Genetic Engineering of Viruses to Infect Cancer for Human Health

Leo Li

Abstract

Genetic engineering is the perturbation of DNA or RNA for research applications, diagnostics and addressing medical needs in health. Genome editing is the modification of genes in a living organism, which can be used, for one example, in an attempt to fight genetic diseases. DNA is the genetic makeup of any organism and can be edited with enzymes, which will cut the nucleic acid, potentially altering the function of the specific gene that was edited. CRISPR is a revolutionary genetic engineering tool that utilizes one of these enzymes that can alter genomes with ease compared to previous tools. It consists of a Cas enzyme paired with a guide RNA and can cut a target locus in the DNA. Cancer remains one of the deadliest genetic diseases to the global human population. Although new treatments have come far, millions die from cancer every year. Viruses are also large killers, but we can alter the DNA of viruses and repurpose them to fight cancer cells. With oncolytic virotherapy, the repurposing of viruses as a therapeutic to attack cancer, we will be able to use certain genetically-engineered viruses to specifically infect cancer cells, helping work towards the eradication of cancer. Genetic engineering is key to the advancement of human health because, with the perturbation of organisms and viruses, we will be able to improve human health. CRISPR's ability to accurately edit genes allows the applicability of virotherapy to fight cancer to become a reality. Being able to convert viruses into a cancer fighting treatment will require further clinical trials as well as additional discoveries of which genes to edit in order for viruses to safely target cancerous cells and avoid healthy ones. If researchers are able to successfully create a virus for cancer treatment, it will only prove CRISPR's applicability as well as reliability in other genetic diseases beyond cancer.

Keywords

Genetic engineering, Cancer, Viruses, CRISPR/Cas9, Therapeutics, Genome editing, Virotherapy

Introduction

Cancer kills millions of people every year, making it one of the world's deadliest diseases.[1] Cancer is a state of uncontrolled cell division and proliferation caused by genetic

mutations in genes that, under healthy conditions, prevent unhealthy cell growth.[2] Cancer-causing mutations often influence the genes responsible for cell cycle checks, which are important for ensuring that only healthy cells are growing and potentially unhealthy or cancerous cells are stopped from continuing to divide.[2] Therefore, there are two types of genes most commonly mutated in cancer cases: oncogenes and tumor suppressor genes, which have opposing functions in the cell. Under healthy conditions, oncogenes promote cell growth, and tumor suppressor genes prevent unwanted or excessive cell growth. In cancer conditions, oncogenes are mutated to act too much, and/or tumor suppressor genes are not active enough.[3]

Current treatments, such as chemotherapy or surgery, are effective to a limited extent. Chemotherapy refers to a class of drugs that inhibits cell growth, specifically in fast-growing cells.[4] It is commonly used in treating cancer, but has severe side effects, suppresses the immune system, causes mutations, and cancer cells sometimes become resistant to chemotherapy drugs, making it ineffective over time.[5] Surgeries are only effective during early stages of cancer before it spreads, or metastasizes, as removing the cancerous cells is only effective when they are all in one place and therefore all removed by the surgery.[6] Both of these treatment options become ineffective as cancer spreads and mutates, so there is a crucial need for more effective, complete treatment options against cancer.

Genetic engineering is the perturbation of nucleic acids, DNA or RNA, for research applications, diagnostics and addressing medical needs in health. Genome editing is the modification of genes in a living organism in an attempt to fight genetic diseases, such as cancer.[7] DNA is the genetic makeup of any organism and can be edited with special classes of nucleases that cut the nucleic acid, altering the function of the specific gene that was edited.[8] Clustered Regularly Short Palindromic Repeats (CRISPR) genome editing is a revolutionary genetic engineering tool, discovered in 2012, that can alter genomes with specificity, efficiency, and ease compared to previous tools, including recombinant DNA technology. This technology consists of a Cas enzyme (Cas9 is the Cas enzyme specifically used to cut double-stranded DNA) paired with a guide RNA and can cut targeted DNA.[9]

Viruses are non-living pathogens with a natural delivery method for putting new nucleic acid into cells and some types of viruses integrate that nucleic acid into the host genome.[10] Using genetic engineering, we can alter the DNA of viruses and repurpose them into fighting cancer cells. With this tool known as oncolytic virotherapy, we could be able to use certain viruses to only infect cancer cells, helping work towards the eradication of cancer.[11] Genetic engineering is key to the advancement of human health. With certain modifications or supplements into our body, immune response towards tumors can be enhanced and magnified, creating more effective resistance against cancer. Although still in research, CRISPR and genetic engineering in concert with oncolytic virotherapy in the clinical field provide much-needed hope as the potential to cure cancer remains on the horizon.

