Sanchez 3:00
Team R06

IMPROVING TREATMENT OF GENETIC DISEASES WITH CRISPR-CAS9 RNA-GUIDED GENOME EDITING

Arijit Dutta ard116@pitt.edu , Benjamin Ahlmark bza2@pitt.edu, Nate Majer ncm41@pitt.edu

Abstract*—Genetic illnesses are among the most difficult to treat as it is challenging to safely and effectively alter DNA. DNA is the basic code for all hereditary traits, so any alteration to DNA risks fundamentally altering the way someone's genes are expressed. This change could lead to unintended consequences for both the individual whose DNA was altered and any offspring they may have in the future, compounding the risk.*

Aiming to overcome this challenge, CRISPR-Cas9 (which stands for clustered regularly interspaced short palindromic repeats) is a technology based on a mechanism found in bacterial immune systems. CRISPR-Cas9 functions by targeting foreign or harmful DNA in the genome of an affected cell and altering the DNA in a way that is beneficial to the organism. Scientists in the laboratory can create a small piece of RNA that acts as a guide sequence for a restriction enzyme (an enzyme used to cut DNA), by binding to a specific DNA sequence in the affected cells' genetic code. Using this specificity, the RNA sequence guides the restriction enzyme to the target location where the enzyme cuts the target DNA strand. Once the affected DNA strand is cut, scientists use the cell's DNA repair mechanisms to edit the targeted gene. The entirety of this process is known as CRISPR-Cas9.

The promising results of CRISPR-Cas9 technology have larger implications in the health field, as scientists and engineers are hopeful that the use of CRISPR-Cas9 will improve treatment of cancers, diabetes, and many more genetic disorders outside the scope of current treatments by making gene editing a sustainable method of treatment. Our research will focus on the mechanisms employed in CRISPR-Cas9-based treatments of genetic disorders. The mechanisms we plan to primarily focus on include rendering malignant DNA harmless and removing the DNA. We will also mention more forward-looking mechanisms for replacing DNA based on the CRISPR method.

Key Words— Cpg-1, CRISPR-Cas9, Gene-editing, Genetic Disorders, Restriction Enzymes

INTRODUCTION: THE WHAT, WHY, AND HOW OF CRISPR-CAS9

What Is CRISPR-Cas9?

CRISPR-Cas9 is an acronym that stands for "Clustered Regularly Interspaced Short Palindromic Repeat", a name that refers to a unique organization of DNA sequences found in genomes of microorganisms. These sequences are an extremely important component of bacterial immune systems, as they are the main defense against viral invasions in bacteria cells. When the virus threatens bacterial cells, CRISPR-Cas9 can defend against the attack by destroying the genetic code of the invading virus. By destroying the viral genome, the CRISPR-Cas9 immune system protects the bacteria from infection as it destroys the virus' ability to replicate and infect other cells. The CRISPR-Cas9 gene editing method is beneficial to the world as it is an extremely effective and reliable way to edit the genomes of cells. This method offers hope to those with genetic illnesses that are otherwise thought to be incurable (diabetes, cancer, cystic fibrosis, etc.) as the medical field now has the ability to remove or replace harmful DNA [1].

Why Is CRISPR-Cas9 Needed?

CRISPR-Cas9 is the most effective method of gene editing available as it has a higher success rate and is much more cost-effective than alternative gene editing methods: Zinc Finger Nuclease (ZFN) editing and Transcription Activator-like Effector Nuclease (TALENs) editing. ZFN editing works by fusing a eukaryotic transcription factor (proteins that allow the eukaryotic cell to alter their cell types and growth patterns in many different ways) with the cleavage domain of the FokI restriction enzyme. The FokI enzyme will bind to the DNA recognition site, where it will then activate

its cleavage domain, where it removes part of the DNA strand. Likewise, TALENs editing fuses the FokI cleavage domain to the binding domain of a TALE effector protein. The binding domain then binds to a specific DNA sequence that is then cleaved by the FokI nuclease domain. These methods of gene editing are significantly less effective and more challenging than the CRISPR-Cas9 method. Both systems demand elaborate design, assembly, and screening of each individual DNA-binding protein for a specific target site. This is due to the weaknesses of each gene editing method, as ZFN proteins struggle to target DNA sequences that lack the DNA base guanine, while TALENs proteins are only able to target DNA sequences with the nitrogen base thymine [2]. This leads to low efficiency as each gene editing type is only able to target and cleave a select variety of DNA strands. The low efficiency and high costs of these other methods of gene-editing have made them unsustainable and have kept them from being implemented. As a result, treatment of genetic diseases tends to focus on managing rather than curing a disease often resulting in an unsustainable model of treatment. CRISPR-Cas9, however, only requires the target DNA strand to be preceded by a protospacer adjacent motif (PAM), which is an RNA sequence that is complementary to the target sequence. PAMs are much more general and can be designed in the lab to compliment specific DNA strands, making CRISPR-Cas9 much more efficient and therefore sustainable [3].

