Treating Chromosomal Abnormalities With CRISPR Gene Editing

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Abstract:

Chromosomal abnormalities, some of the most prevalent genetic diseases in the world, are caused when chromosomes are present in the wrong number or configuration. Some common types include duplications (more chromosomes than normal), deletions (less chromosomes than normal), and other structural issues (deletions, duplications, or inversions within one chromosome). Unfortunately, there are few available treatments for any of these diseases today. However, there have been several studies based on the possibility of using CRISPR gene editing techniques to treat these conditions. These methods range from using traditional CRISPR-Cas9 for treating duplications, utilizing integrase proteins to treat deletions, and employing gold nanoparticles to deliver CRISPR directly into the brain. CRISPR gene editing methods have the potential to become a widespread curative technique for chromosomal abnormalities.

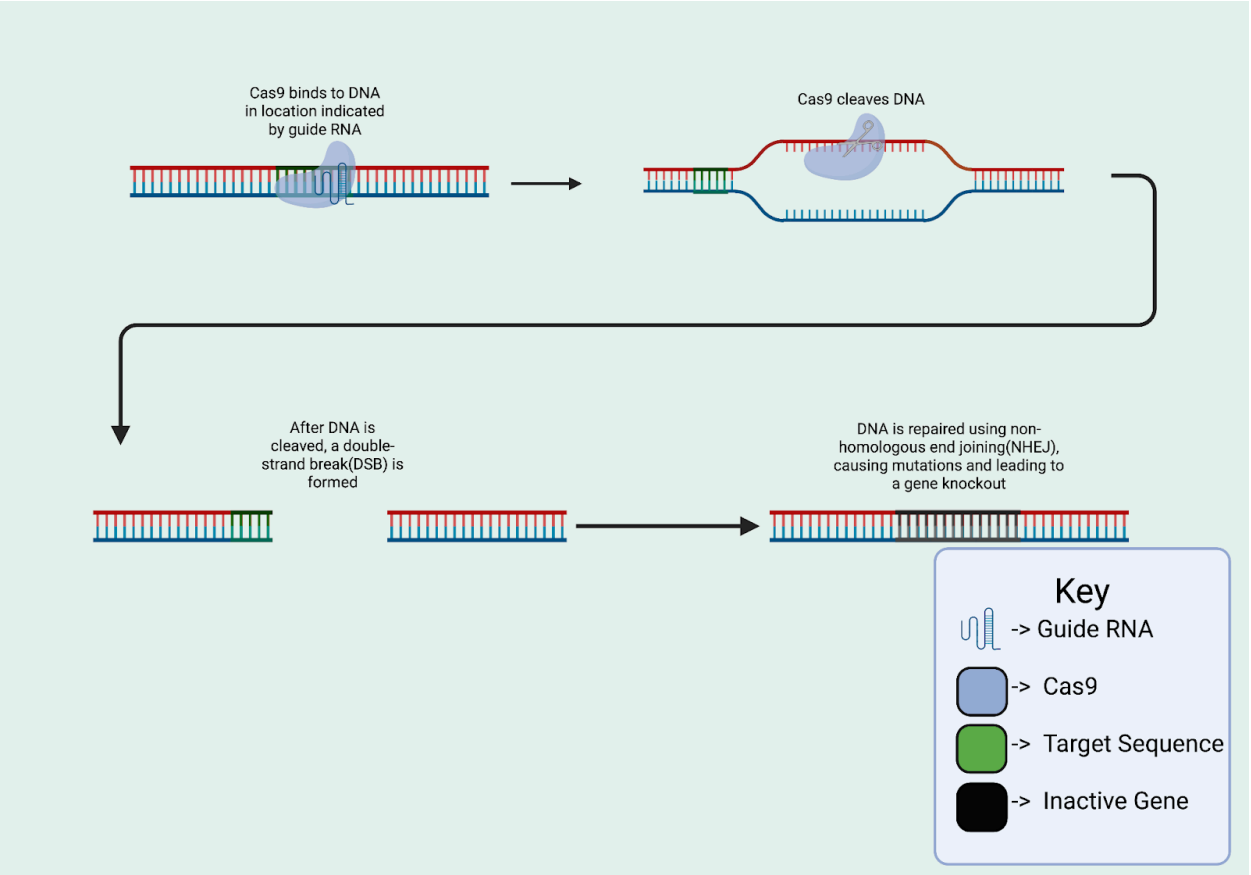

Figure 1. CRISPR Overview. This figure depicts the process of knocking out a gene with Cas9, starting with a section of DNA and ending with a knocked out gene.Figure created in biorender.com.