CRISPR/Cas9 for genome editing

The CRISPR/Cas9 system was discovered from bacteria's natural defense mechanism against viruses. Through evolution, prokaryotic cells evolved an immune defense mechanism, CRISPR, in order to protect the cell from viral infections. CRISPR sequences in the bacterial genome were identified when they noticed short, palindromic repeats interspaced with other sequences, which they later realized to be viral.[9] Those in-between sequences were actually transcribed into RNA that was used to guide a newly identified CRISPR-associated enzyme, Cas9 (an endonuclease-an enzyme that cleaves nucleotides- guided by RNA), to the complementary sequence in the viral genome.[9] In the bacteria, two types of short RNA are produced when virus DNA is detected, trans-activating CRISPR RNA (tracrRNA) and CRISPR (crRNA).[9] TracrRNA base-pairs at the end of crRNA, known as the complementary strand, creating a structured complex that helps Cas9 recognize what DNA to edit.[9] The two strands form a guide RNA (gRNA), which matches the virus DNA. The RNAs form a complex with a nuclease called Cas9, a type of enzyme that can cut DNA based on sequence complementarity to the guide RNA, making it a sequence-specific system that therefore targets specific places in the genome.[9] When the guide RNA matches with the DNA, Cas9 cuts the viral DNA, preventing a viral infection.[9,12] From this, researchers realized that the guide RNA can be engineered so Cas9 cuts any DNA sequence, not just the bacteria-infecting viruses it was evolutionarily intended to defend against.[9] The CRISPR/Cas system locks onto the protospacer adjacent motif (PAM), a short sequence of DNA at the site of cleavage.[9,13] Using the guide RNA, Cas9 is able to recognize which DNA sequence to cut because of complementary base-pairing rules (adenine-thymine/uridine, guanine-cytosine),[14,15] completely revolutionizing genetic engineering.[9] CRISPR is highly accurate because the guide RNA is sequence-specific and is limited to a small number of sites.[9] Previous genetic engineering tools such as Zinc finger nucleases (ZFN) and Transcription Activator Like Effector Nucleases (TALEN) were more inaccurate and ineffective in editing DNA compared to CRISPR, making it one of the most groundbreaking discoveries in genome history.[9,12]

After cleavage, researchers can either allow nonhomologous end joining (NHEJ) to repair the DNA, disrupting any function of this DNA sequence. NHEJ is a natural method for cells to fix damaged DNA. NHEJ combines the two ends of the broken DNA strand, gluing them together.[12,16] In this case, cleaving the DNA causes gaps between the DNA and NHEJ closes the void by reconnecting the two broken ends, also known as "knock-out," by adding or remove random base pairs, causing what is known as a frameshift.[12] Due to these frameshifts, NHEJ ruins the codon sequences, disrupting gene functions and silencing it. For research purposes, nonhomologous end joinings can be beneficial.[17] These mutations often disrupt the functions of the targeted DNA sequence, allowing researchers to study the role of the gene, because they can observe what happens or possibly what goes wrong without the function of that gene. Homology-directed repair (HDR) uses a single strand of DNA as a "template" to teach the cell to repair its own DNA.[12] By using this template, it specifically guides the cell to use certain base

pairs, also known as “knock-in,” ensuring accuracy of desired base pairs. With HDR, we can add full sets of genes, avoiding the randomness in NHEJ.[18] Using CRISPR and these two methods of genome editing, we can disrupt genes in cells in the lab, allowing researchers to investigate the functions of those genes in the cancer context. This can be applied, for example, to the identification of drug resistant genes, improving the effectiveness of current cancer treatments.