How CRISPR-Cas9 Works: The Origins and Mechanics

There are three basic steps that the CRISPR defense mechanisms follow when defending bacteria from invading viruses. The first step, adaptation, is when the DNA from an invading virus is processed into short segments that are inserted into the CRISPR sequence as new spacers. A spacer is a short variable sequence that is similar to the DNA repeats of bacterial CRISPR that is found scattered between said DNA repeats. These spacers serve as a genetic memory of previous infections, which will destroy any viral DNA sequence that matches the spacer sequence, effectively protecting the bacterium from another viral attack. The next step is the production of CRISPR RNA, where CRISPR repeats and spacers in the bacterial DNA undergo transcription. Through the transcription process, in which DNA is used to produce RNA by RNA polymerase, the bacterial DNA produces a single-chain molecule RNA. This RNA chain is then cut into shorter pieces known as CRISPR RNAs. The final step, targeting, is when the CRISPR RNAs guide the bacterial molecular machinery, Cas9, to destroy the viral material. Due to the RNA sequences being copied from the viral DNA sequences during adaption, they are exact matches to the genetic code of the virus, which allows them to be extremely efficient and effective guides to the molecular machinery. While this is the process for CRISPR in bacteria, it is essentially the same process that scientists in the lab use when editing genetic code. The only difference is, rather than relying on the CRISPR bacteria to create the CRISPR RNA, scientists will first synthesize short RNA molecules that match specific DNA sequences. This synthetic RNA serves the same purpose as the CRISPR RNA, as they are designed to match specific DNA sequences and therefore are perfect guides for the molecular machinery [1]. Figure 1, as provided by Harvard graduate student Ekaterina Pak's article "CRISPR: A game-changing genetic engineering technique," shows the bacterial defense system from which CRISPR-Cas9 was derived in action in a much more understandable format.

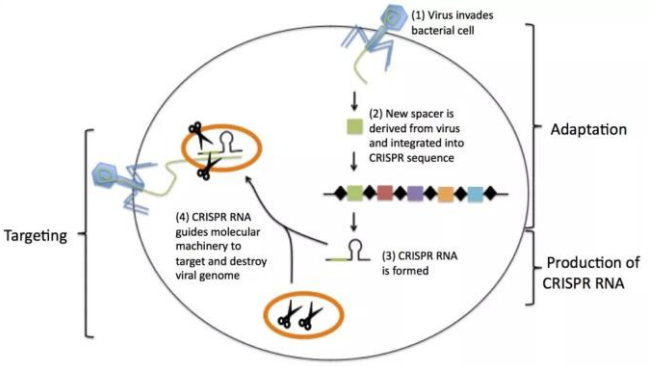

FIGURE 1 [1]
The mechanics of the CRISPR defense system function against an invading virus in a bacterial cell

TECHNOLOGICAL OVERVIEW

This section will give a technological overview of CRISPR-Cas9 technology. More specifically, it will compare CRISPR-Cas9 as an improvement over the other gene editing technologies currently available. This is important to discuss because, while there are several gene editing technologies, CRISPR-Cas9 stands out in its practicality. To defend CRISPR-Cas9's superiority, this section will discuss how it is more effective, more cost-efficient, and simpler to use when compared to other gene editing technologies.

CRISPR-Cas9's Technical Superiority

CRISPR-Cas9 is a much more effective tool compared to other gene editing techniques because of its versatility. Besides CRISPR-Cas9, two of the most popular means of genome editing are TALEN and ZFN. Both technologies employ programmable nucleases, which target DNA based on protein-DNA interactions [2]. These protein-DNA interactions are limited in their versatility in comparison to CRISPR-Cas9. For example, as stated by Michael Fan of BCC research, it is "difficult to target non-[guanine] rich

sequences" with ZFN, and the 5' targeted base for TALENs must be thymine for each TALEN monomer [2]. These limitations mean that ZFN and TALEN methods cannot be used to edit every DNA strand because many sequences do not meet those specifications. In such situations, the ZFN and TALEN methods are useless. While CRISPR-Cas9 does have limitations, such as requiring targeted sequences to precede PAM, this requirement is less of an obstacle because of how common such sequences are in DNA sequences [2]. Although this limitation on CRISPR-Cas9 does exist, it is not nearly as large of a limitation as for the ZFN or TALEN methods of genome editing. So, one area where CRISPR-Cas9 stands out from competing genome-editing methods, ZFN and TALEN, is greater versatility of application.

Additionally, simplicity involved in targeting any particular DNA sequence with CRISPR-Cas9 promises to make it easier to use than any prior discovered gene editing methods. Other genome editing methods are based on protein-DNA interactions, which require "elaborate design, assembly, and screening of each DNA-binding protein for a particular target site," as stated by Mike Fan in a BCC Research report [2]. Because such methods require such specificity and are so involved for the whole sequence of DNA, they take a long time to process and are very expensive ways to edit genomes. In fact, the cost associated with these methods has been the primary roadblock to genome editing.