Introduction:

Chromosomal abnormalities are some of the most common and debilitating diseases in the modern world. These include aneuploidy (having the incorrect number of chromosomes in the cell) or chromosomal damage such as deletions, duplications, and inversions. Common symptoms of these diseases include mental disability, short stature, and other physical defects. One other treatment currently in development is chromosome transfer, which involves lab-growing a copy of the missing chromosome and then implanting it into the cell [2]. Unfortunately, this has the potential to lead to trisomy, as there is no guarantee that the offending copy of the chromosome will be removed [2]. In addition, symptomatic relief drugs are widely available mechanisms for combating these diseases. However, these drugs only provide temporary relief for patients without actually treating the underlying disease. For instance, if a patient has Down syndrome, no drug they take will cure them of that condition, even if it does alleviate their symptoms.

However, CRISPR/Cas9 gene editing is a potential candidate for safe and efficient treatment of these conditions. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a bacterial defence mechanism against bacteriophages that involves the cutting of viral DNA by different types of Cas proteins, and more importantly, the copying of the viral DNA into the bacteria's genome [1]. These features can be leveraged to edit the genome of human cells. The Cas protein (often Cas9) will make its cuts in the sections of DNA indicated by the guide RNA, which leads to the two main applications of CRISPR. Firstly, this cut, also known as a double-strand break (DSB), can be used to alter the genome if a template sequence is provided to use in place of the old one (Figure 1). The cell will use this sequence to patch the hole in its DNA, which can lead to the alteration of the targeted gene. On the other hand, if the cell is not provided with a template sequence, the cell will attempt to repair the DSB on its own using a process called non-homologous end joining (NHEJ). However, this often results in the "knock-out", or deactivation, of a gene as NHEJ is error-prone and will often introduce mutations into the genetic sequence (Figure 1).

A currently available CRISPR treatment is for sickle cell anemia (caused by a 1-base substitution in the beta-globin gene) and beta-thalassemia [3]. CRISPR has shown its viability as a treatment for those suffering from a wide range of genetic disorders and is also well on its way to being feasible for large scale treatment of a variety of chromosomal abnormalities, including duplications, deletions, inversions, and translocations.