There are many potential applications of CRISPR in cancer as cancer is a genetic disease. Cancer is caused by mutations that promote uninhibited, uncontrollable cell growth. Inhibition of specific genes can cause apoptosis, which can fight cancer.[11] Apoptosis is programmed cell death caused by some problem in the cell, such as damaged DNA during mitosis or viral infections.[19] In one study, the knock-out and inhibition of the Myeloid Cell Leukemia (*MCL-1*) gene induced apoptosis in Burkitt lymphoma cells, a cancer in the lymphatic system (part of the immune system).[20] In healthy cells, the *MCL-1* gene produces the MCL-1 protein that inhibits apoptotic proteins, preventing cell death.[20] MCL-1 controls unnecessary apoptosis in cells, but when a cell becomes cancerous, it will evade apoptosis and divide uncontrollably,[2] leading to a cancer, which is unhealthy for the organism. Mechanisms to promote cell death in cancer cells are crucial to fighting cancer. Inhibiting this gene can allow cancerous cells to commit apoptosis before spreading and becoming a tumor.[21] Additionally in colon cancer, protein kinase C (PKC)--an enzyme in a phosphorylation signaling pathway–was inhibited by a cancer-causing mutation.[22] *PKC* has been recognized as a tumor suppressor gene, so because of its inactivation, a tumor developed[21]. CRISPR/Cas9 may be harnessed to correct this mutation, allowing it to fight tumors. Furthermore, stromal cells make up connective tissues between organs and modulate inflammation in the body.[23] However, in cases of tumor growth, normal stromal cells have been found to have a symbiotic relationship with cancer cells, allowing tumor growth.[23] For example, in pancreas cancer, the vitamin D receptor in stromal cells promotes tumor growth. Cas9 can knock down genes like the vitamin D receptor to make cancer more treatable.[21] One main benefit of edited stromal cells is that they do not affect the rest of the body, ensuring no side effects.

CRISPR/Cas9 genome editing may be the key to the future of the cancer field as it offers many promising solutions on fighting this deadly killer. One of the key issues with cancer is its variability. The reality is that every tumor in every patient is different and has different genetic mutations. In other words, researchers would need to discover the correct guide RNA sequence to use in every scenario. As mentioned above, if the patient’s cell had a mutation that caused the inhibition of *PKC*, researchers and oncologists would have to work together in order to discover which specific sequence of the DNA caused this inhibition. Unfortunately, each individual case may be a result of different mutations, even within the same type of cancer, which is where CRISPR comes into effect. Even niche mutations that are rare can be resolved via CRISPR as long as researchers are able to identify which mutation caused the tumor. By specifically targeting the cell type that is developing cancer with the Cas9 enzyme (alongside the gRNA and donor sequence), it can nullify and delete or correct mutations, returning the cell

into its healthy state. The only key problem that remains is the safety and efficacy of Cas9 within drugs and how to deliver these enzymes to the cells that need it. Such methods can be optimized through testing of artificial cells resembling a patient's tumor and later, clinical trials to ensure the safety of not our patients, but the safety of the participants in these clinical trials.

Oncolytic virotherapy

Oncolytic virotherapy is a new approach being investigated for potential clinical purposes against cancer. It relies on genetically modified viruses to attack and kill cancer cells. While some viruses cause illness and can even lead to cancer, such as Human Papilloma Virus, a modified HPV virus was surprisingly found to promote tumor regression.[23] Randomly, many patients were discovered to have tumor regression after certain viral infections. The first report of using virotherapy was in 1949: 22 patients with Hodgkin's disease, a lymphatic cancer, were treated with tissue extracts containing hepatitis [25] (note: The different types of hepatitis were discovered in the late 20th century[26], therefore it is unknown which hepatitis virus was used in this case). It appeared that researchers were well aware of the lower prevalence of Hodgkin's disease whenever the patient had some sort of liver disease. Although not specifically stated, we believe that hepatitis was used to treat these patients because researchers were aware that patients who had liver disease had a lower prevalence and severity of Hodgkin's disease.[27] Over the course of the next several decades, from the 1950s-80s, several clinical trials attempted to use viruses to fight cancer, but viruses were not deemed effective against cancer because of the lack of the ability to clinically control the virus because it does not specifically target cancer cells.[11] However, with our current understanding of cancer, researchers discovered that most cancer cells had impaired protection against viruses.[28] This is because some cancer cells express higher levels of receptors, making it easier for viruses to infect the cell.[29] This inspired virotherapy, a technique where viruses are engineered to specifically target cancer cells. Every virus has its own method of infection, each controlled by different genes in the virus and different protein interactions on the surface of the infected cell.[30] Because of this, there isn't yet a definitive solution to cancer using virotherapy.