On the other hand, CRISPR-Cas9 is based on DNA-RNA-Protein interactions, with the Cas9 nuclease interchangeably attached to gRNA sequences. This makes targeting a new sequence of DNA much easier. The key to the CRISPR-Cas9 system is that only the RNA sequence used by Cas9 to target the DNA sequence has to be modified [2]. It is much easier to sequence RNA than it is a protein, so targeting a new sequence of DNA is much easier with CRISPR-Cas9 than with other genome editing techniques like ZFN or TALEN. This has resulted in the expansion of genome editing as CRISPR-Cas9 has made the process affordable with respect to money and manageable with respect to time. The technical aspects of CRISPR-Cas9 have made it more economically practical and user-friendly.

CRISPR-Cas9's Economic Practicality and User-Friendliness

The overall effects CRISPR-Cas9 has had in the lab indicate that it is a user-friendly, effective, and economically practical method of genome editing. First of all, CRISPR-Cas9's user friendliness has made it incredibly practical for use in labs. According to a report by biologists working with genome editing in mice, they were able to prepare the CRISPR-Cas9 system for a specific gene and inject the gene in embryo in one day [4]. The setup time of CRISPR-Cas9 is so short because only a gRNA strand has to be synthesized, which, as mentioned above, is easier than synthesizing a protein to guide the nuclease to the target gene. As a result, significantly less time and money must be spent redesigning the system for any gene. This has brought "genome editing within the budget of any molecular biology laboratory," according to a BCC report which has further contributed to the exponential growth of studies with CRISPR-Cas9 [2].

Another beneficial consequence of the DNA-RNA-Protein interactions is that lengthy DNA sequences, which would have been almost impossible to target with DNA-Protein systems, are now practically targetable and therefore affordable. This puts genetic diseases based in very long genes within the reach of gene therapy, which could help millions of people affected by genetic illnesses. Additionally, the DNA-RNA-Protein interaction has proven in studies to be more efficient, as measured by the amount of desired mutations achieved in a sample. Studies have shown up to 70% efficiency in genome editing of Zebrafish and plants, while ZFN and TALEN could only achieve an efficiency range of 1-50% in studies with human cells [2]. Because DNA is essentially the same in all living things, this higher efficiency would carry over for any sample, highlighting that CRISPR-Cas9 is more effective than other gene editing methods. Connecting this heightened efficiency in targeting DNA with the lower costs of production, and greater versatility of CRISPR-Cas9, it becomes apparent how much more efficient this new method will make genome editing. This practicality is truly the source of all the recent work that has been done with CRISPR-Cas9 and is the reason scientists are so optimistic about the future of genome editing.

The overarching results of this innovation include affordable gene-editing and therefore, exponential growth in studies on genome editing. These studies in the laboratory have had promising results in treating genetic diseases in animal and human tissue samples.

CURRENT APPLICATIONS

In this section on current applications of CRISPR-Cas9 technology, the results of contemporary research scientists have had using the CRISPR-Cas9 process in the lab will be discussed. This section will be driven by the lab results of two different studies: hSERPINA1 gene in mice and the MIEN1 gene in breast cancer cells.

First Study: hSerpina Gene in Mice

The first study that will be examined is the reversal of the effect of the genetic mutation of the hSERPINA1 gene in affected mice. A1-Antitryspin (AAT) is a serine protease inhibitor (an enzyme that breaks down proteins and is a constituent of most proteins) that is primarily secreted by the liver and protects against tissue damage in the lungs. The point mutation (PiZ mutation), a mutation caused by a single nucleotide being changed, of the hSERPINA1 gene results in

an amino acid substitution, which causes a protein misfolding and aggregated protein retention in liver cells. This mutation causes affected persons to be subject to an increased risk of clinical liver diseases due to the accumulation of proteins in the liver. The mutation also inhibits the release of AAT, resulting in lower levels of AAT and subsequently impaired lung function. In this study conducted by researchers from the Biomedical Department in Cornell University in 2014, a specific guide RNA molecule was designed and used for the hSERPINA1 found in the liver of PiZ mutated mice in an attempt to disrupt or silence the gene, thus reversing the effects of the PiZ mutation. The experiment begins with the designing of the guide RNA, which was done using online software tools. A surveyor nuclease assay was then used to detect insertions or deletions of bases in the genome that were introduced by the hSERPINA1 specific guide RNAs. A standard gel electrophoresis kit was used to infect a cell with a plasmid containing the Cas9 and hSERPINA1 specific guide RNAs. Off-target sites for the hSERPINA1 guide RNA in the mouse genome were then identified and primers for the target sites were designed. The guide RNAs then guided the Cas9 enzyme to the target sites, where the restriction enzyme began cleaving affected areas of DNA. Aside from the deletion of the affected areas, no other structural variation was seen in the liver, showing the accuracy of the CRISPR-Cas9 gene editing model. After the experiment was completed and the liver was analyzed, a reversal of the PiZ mutation was observed, including reduced liver fibrosis and protein accumulation. Additionally, liver histology was improved as inflammation decreased in PiZ mutated mice. CRISPR-Cas9 was able to successfully, and with great accuracy, edit the DNA of the liver cells of mice by deleting the mutated DNA strands, effectively reversing the effects of the PiZ mutation [5].