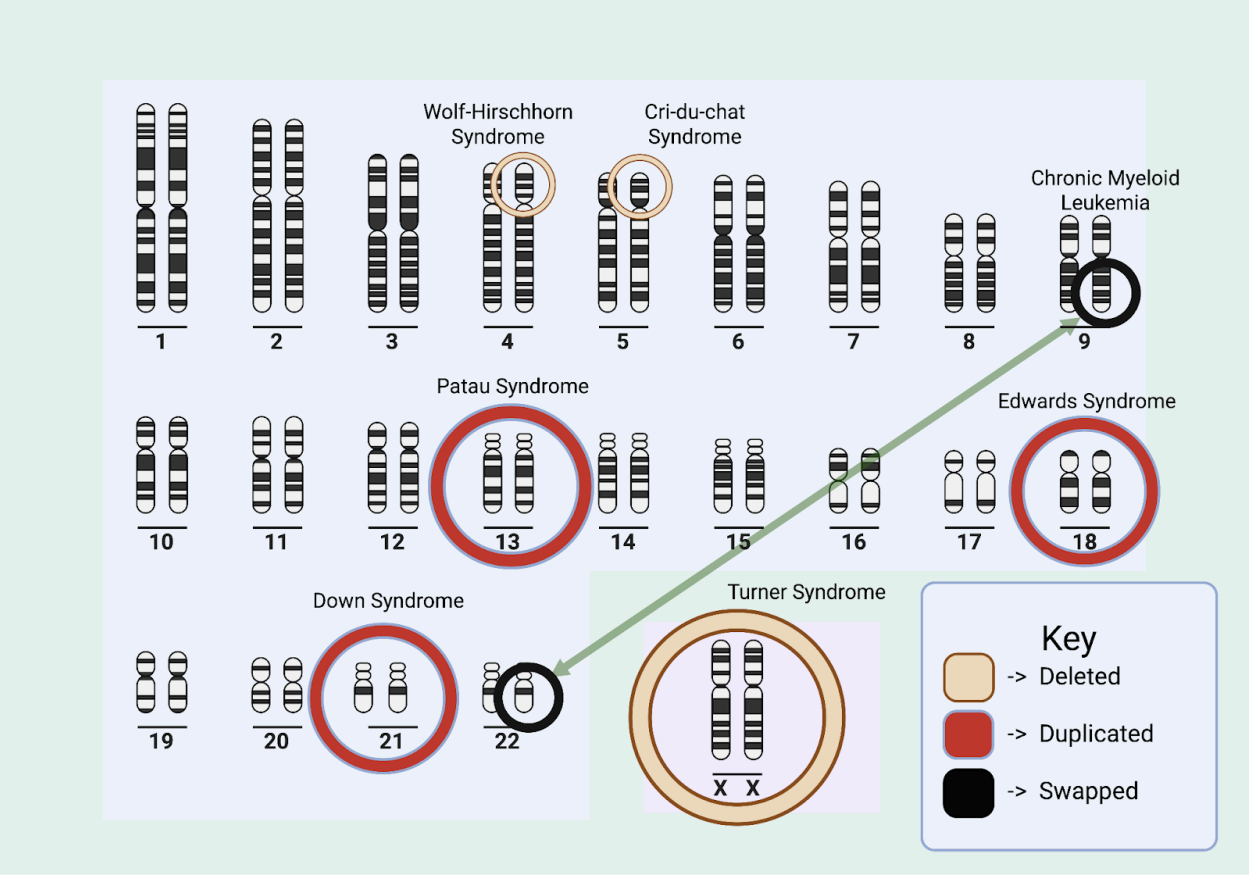

Figure 2. Normal Human Karyotype and Diseases. The normal karyotype is shown. Deletions, duplications, and translocations are noted with colored circles. For deletions, large circles represent complete deletions and small circles represent partial deletions. The green arrow depicts the pieces of chromosomes that are swapped in chronic myeloid leukemia (CML). Figure created in Biorender.com.

Treating Duplications:

One common type of chromosomal abnormality is chromosome duplication. This leads to aneuploidy, when there are the incorrect number of chromosomes in a cell. Specifically, many duplications lead to trisomy, where there are three copies of a chromosome instead of the normal two. These duplications typically form when gametes are performing meiosis. If the chromosomes do not separate correctly in Anaphase II, there will be a cell with three rather than two chromosomes and a cell with one chromosome instead of two. The most common chromosomes to be duplicated are 15, 16, 17, and 21. Some of the diseases caused by these duplications are Down syndrome (Trisomy 21), Patau syndrome (Trisomy 13), and Edwards syndrome (Trisomy 18) (Figure 2).

Down syndrome is one of the oldest recorded chromosomal abnormalities, first described in 1866 by John Langden Down. It causes mental disability, short stature, and heart defects. While it does not directly decrease life expectancy, the quality of life can be drastically reduced.

Common treatments today are symptom relief methods including surgery and special education [3].

Another disease caused by a chromosomal abnormality is Patau syndrome. Discovered in 1960 by Klas Patau, it is often immediately fatal after birth, with the average lifespan being just a few days and only 5-10% of babies reaching one year of age. It leads to malformed eyes, weak hearts, cleft lips, and poor muscle tone. Again, the only treatment is surgery for cleft lips and heart issues [4].

Finally, Edwards syndrome is another chromosomal abnormality that is immediately fatal and has no cure. It was discovered in 1960 by John Hilton Edwards. Edwards syndrome causes slow prenatal growth, mental and physical disability, and clenched hands [5].

To sum up the current methods of treatments for chromosomal disorders, the only solution that we are turning to is to relieve the symptoms of the patient. However, this does not treat the underlying disease. CRISPR/Cas is currently emerging as a possible candidate for deleting an extra copy of the offending chromosome and curing the duplication. The basic idea of this method is to fragment the chromosome and cause it to truncate, or break apart.

