and microinjection is limited to only cultured cells, whereas plasmids containing CRISPR toolkit has found difficult to be delivered due to large size and also have a delayed therapeutic effect.[25] Overall, every delivery system available so far has its advantages and challenges.

Recent research studies on different non-viral systems come up with new techniques which could be used in clinical translation of CRISPR gene therapies in near future without any safety concerns. Liposomes are one of the most widely studied CRISPR/Cas9 carriers because of

its excellent biocompatibility and biodegradability. Out of different types of liposomes, neutral liposomes are found to be efficient for in vivo applications in terms of endosomal escape and tumor accumulation but their cellular uptake rate and CRISPR/Cas9 loading is still need to be improved.[24] In 2017, Chen et al could demonstrate the efficiency of synthesized liposome-templated hydrogel nanoparticles (LHNPs) in the target delivery of CRISPR/Cas9 in brain cancer cell lines.[26] Administration of LHNPs in mouse models with brain tumor showed considerable decrease in tumor cell proliferation which is promising in the advancement of CRISPR delivery systems.[26] Most recently, researchers from University of New South Wales (UNSW) Sydney modified liposomes with a photosensitizing drug called verteporfin on the membrane which triggers the release of CRISPR only under the light illumination of 690 nm wavelength.[27] This interesting strategy establishes a control over the expression of CRISPR/Cas9 and makes the gene editing technology safer in clinical applications. Hopefully, this will be commercially available soon to treat various diseases.

Apart from liposomes, other nanocarriers such as polymers, inorganic nanoparticles, peptides and LNPs are also being widely subjected to clinical studies for in vivo CRISPR-Cas9 delivery. Although polymer and lipid-based strategies were used for CRIPR-Cas 9 delivery in clinical trials of various diseases, their efficiency is still under question when compared to viral vectors.[28] Recently, a novel modifiable LNP platform called selective organ targeting (SORT) drew the attention of researchers due to its possible potential applications in delivering CRISPR-Cas9 cargoes in specific tissues such as heart, kidney and brain.[29] In 2020, Intellia Therapeutics' ongoing clinical trial named NTLA-2001 has used LNP based CRISPR-Cas9 in patients suffering from transthyretin amyloidosis. The first clinical data from the trial shared by Intellia Therapeutics in June 2021 shows promising results in the management of disease along with excellent safety profile. As the safety and efficiency of gene editing tools depend on its mode of delivery in cells to a greater extend, further developments in the area of non-viral vectors are necessary to increase the number CRISPR based gene therapy with 100 percentage safety assurance in medical field.[30]

In 2016, the first human clinical trial involving CRISPR-Cas9 was conducted in China for the treatment of metastatic non-small cell lung cancer. In that ex vivo cell-based gene therapy, CRISPR was used to knockout PD1 gene as this would help cells to reactivate immune response.[31] Followed by this, FDA granted approval for U. S's first Phase 1 clinical trials of CRISPR based gene editing in 2018. It allowed scientists to edit T cells collected from patients suffering from different type of cancers including melanoma, multiple myeloma, and synovial sarcoma and use these manipulated healthy T cells for immunotherapy.[1] Since then, several CRISPR based trials have been opened up, most of which is still for cancer immunotherapy. Other than cancer, FDA also approved CRISPR mediated human trial for diseases such as sickle-cell anemia, and β-thalassemia in 2018.[32] EDIT-101 also known as AGN-151587 is the first in vivo CRISPR gene therapy in which was approved by FDA in 2018. EDIT-101 is designed to correct a point mutation in CEP290 for treating blindness.[33] Ongoing in vivo CRISPR based therapies such as EDIT-101 and NTLA-2001 shows promising initial results in terms of efficiency and safety.[34] This marks an important breakthrough in CRISPR technology. Successful completion of these trials might open up a new era in the medical filed where treatments for all genetic abnormalities are possible. To date, there are more than 20 CRISPR therapies are listed in clinicaltrials.gov.[35]