Second Study: MIEN1 Gene in Breast Cancer Cells

Results of similar studies with CRISPR-Cas9 indicate it has real potential to cure genetic diseases like breast cancer. For example, researchers, from the University of North Texas Health University Science Center, targeted an oncogene known as Migration and Invasion Enhancer (MIEN1), which increases the ability of cancer cells to move, leading to disease progression. These researchers sought to delete the MIEN1 gene using CRISPR-Cas9, to cleave the DNA sequence. The researchers found guide RNA sequences, that would match the DNA in the target where two or three functional domains were located, using a biology software known as Benchling. By using a software to quickly find and synthesize gRNA, these researchers exemplified the user friendliness of CRISPR-Cas9.

They were then able to associate a Cas9 nuclease to the guide RNA, preparing the system for the DNA-RNA-Protein interaction characterizing CRISPR-Cas9. When introduced to a cell culture affected by the MIEN1 gene the guide RNA sequence guided the Cas9 enzyme to the DNA in two places. Both were cut, employing Cas9's function as a nuclease, and the target MIEN1 gene was separated from the DNA sequence. The non-homologous ends were then joined by the cells repair mechanisms, highlighting the clever use of the targeted cell's own repair mechanisms that make CRISPR-Cas9 so powerful. The MIEN1 gene was thus deleted from the genome, and the rest of the DNA was left undamaged.

The results of the study saw that 85% of the treated cells did not express the MIEN1 gene, in other words the treatment was effective in 85% of cells. This high efficiency rate displays the success scientists have found with CRISPR-Cas9 and reflects positively on the technology. The study then compared the treated cell line to a control cell line of MIEN1 affected cells and found that besides negating the expression of MIEN1 the treatment with CRISPR-Cas9 had no adverse effects on the morphology or health of the treated cells. This is a very important finding because it points to no unforeseen adverse effects of treatment with CRISPR-Cas9 genome editing. While adverse effects may be found in the future, this study supports the apparent safety of CRISPR-Cas9 [6].

These studies exemplify many of the reasons CRISPR-Cas9 has improved genome editing, including user-friendliness, efficiency, and safety. They also exemplify how close we are to using CRISPR-Cas9 to edit the human genome as a treatment for genetic illnesses. While this cause is noble, it is worthwhile to consider other implications of being able to edit and "improve" the genomes of humans and other species.

ETHICAL EVALUATIONS

In this section on the ethical ideas of CRIPSR technology, we will discuss and evaluate the ethical concerns regarding CRISPR-Cas9. As with any developing new technology, its obvious benefits often obscure the moral implications that widespread application entail. An analysis will be conducted, using two examples, on whether the benefits of CRISPR-Cas9 genome editing technology outweigh the potential harm it may cause to determine if there is truly a benefit to be had. Possible solutions to make CRISPR-Ca9 more ethical will also be described.

Designer Babies

The first ethical issue regarding CRISPR-Cas9 technology is the capability for individuals to use CRISPR-Cas9 for "designer babies." This concept of being able to design the genetics of your offspring to select certain features – from their height to skin color to body shape – has been shown in many futuristic movies and books. With the further

improvement of CRISPR-Cas9 technology, manipulation of germline cells (sperm and egg cells) and embryonic genomes, it will not be for very long before these fantasies become reality [7].

While the ability to design babies can be useful, in terms of how genetic diseases can be edited out, it also has its faults. Giving parents the ability to design their baby will most likely lead to them choosing the most superior traits in terms of intelligence and physique as they will want the best for their children [8]. However, other parents who want to have a natural child will not be able to ensure that their children have superior traits and so the children will obviously have some faults and be at a clear disadvantage. As a possible theory from Sarah Ly of the Embryo Project at Arizona State University stated, the creation of the superior children will eventually result in two divided classes: genetically designed individuals and naturally born individuals. The natural individuals will most likely be viewed as lesser because of their natural born faults and so will be at a disadvantage in terms of education and job prospects. Such a divide is sure to lead to tensions between the two groups similar to that of racial conflicts as the natural born will feel discriminated against and the designer born will feel superior [9].