The first method uses Cas9 proteins to target the centromere of the chromosome. By finding repeating fragments at the centromere, the Cas9 can be used to cut the DNA at multiple points in that area [6]. In a 2017 study by Adikusuma et al., two different gRNAs were found to make 140 and 41 cuts in the centromere area of the Y chromosome in mice embryos. This method could likely be extended to human embryos [6]. As the centromere is vital for mitosis, deleting it will cause the chromosome to truncate and be lost. Another way to induce chromosome fragmentation is to target the long arm of the chromosome. The long arm contains many important pieces of DNA, so cutting it there would induce the chromosome to break apart. Again, this approach was tested on mice embryos but could be extended to human ones.

A different approach entirely would include the use of a Cas3 protein in the place of a Cas9 one [8]. The Cas3 protein relies on a CASCADE (CRISPR-associated complex for antiviral defense) complex to guide it to the correct area, rather than a guide RNA. The CASCADE complex is made up of multiple proteins that allow it to bind and interact with the target DNA area, including Cas5, Cas6, Cas7, and Cse1 [7]. The Cas5 is responsible for binding to the guide RNA and forming the backbone of the whole CASCADE complex. The Cas6 is used for cleaving the crRNA into smaller pieces. The Cas7 makes up the backbone of the complex, protecting the crRNA. The Cse1 recognizes the PAM (protospacer-adjacent-motif) on the DNA sequence and facilitates the formation of the R-loop, a vital component of Cas3 [7]. Once the whole complex is bonded to the DNA, instead of making a double-strand break like the Cas9, the Cas3 unwinds the helix of the DNA and moves along it, shredding it as it goes (Figure 4) [7]. This makes it far more suited to larger deletions than Cas9. However, these deletions can be large and unpredictable, so safety measures are absolutely necessary for safe usage of this specific Cas protein [7]. A common mechanism is to implant Anti-Cas (ACR) proteins into the genome (Figure 4) [9]. These proteins interfere with the Cas3 mechanisms, leading to it being unable to pass beyond that section. These proteins are taken from the bacteriophages that use them to combat CRISPR defences.

Overall, the future of CRISPR being used to treat chromosomal abnormalities looks bright, but there are still a few problems that need to be worked out. Namely, Cas3 CRISPR can still be very unpredictable and lead to catastrophic deletions in the cell. Secondly, the Cas9 still suffers from off-target edits. Across both treatments, an important issue is that the guide RNA will have to be custom-made for each and every patient.