Countless efforts have been going on all around the world to improve the efficiency CRISPR technology since its discovery. CRISPR technology basically utilizes cell's natural DNA repair machinery which is triggered by double stranded breaks (DSBs) generated by Cas9 to manipulate the genome. Interestingly, recent advancements in CRISPR research proposes two new strategies in the technology to increase the precision in gene editing without creating DSBs. They are prime editing and base editing. Base editing is good in introducing point mutations in the genome, whereas prime editing is more versatile as it can delete, replace and insert any desired stretch of sequence in the specified location of genome.[36] With the help of a proper delivery system, these two novel strategies could create significant advancements in the therapeutic world.

In the new realm of medical research field where impossible no more seems to remain as it is for long, CRISPR edited human embryos are not a miracle. Although it is still highly controversial, He Jiankui might have already confirmed the power of CRISPR to rewrite human future. He announced the birth of world's first gene edited twin babies who are said to be HIV resistant in October 2018.[37] Although there is no strong evidence supporting his claim of gene edited babies, the news has created a buzz in the research world.

CONCLUSION

With a proper deliver strategy in place, CRISPR-Cas 9 based gene editing could make remarkable developments in the field of gene therapy. Research studies on the use of non-viral systems for gene therapy is progressing so that the safety concerns associated with viral vectors can be eliminated. Moreover, novel strategies including prime editing and base editing is also very promising in terms of efficiency. Overall, CRISPR technology is about to revolutionize the medical filed sooner or later.