So, the question must be answered: does the CRISPR-Cas9 technology uphold its ethical values in regard to 'designer babies'? In terms of saving lives through the correction of faulty genes that lead to diseases, CRISPR-Cas9 upholds these values because it can be used to treat genetic illnesses. However, giving people the ability to produce genetically superior children is not ethical. This is because, as mentioned before, there will be an eventual clash of artificial and natural people. In this instance, the use of CRISPR-Cas9 is very unethical in that it is essentially setting up society for a long and enduring conflict, and many people, specifically the natural individuals, will suffer as a result [9]. Thus, though unintentional, CRISPR-Cas9 could be misused to violate ethical principles by indirectly causing a widespread conflict.

However, with regulation, CRISPR-Cas9 can remain ethical. This regulation would require strict limits placed on the types of gene editing that would be allowed. For example, federal and international laws could be put into existence such that gene editing is only legalized for removing life-threatening/impairing genetic diseases. Any further manipulation of a person's genes would be considered illegal. By doing this, the unethical aspect of CRISPR-Cas9 is removed as "designer babies" would not be allowed and only genetic diseases would be allowed to be edited. CRISPR-Cas9's use in modifying genes would be limited to saving and improving life by curing genetic diseases before birth.

Biological Weapons

Another idea that can be ethically evaluated with respect to CRISPR-Cas9 is biological warfare. As mentioned, several times throughout this paper, one of the main benefits of CRISPR-Cas9 is the fact that it is very easy to use and can edit genes at a fast and easy rate. However, this can easily be turned into a deadly weapon as bacteria or viruses can be genetically engineered to have deadly diseases, like anthrax, which can cause massive human death and crop damage. Though it seems like a far-fetched idea, the low-cost of the technology will make it so that even small terrorist groups can utilize genome editing to cause harm [8].

As seems evident, creating a possible weapon for biological warfare would be an unethical application of CRISPR-Cas9 technology as it would directly cause harm to millions of people in terms of infecting them with deadly diseases or starving them by destroying crops. Obviously, the initial goal for this technology is to only benefit people by removing genetic defects and improving society. However, such a powerful and easy to use technology could eventually fall into the wrong hands and be weaponized. A similar example in history can be seen in Fritz Haber, a German chemist, who discovered a faster way to complete the nitrogen-fixation process, improving fertilizer and boosting agricultural yields everywhere [10]. However, as World War I began, he used the same process for nitrogen fixation to create the many poisonous gasses used during the war [10]. Thus, a technology initially created for good, which was also ethically sound, eventually became unethical as it turned into a weapon.

All in all, CRISPR-Cas9 has the potential to save millions from life-threatening genetic deficiencies when used in the right hands, but as soon as it falls into the wrong hands, the harms quickly outweigh the benefits. To ensure that CRISPR-Cas9's use remains ethically sound, the government should monitor sales of the technological components, such as software used to find guide RNA sequences. While this may limit the number of people working on CRISPR-Cas9 and slow the progress of this technology, it is necessary to ensure that terrorist organizations are not able to easily get this technology to weaponize. And so, with the possibility of being turned into a weapon removed, CRISPR-Cas9 will remain as a much more ethical technology intended to improve lives only.

This ethical evaluation of CRISPR-Cas9 reminds us that although this technology has great potential to improve the human life and treat illnesses, it also raises ethical dilemmas in ways that are not easily foreseen. Ultimately, when any individual handles a technology with such great power, it is important that the individual acts with an even greater responsibility.

FUTURE APPLICATIONS

CRISPR-Cas9 has currently not been used clinically to treat a genetic disease officially. Thus, no live human trials have been conducted using the technology. And so, the next

step after live trials with animals and human tissue samples is to edit genes in live human cells.

As mentioned before, the sheer number of genetic illnesses means we have only scratched the surface of genetic illnesses we have studied in the laboratory to eventually develop treatments. While such feats are impressive, it must be viewed with respect to the fact that there are hundreds of genetic diseases and only so many have been dealt with so far with CRISPR-Cas9. With continued breadth and depth of research studies, it is expected that CRISPR-Cas9 will start to be used to treat more common genetic diseases, like hemophilia and sickle cell anemia, as well as complex diseases like the human immunodeficiency virus (HIV) [7]. Luckily, due to the CRISPR-Cas9's relatively low cost and easy usability, it can be used in greater quantities than other gene editing tools, reducing the amount of time before the more common genetic disorders can be treated. This advancement will continue also as a result of labs transitioning from human tissue samples and live animals to live human trials. The progression onto live humans will enable scientists to determine what works and what does not work with the CRISPR-Cas9 in actual humans rather than just speculation from animal tests.