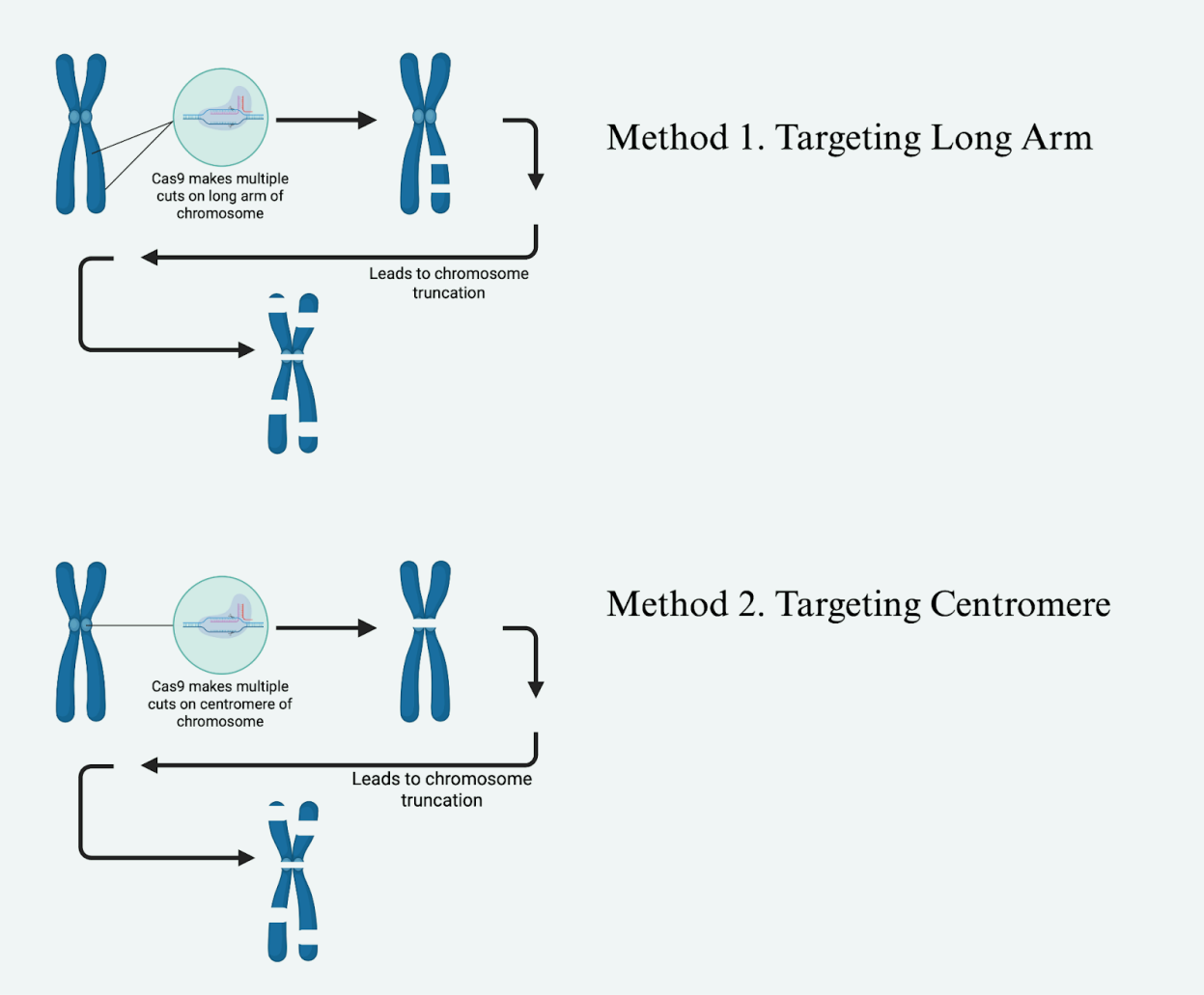

Figure 3. Deleting Chromosomes by Using Cas9 at Different Locations. Both of the strategies listed above begin with a copy of the extra chromosome that needs to be deleted. After this, the Cas9 protein attaches to and makes cuts at either the centromere or the long arm of the chromosome. This then leads to the chromosome truncating, or breaking apart. Figure created in Biorender.com.