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REFERENCES

1. Uddin F, Rudin CM, Sen T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. Front Oncol. 2020;10:1387.
2. Zhu H, Li C, Gao C. Applications of CRISPR-Cas in agriculture and plant biotechnology. Nat Rev Mol Cell Biol. 2020;21(11):661-77.
3. Schmidt F, Platt RJ. Applications of CRISPR-Cas for synthetic biology and genetic recording. Curr Opin Systems Biol. 2011;5:9-15.
4. Xie Y, Yang Y, He Y, Wang X, Zhang P, Li H, Liang S. Synthetic Biology Speeds Up Drug Target Discovery. Front Pharmacol. 2020;11:119.
5. Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacteriol. 2018;200(7):e00580-17.
6. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biology Direct. 2006;1(1):1-26.
7. Shah SA, Erdmann S, Mojica FJ, Garrett RA. Protospacer recognition motifs: mixed identities and functional diversity. RNA Biology. 2013;10(5):891-9.
8. Fridovich-Keil JL. "Gene editing". Encyclopedia Britannica. 2019. Available at: https://www.britannica.com/science/gene-editing. Accessed on 27 October 2021.
9. Ledford H, Callaway E. Pioneers of CRISPR gene editing win Chemistry nobel. Nature. 2020;586(7829):346-7.
10. Gaj T, Sirk SJ, Shui SL, Liu J. Genome-editing technologies: principles and applications. Cold Spring Harbor perspectives in biology. 2016;8(12):a023754.
11. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal transduction and targeted therapy. 2020;5(1):1-23.
12. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Investig. 2014;124(10):4154-61.
13. Mitha F. The Return of Gene Therapy. [online] Labiotech.eu. 2019. Available at: https://www.labiotech.eu/in-depth/gene-therapy-history/. Accessed on 27 October 2021.
14. Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. Gene. 2013;525(2):162-9.
15. Gene therapy needs a long-term approach. Nature Medicine. 2021;27(4):563.
16. Belete TM. The Current Status of Gene Therapy for the Treatment of Cancer. Biologics: Targets & Therapy. 2021;15:67.
17. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: An update. J Gene Med. 2018;20(5):e3015.
18. Cockroft A, Wilson A. Comparability: what we can learn from the review of advanced therapy medicinal products. Regenerative Medicine. 2021;16(07):655-67.
19. FDA Approves First Cell-Based Gene Therapy for Adult Patients with Multiple Myeloma. FDA. 2021. Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-cell-based-gene-therapy-adult-patients-multiple-myeloma. Accessed on 27 October 2021.
20. Szebik I, Glass KC. Ethical issues of human germ-cell therapy: a preparation for public discussion. Academic Medicine. 2001;76(1):32-8.
21. Brunn C. Challenges and opportunities in gene therapy development. 2021. Available at: https://www.drugtargetreview.com/article/92596/challenges-and-opportunities-in-gene-therapy-development/. Accessed on 27 October 2021.
22. Coopman K, Medcalf N. From production to patient: challenges and approaches for delivering cell therapies. StemBook. 2014.
23. Cooper E. Five-month old baby becomes first patient to receive world's most expensive drug. Pharmafield. 2021. Available at: https://pharmafield.co.uk/pharma_news/five-month-old-baby-becomes-first-patient-to-receive-worlds-most-expensive-drug/. Accessed on 27 October 2021.
24. Zhen S, Li X. Liposomal delivery of CRISPR/Cas9. Cancer gene therapy. 2020;27(7):515-27.
25. Li L, Hu S, Chen X. Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. Biomaterials. 2018;171:207-18.
26. Chen Z, Liu F, Chen Y, Liu J, Wang X, Chen AT, et al. Targeted delivery of CRISPR/Cas9-mediated cancer gene therapy via liposome-templated hydrogel nanoparticles. Advanced functional materials. 2017;27(46):1703036.
27. Aksoy YA, Yang B, Chen W, Hung T, Kuchel RP, Zammit NW, et al. Spatial and Temporal control of CRISPR-Cas9-mediated gene editing delivered via a light-triggered liposome system. ACS Applied Materials & Interfaces. 2020;12(47):52433-44.
28. Yip BH. Recent advances in CRISPR/Cas9 delivery strategies. Biomolecules. 2020;10(6):839.
29. Behr M, Zhou J, Xu B, Zhang H. In vivo delivery of CRISPR-Cas9 therapeutics: Progress and challenges. Acta Pharm Sin B. 2021;11(8):2150-71.
30. Intellia and Regeneron Announce Landmark Clinical Data Showing Deep Reduction in Disease-Causing Protein After Single Infusion of NTLA-2001, an Investigational CRISPR Therapy for Transthyretin (ATTR) Amyloidosis. Intellia Therapeutics. 2021. Available at: https://ir.intelliatx.com/news-releases/news-release-details/intellia-and-regeneron-announce-landmark-clinical-data-showing. Accessed on 23 October 2021.

31. Cyranoski D. Chinese scientists to pioneer first human CRISPR trial. Nature News. 2016;535(7613):476.
32. FDA approves first test of CRISPR to correct genetic defect causing sickle cell disease. [online] Berkeley News. 2019. Available at: https://news.berkeley.edu/2021/03/30/fda-approves-first-test-of-crispr-to-correct-genetic-defect-causing-sickle-cell-disease/. Accessed on 23 October 2021.
33. Sheridan C. Go-ahead for first in-body CRISPR medicine testing. Nature Biotechnology. 2018;36:907-8.
34. Philippidis A. Editas Early Data for CRISPR Therapy EDIT-101 Shows Efficacy "Signals" in Two Patients. [online] GEN - Genetic Engineering and Biotechnology News. 2021. Available at: https://www.genengnews.com/news/editas-early-data-for-crispr-therapy-edit-101-shows-efficacy-signals-in-two-patients. Accessed on 27 October 2021.
35. Find Trials. Available at: https://clinicaltrials.gov/. Accessed on 27 October 2021.
36. Antoniou P, Miccio A, Brusson M. Base and prime editing technologies for blood disorders. Frontiers in Genome Editing. 2021;3:1.
37. Cyranoski D. The CRISPR-baby scandal: what's next for human gene-editing. Nature. 2019;566(7745):440-3.

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