In order to fix some of the currently incurable genetic diseases, a method must be developed for CRISPR-Cas9 to remove and replace genes in cells. Figure 2 displays two methods of gene editing: the already discovered gene silencing technique as well as the less developed gene replacement technique. Current studies have primarily been conducted about silencing genes rather than replacing them despite the two techniques not being that much different, as seen in Figure 2 [5][6]. This limits the applications of CRISPR-Cas9 as faulty genes cannot be replaced with functioning genes. Therefore, CRISPR-Cas9 only has the ability to cure diseases in which silencing the gene cures the subject. In order to cure genetic disorders such as muscular dystrophy, which is where the affected subject does not produce enough of the protein dystrophin (causing excessive muscle loss), the gene would need to be replaced with a functional version that can produce sufficient amounts of the protein. Silencing the gene would not suffice, as it would prevent the affected person from producing the required protein, leaving them affected by the disease. Such an advancement would save many more lives due to an increase in the amount of disorders that will become treatable through the CRISPR-Cas9 method.

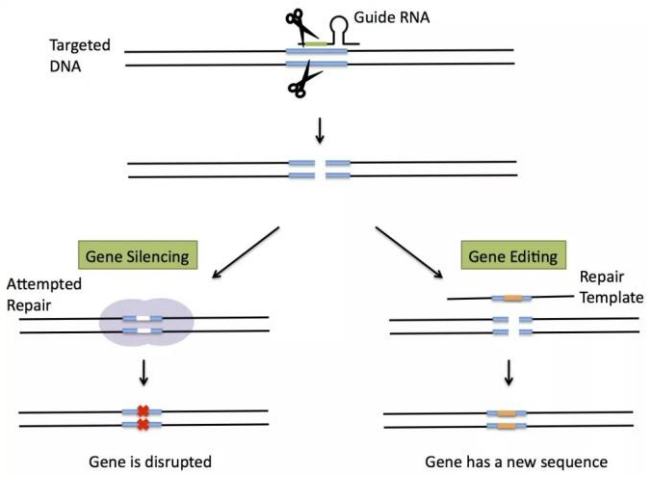

FIGURE 2 [1]
Two of the possible applications of CRISPR-Cas9 gene editing. Gene repairing is being developed further.

Whether improving current techniques of CRISPR-Cas9 or creating new techniques, this new genome editing technology will make great strides in improving the health of currently living humans and possibly more. Mo Ebrahimkhani, assistant professor at Arizona State University, states that in the near future scientists will be able to "integrate CRISPR technologies with human stem cells, tissue engineering, and synthetic biology to generate personalized human tissue in vitro" [11]. Essentially, scientists will eventually be able to silence, replace, and or remove genes from babies before they are even born. This opens a new realm of possibilities as babies will be able to be identified and cured of genetic diseases before they are born. Genetic diseases will be removed from the scope of the world entirely in coming generations as they would be removed before birth. However, associated with this solution is the fact that it will lead to the possibility of "designer babies" as it would be possible to add and remove any specific gene [7]. Such an idea would be unethical because of possible discrimination that would occur as a result of genetically perfect children, as stated in the Ethical Evaluation section. With proper regulation, however, the genetic manipulation of babies would only be allowed for genetic diseases, removing the possibility of "designer babies."

To conclude, the future looks very bright for possible applications of CRISPR-Cas9. With live human testing and new methods of using CRISPR-Cas9, more genetic diseases will become curable than ever before. Additionally, prenatal gene editing will become possible, essentially curing any genetic disease before the affected person is born.

SUSTAINABILITY

The coming introduction of CRISPR-Cas9 to the clinical treatment of genetic diseases promises to introduce a level of sustainability to the treatment of genetic disease, a field that has long been subject to complaints about a lack of sustainability.

In regard to sustainability in treating disease, three criteria must be evaluated: quality of life, treatment efficacy, and financial cost. The first, quality of life is marked by how a patient's physical and emotional experience has improved or worsened due to the treatment. Tied to a patient's quality of life is the efficacy of treatment. That is, how likely is it that the specific treatment will have the expected results on quality of life. The marginal utility of treatment for the average patient is roughly proportional to both the efficacy and improvement in the quality of life. On the other side of the coin, the financial cost of a treatment represents the amount of money a patient will have to pay due to the treatment. This monetarily represents the cost faced by a patient and marginal utility of treatment for the average patient is inversely proportional to the financial cost of the treatment. By evaluating these three criteria in current treatment we can see the ways CRISPR-Cas9 will make the treatment of genetic diseases more sustainable

Current treatment of genetic diseases particularly in the United States, remains relatively unsustainable for a number of reasons. The main reason for this unsustainable treatment being that current treatment tends to focus on management of the disease rather than a "cure" due to former high costs and difficulty associated with altering genes.