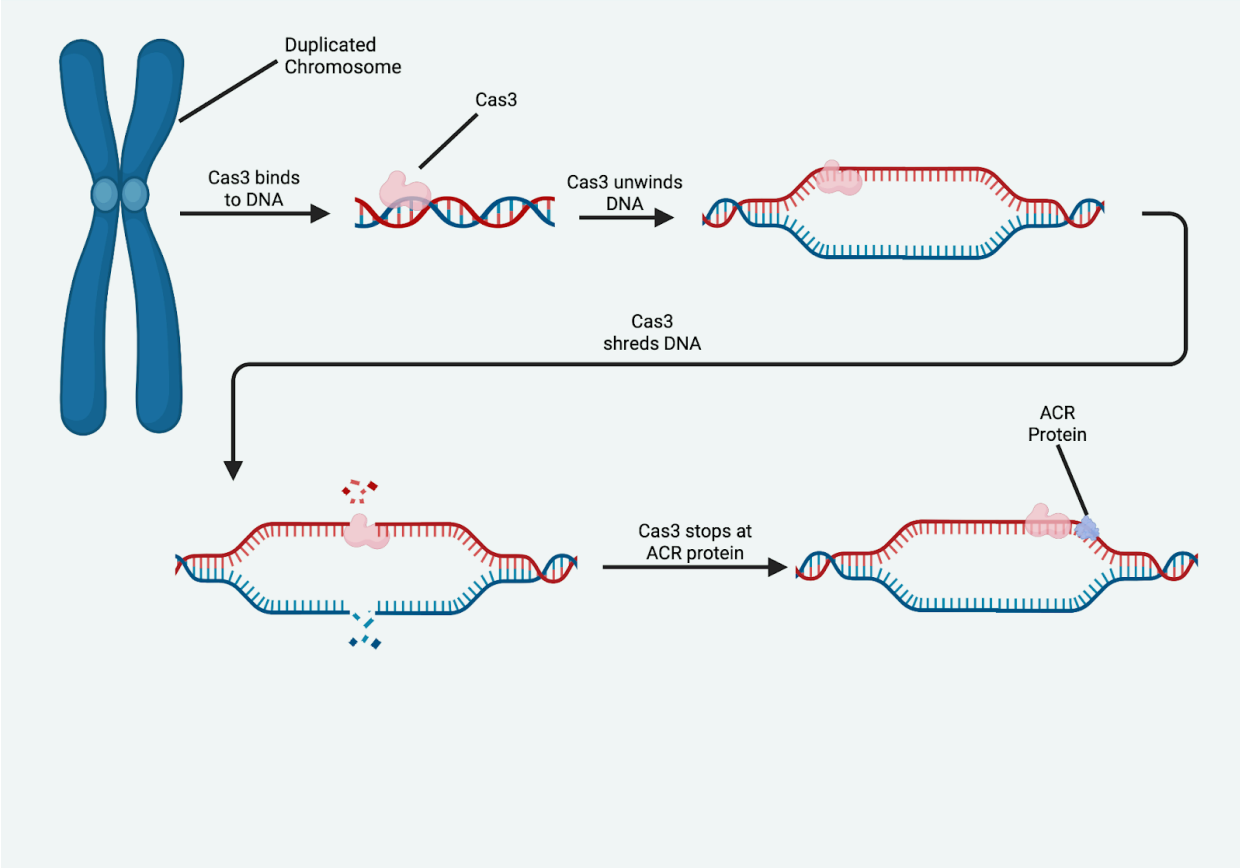

Figure 4. Using Cas3 to Delete Chromosomes. In this figure, a Cas3 protein binds to the DNA on a duplicated chromosome and begins by unwinding the DNA. After this, the Cas3 moves along the DNA, shredding it as it goes. Once the Cas3 reaches the anti-CRISPR(ACR) protein, it is stopped from progressing any further, helping to reduce the risk of uncontrollable deletions. Figure created in Biorender.com.

Treating Deletions:

Deletions are another type of common chromosomal abnormality, and unfortunately they are far more fatal than duplications. In fact, the only complete monosomy (only one copy of a chromosome due to a deletion) that is not fatal in utero is Turner syndrome, which involves the loss of one X chromosome. However, there are several other partial monosomies that include a partial loss of one of the chromosome arms.

Turner syndrome, discovered in 1938 by Henry Turner, is characterized by the loss of an X chromosome. Patients will appear similar to people with an XX karyotype, but suffer from infertility, webbed neck, short stature, and swelling of the extremities. Additionally, 50-60% of patients with Turner syndrome have congenital heart diseases, typically narrowing of the aorta or issues with the aortic valve. Common treatments today include hormonal therapy and surgery, but the condition cannot be cured [10].

One of the most common partial monosomies is Cri-du-chat syndrome. It is caused by a deletion in the p arm of chromosome 5. It was discovered in 1963 by Dr. Jérôme Lejeune. It

causes poor muscle tone, small head size, low birth weight, and speech disability. In French, Cri-du-chat means “cry like a cat”, which resembles the cry of a baby with this condition. The only treatments today are physical and speech therapy [11].

Wolf-Hirschhorn syndrome, caused by a deletion in the p arm of chromosome 4, is yet another partial monosomy condition. It was discovered in 1961 by Herbert L. Cooper and Kurt Hirschhorn. It leads to delayed development, low birth weight, underdeveloped muscles, scoliosis, and kyphosis (forward curvature of the spine). Current treatments include physical therapy for muscles and surgery to correct the spinal deformities [12].

Again, there are no cures for these diseases today, but CRISPR has the potential to cure them in the future. However, instead of the typical Cas9 double-strand breaks made in traditional CRISPR treatments, there are other methods for inserting DNA into the genome (Figure 3).