Quality of Life

The first aspect of sustainability, as mentioned before, is quality of life. Current treatments of genetic diseases have only resulted in the successful management of the diseases rather than actually curing the diseases. For example, sickle cell disease, a genetic disorder in which the person afflicted has malformed red blood cells that will often clog blood vessels causing strokes, infections, and periods of intense pain is currently treated with pain medications and symptom reducing medicines (hydroxyurea). Currently the only true cure for the disease is a bone marrow transplant which is very challenging because of the difficulty of finding a matching donor and the deadly complications that can result once the transplant is completed [12]. Thus, with no viable cure discovered yet, those with this disease can only reduce the symptoms of it. The "managed" person, still enduring the pain from the disease, will not see their quality of life restored to that of an individual without the disease. Medications only lessen pain rather than fixing the disease. Thus, current treatments of genetic diseases based on management, do not do a perfect job of improving the quality of a patient's life.

On the other hand, by targeting and altering faulty genes, CRISPR-Cas9 will be used to cure the root of the problem in a way hitherto unseen. In doing so, the quality of life for people afflicted with genetic diseases will be improved, due to the gene-editing capabilities of CRISPR-Cas9. In this manner, CRISPR-Cas9 offers a chance to make treating genetic disease a much more sustainable venture.

Efficacy

Tied very closely to the quality of life aspect of sustainability is efficacy. In the sense that the more efficient a treatment method is, the better the improvement of quality of life. Most genetic diseases are currently being treated in a method that only manages the symptoms rather than fixing the actual cause of the disease. Using the sickle cell disease example from before, with no viable cure, the only treatment for the disease is the management of its symptoms through pain medications [12]. Another example is cancer. With no cures for cancer currently, the only solution for people with genetic predispositions to cancer is to get regular screenings for any tumor growth. If there is any growth, chemotherapy or radiation therapy will be used to try to remove the tumor [12]. However, this is not a certain cure as such therapy may not work, and the treatment is often ongoing. Thus, as shown with sickle cell disease and cancer, current treatments of genetic diseases have little efficacy in actually curing the disease. And so, in this respect, current genetic disease treatment methods are not sustainable. With CRISPR-Cas9 technology, the actual cause of the disease can be treated rather than trying to manage its symptoms. By changing the DNA code within the afflicted person to deactivate a gene, the disease is rendered harmless at its roots. Thus, CRISPR-Cas9 will have a high efficacy compared to current treatments of genetic diseases and so is sustainable in this aspect.

Financial Viability

The last aspect of sustainability is financial viability. As mentioned before, current treatment of genetic diseases heavily involves the idea of managing the disease rather than curing disease because of the high costs and difficulty in altering genes. Managing disease symptoms has its own problems in that it does not improve the quality of life much and is an ongoing cost. The ongoing costs of managing genetic diseases add up to a large amount, compounding the high expenses of the treatment. For example, the United States spent around 17% of its GDP on healthcare in 2017 or $10,224 per capita [13]. With such a large amount of financial resources being spent on management of the diseases, does it not make sense to instead invest in a possible cure that would only be needed once rather than continuous treatments?

CRISPR-Cas9, on the other hand, stands out from current methods of managing disease in that it is very financially viable. CRISPR-Cas9 is so financially viable because it actually edits the faulty genes so ongoing

management can be eliminated. Thus, the financial burden of management would be alleviated by the introduction of CRISPR-Cas9. Additionally, as mentioned earlier, compared to other gene editing methods, CRISPR-Cas9 is cheaper because of its relatively low cost of production and ability to target very specific DNA sequences [2]. Thus, by providing a potential cure for currently untreatable diseases and being the most effective known method of gene therapy, CRISPR-Cas9 is very financially viable and sustainable.

CONCLUSION: A REFLECTION ON CRISPR-CAS9

In conclusion, CRISPR-Cas9 gene editing has the potential to change the scope of modern medicine as it would allow for the reversal of genetic disorders. This technology would allow for the eventual eradication of many of the most severe diseases known to man, including diabetes, cancer, muscular dystrophy, cystic fibrosis, and many more. While current applications of CRISPR-Cas9 are not as high profile as the diseases it could potentially be used to cure, it is showing significant promise in the laboratory. In the human-mice model study, CRISPR-Cas9 was able to successfully reverse the effects of a PiZ mutation. While this seems like a small-scale success, the gene they were able to silence was very similar to genes in humans (hence the human-mice model). Additionally, an experiment involving the MIEN1 gene, which is a gene that allows the cancer cell to spread rapidly, saw a high success rate of removing the MIEN1 gene from cancer cells, significantly decreasing its ability to spread throughout the body. However, with a gene editing method as effective as CRISPR-Cas9, there is bound to be ethical concerns about the future applications of the method.

As with any new technology, consumers will be worried about the ethical implications of CRISPR-Cas9, including biological weapons and designer babies. Due to the effectiveness of the CRISPR-Cas9 model, viruses and bacteria can be modified to be deadly and spread at concerning rates. Similarly, babies can be "designed" to have certain genes, and lack other genes, which would lead to an issue of discrimination towards the designer babies. However, with proper regulation of gene editing, the realization of both of these unethical ideas will be avoided.

Having taken into account these ethical dilemmas, the potential CRISPR-Cas9 has to be used for good persists. Beyond the obvious goal of curing genetic diseases and benefitting humanity, CRISPR-Cas9 also offers a chance to make healthcare more sustainable for people with genetic disorders. CRISPR-Cas9's potential to improve quality of life, efficacy of treatment, and financial viability are clear indicators of the sustainability CRISPR-Cas9 will add to the field of medicine.

Ultimately, the CRISPR-Cas9 technology is the most effective gene editing approach that has been introduced to the industry and has the potential to be revolutionary worldwide. However, its potential could be harnessed for the wrong reasons and cause severe ethical dilemmas and devastation throughout the world. But if these issues are dealt with accordingly, the entire human race will benefit greatly.

SOURCES

[1] E. Pak. "CRISPR: A game-changing genetic engineering technique." Science in the News. 07.31.2014. Accessed 01.15.2019. http://sitn.hms.harvard.edu/flash/2014/crispr-a-game-changing-genetic-engineering-technique/

[2] M. Fan. "Genome Editing: Technologies and Global Markets." BCC Research. 03.01.16. Accessed 02.16.19. https://www.bccresearch.com/market-research/biotechnology/genome-editing-bio146b.html

[3] "What is a PAM Sequence and where is it located?" Integrated DNA Technologies. 2019. Accessed 03.06.2019 https://www.idtdna.com/pages/support/faqs/what-is-a-pam-sequence-and-where-is-it-located

[4] E. Bolcun-Filas, J. Schimenti, P. Singh. "A Mouse Geneticist's Practical Guide to CRISPR Applications." Genetics. 01.01.2015. Accessed 01.16.2019. http://www.genetics.org/content/199/1/1.full

[5] M. Bohlooly, M. Bjursell, J. Wiseman, et al. "Therapeutic Genome Editing with CRISPR-Cas9 in a Humanized Mouse Model Ameliorates a1-antitrypsin Deficiency Phenotype." The Lancet. 03.2018. Accessed 01.16.2019. https://www.sciencedirect.com/science/article/pii/S2352396418300690?via%3Dihub p.104-111

[6] T. Treuren, J. Vishwanatha. "CRISPR Deletion of MIEN1 in Breast Cancer Cells." PLOS ONE. 10.04.2018. Accessed 01.16.2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0204976

[7] "What are Genome Editing and CRISPR-Cas09?" Genetics Home Reference. 01.22.2019. Accessed 01.26.2019. https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting

[8] K. Speights. "Is Gene Editing Dangerous? 4 Things You Should Know." The Motley Fool. 06.18.2018. Accessed 1.26.2019. https://www.fool.com/investing/2018/06/18/is-gene-editing-dangerous-4-things-you-should-know.aspx

[9] S. Ly. "Ethics of Designer Babies." The Embryo Project Encyclopedia. 03.31.2011. Accessed 3.07.2019 https://embryo.asu.edu/pages/ethics-designer-babies

[10] T. Kealey. "Gene Editing Babies is Unethical: Biochemist." USA Today. 11.27.2018. Accessed 03.04.2019.

https://www.usatoday.com/story/opinion/2018/11/27/gene-editing-babies-china-aids-hiv-designer-ethics-crispr-column/2125880002/
[11] K. Joseph. "Mo Ebrahimkhani on CRISPR technologies." American Institute of Chemical Engineers. 10.25.2018. Accessed 01.17.2019. https://www.aiche.org/chenected/2018/10/mo-ebrahimkhani-on-crispr-technologies-interview
[12] "How are Genetic Conditions Treated or Managed?" Genetics Home Reference. 03.19.19. Accessed 3.27.19 https://ghr.nlm.nih.gov/primer/consult/treatment
[13] C. Cox, B. Sawyer. "How does Health Spending in the U.S. Compare to other Countries." Petersen Center on Healthcare. 12.07.18. Accessed 3.26.19 https://www.healthsystemtracker.org/chart-collection/health-spending-u-s-compare-countries/#item-u-s-health-spending-growth-higher-1980s-similar-since

ADDITIONAL SOURCES

"Duchenne Muscular Dystrophy." Muscular Dystrophy Association. 2019. Accessed. 03.05.2019 https://www.mda.org/disease/duchenne-muscular-dystrophy
J. Doudna. "How CRISPR lets u edit our DNA." TED TALK. 5.12.2015. Accessed 2.15.2019 https://www.youtube.com/watch?v=TdBAHexVYzc. 0:00-15:53

ACKNOWLEDGMENTS

We would like to first thank Lacey Kostishack and Tyler McAndrew for their advice on possible edits to improve the quality of this paper. We also would like to thank all of the people in charge of hosting the First Year Engineering Conference as we would not have this opportunity to showcase our research abilities without them.