One of the methods, PASTE (Programmable Addition via Site-specific Targeting Elements), relies on viral integrase proteins instead of Cas double-strand breaks to deliver DNA [13]. Integrase is a protein commonly found in viruses, and its purpose is to facilitate the insertion of the phage’s DNA into the host’s. However, the integrase needs a specific landing site, called an AttP [13]. The CRISPR component can be used to deliver the AttP to the target sequence, and the integrase, connected to its DNA payload, can later dock there to insert the DNA into the sequence. This method is far more efficient than traditional CRISPR methods, with a DNA payload of up to 36 kilobases (kb), allowing it to facilitate larger gene additions. This method has been tested on mouse liver cells to insert genes into those cells, with considerable success [13].

Another approach is to use transposons, or jumping genes, to insert DNA where it needs to go. Transposons are genes that can “jump” and move across sections of the genome [14]. They can lead to disease and cause damage to the genome, but here they are leveraged to form a method for quick and effective insertion of large sections of DNA into the genome. An enzyme called transposase finds the repeats at both ends of the transposon and cleaves them there, freeing it from the genome. Then, sticky ends are made at the genome insertion point by creating a staggered cut rather than a straight one. This allows more DNA to join at either end because it leaves overhanging DNA that can be paired with the transposon, meaning that the jumping gene has been effectively taken from one area and used to patch another one. This is also less risky than Cas9 because the break is at a non-vital place in the genome, and it also has a much higher DNA payload [14].

All in all, these two methods are very promising gene insertion tools. However, PASTE methods can sometimes run into DNA binding issues, where the integrase is unable to connect to the AttP landing pad. One limitation of the transposon method is that it can lead to off-target edits.

Treating Structural Aberrations:

Structural aberrations, or chromosomal damage, can include translocations and insertions. These abnormalities are typically not as visible on a karyotype as deletions or duplications, but they can still cause illness. A translocation is when a section of a chromosome breaks off and reattaches itself to a different chromosome, leading to gene fusion mutations. This can either be a reciprocal translocation (when the genetic material is swapped) or a Robertsonian translocation (where genetic material is added to one chromosome and taken

away from another). An insertion is when a section of DNA is inserted into the chromosome, such as a nucleotide repeat.

An example of a disease caused by a translocation is chronic myeloid leukemia. It occurs when sections of chromosomes 9 and 22 swap, causing the BCR gene on chromosome 9 to fuse with the ABL1 gene on chromosome 22, forming the BCR-ABL1 gene [15]. Both the BCR and ABL1 gene provide instructions for cell growth and development. However, the mutated BCR-ABL1 gene facilitates uncontrolled growth of white blood cells, which leads to leukemia [15]. This gene can be knocked out using traditional CRISPR/Cas9 techniques, and it has been tested in mice.

As for insertions, an example of a condition would be Fragile X syndrome. The X chromosome has nucleotide repeats of CGG in the FMR1 gene, and abnormal repetition of this section can lead to the disease. This condition causes mental disability and distinctive physical features such as sunken chest, large ears, and flat feet. The repeat on the X chromosome disrupts the function of the FMR1 gene, which is vital for brain synapses. The synapses are what carry the electrical signals throughout the brain and problems with these are what lead to mental disability. Cutting out this repeat would be an effective way of treating this disease.

Trials in mice have been conducted with a nonviral vector known as CRISPR-Gold [16]. This nonviral vector gets around the issues of immune responses by using a gold nanoparticle with the CRISPR machinery attached to it. This complex is then injected into the brain of the mouse, which leads to the repeat being cut out by the CRISPR [16].

These two methods have been some of the most advanced techniques used to treat these chromosomal aberrations, and they will surely be utilized in the future.

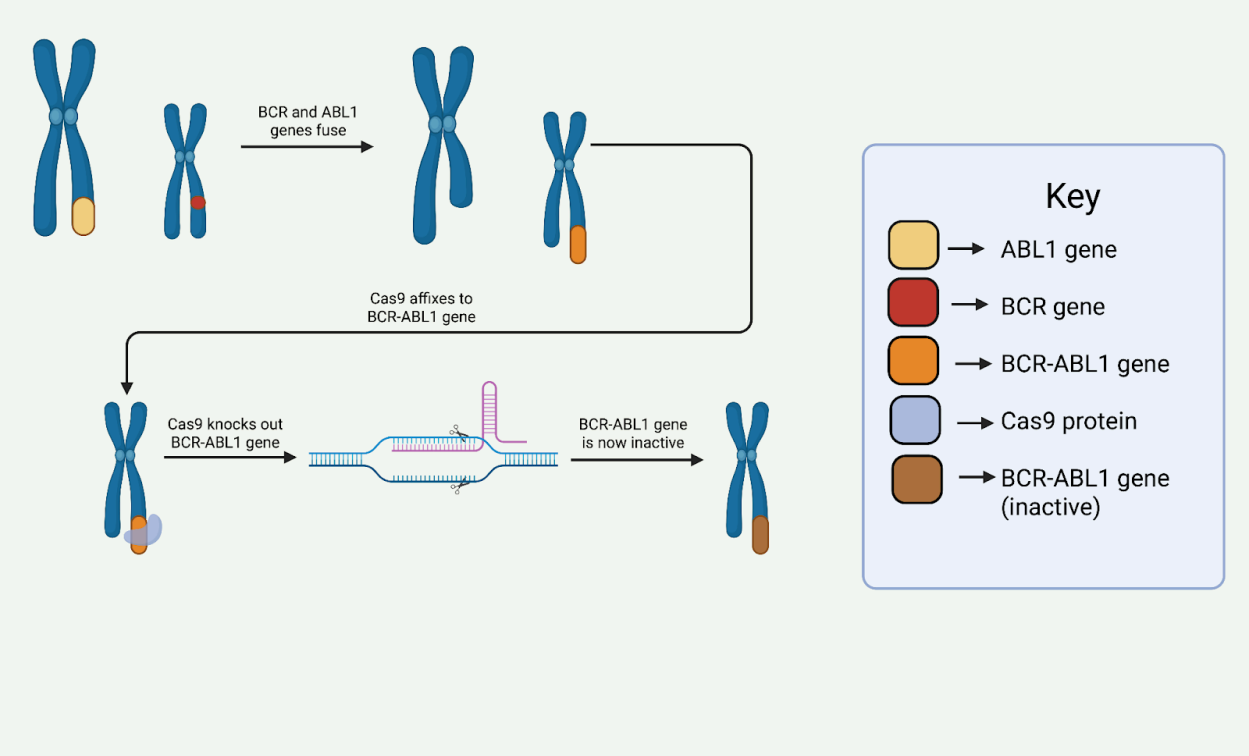

Figure 5. Using Cas9 to Treat Chronic Myeloid Leukemia. CML is caused when sections of chromosomes 9 and 22 swap, fusing the BCR and ABL1 genes together. This results in the mutated BCR-ABL1 gene. To treat this condition, Cas9 is used to knock out the BCR-ABL1 gene and deactivate it. Figure created in Biorender.com.

Discussion:

CRISPR gene editing is a promising method for treating chromosomal abnormalities. There are many methods of doing so, including using Cas9, Cas3, and integrase proteins to treat duplications, deletions, and inversions. Unfortunately, these methods are not without difficulties. Cas9 CRISPR can cause off-target edits leading to catastrophic mutations, while Cas3 CRISPR can delete vast swathes of vital DNA.

In addition, all of these methods have only been tested in mouse embryos. These editing techniques would be far riskier in human embryos, and even more so in a living patient. On top of all this, the process itself is very tedious and labor-intensive. Every patient will need a custom treatment due to differences in their DNA. This could possibly drive up costs, leading to an insurmountable barrier in access for some individuals. The cost for CRISPR sickle cell treatment, the only commercially available sickle cell cure, can be as high as 2.2 million dollars per patient. This price can be a large burden on patients, hospital staff, and insurance companies [17].

Another potential barrier for CRISPR viability is the complexity of getting the CRISPR/Cas complex into all of the cells of the patient. A way to get around this is to use the CRISPR in an embryo, so that there is only a single cell that needs to be edited. However, in order to treat the embryo, prenatal genetic testing to diagnose any possible diseases would be necessary.

However, CRISPR is not just a tool for curing diseases. It can also be used to induce various abnormalities into cells for the purpose of studying them. There are several methods to cause insertions, deletions, duplications, and more in cells [18].

In conclusion, CRISPR/Cas gene editing techniques are being tested to treat and cure chromosomal abnormalities, including duplications, deletions, and inversions. It does this by use of different proteins, including Cas9, Cas3, integrase, and more. Though it is not without its challenges, CRISPR gene editing shows promise to be a curative technique for chromosomal abnormalities.

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