Regardless, a promising discovery was made in the late 1990s. Researchers wanted to engineer a virus which would only target cancer cells, allow it to reproduce and infect other tumors.[11] The main challenge was preventing viral infections to our healthy cells, yet maintaining its effectiveness in infecting and killing cells.[11] That problem was solved with TVEC,[11] a double mutated herpes simplex virus (HSV)-1.[31] As of right now, TVEC is the only approved virus used for treatment against cancer, specifically melanoma(a type of skin cancer) in the US. One of the key aspects of TVEC that makes it so promising are the deletions in the HSV-1 viral *y34.5* and *a47* genes.[11] The main function of the *y34.5* gene is to prevent the host cell from shutting off its protein synthesis.[11] By inhibiting this gene, TVEC would be unable to infect normal cells because protein synthesis is inhibited, preventing replication.[11] Cancer cells, on the other hand,

do not have the ability to shut off protein synthesis, meaning TVEC can only replicate in these cells.[11] The *a47* gene normally functions to disrupt the transporter of the host cell.[11] If this gene is inhibited, then the cell would not prevent the *MHC* class 1 expression, which in turn promotes antitumor immune responses such as *MHC-1* expression (produces antigens used by T cells).[11,32] This groundbreaking advancement paves way for the potential of virotherapy to fight cancer as an effective and safe treatment. If research continues in this direction, genetic engineering promises a major victory against cancer.

With the advent of CRISPR/Cas9 genome editing this technology could be even more widespread to engineer other viruses to target cancers. The duality of cancerous cells versus healthy cells is very thin. Viruses will target cancerous cells because they are still part of the human body, making them seem like the perfect method of treatment. Also, the rapid rate of cell division within cancerous cells allows viruses to be one of the greatest tools possible. Simply put, the more cancer cells, the more host cells, which in turn allows the viruses to spread further and eradicate tumors. Furthermore, there are many viruses that target a specific cell in our body; for example, chickenpox specifically targets the skin[33], so the varicella-zoster virus may be a potential starting point in creating a virus that fights skin cancer. Even though viruses may seem like the perfect solution to cancer, its viability in the current field is limited because much further research is required, specifically using other viruses to infect cancer cells in other organs, not just TVEC for melanoma.

Challenges and Limitations

Although promising, there are many challenges that remain with any new technology. One common difficulty that appears in most attempts to genetically modify DNA is off-targeting. Off-targeting is the phenomenon that takes place when the wrong area of the DNA is targeted by the genome editing tools, making an unwanted DNA break or genomic edit in the cell.[34] This is possible in both CRISPR and virotherapy, which is why research must focus on addressing these problems. In CRISPR and virotherapy, because DNA is so large on the micro level, there are loci in the genome that have the same or very similar DNA sequences, even if they are in or near different genes, or on different chromosomes. As a result, the guide RNA may guide the Cas-9 enzyme to the wrong site.[12] Off-targeting or unintended editing may occur at different parts of the DNA which can be harmful to the cell. If anything, this can result in damaged and mutated DNA or even worse, develop into cancer.[35] Key solutions may include culture experiments that focus on how these edited viruses work on the molecular level, both against healthy and cancerous cells to ensure that the virus is properly edited to attack cancerous cells only. These viruses should not be attempted in clinical trials unless they have been verified through experimentation to be safe and effective, which can be accomplished by exposing these viruses to a small subculture of cells, imitating a tumor in a human body. This ensures that we can test the virus on a smaller scale to avoid any dangers before testing it on an actual person.

Viruses can be dangerous to control. Because of their natural rapid replication rate, viruses are more prone to mutations. This means that even if we are able to perfectly modify a virus, there is still a chance for it to mutate and reverse the modification or develop new, unexpected mutations, potentially resulting in uncharacterized impacts on the cell and body.[36,37] In order to avoid this, development of replication-incompetent viruses in the lab for treatment purposes may be useful in testing out the effects of certain viruses on different tumors.[38] The effectiveness of these treatment options will also be impacted because modifying the genetic material of either a cell or virus results in a less fit version. Even with viruses that cannot replicate, there are limitations. For example, the amount of viruses that would be engineered may vary from patient to patient. These viruses should be able to help the goal of using a virus to fight cancer because it resolves the potential of mutations as well as the rapid spread of viruses that would normally occur in a human body, and instead, keep these viruses near the target site. This can be resolved through multiple rounds of treatment, so medical professionals can monitor the effects a certain number of these viruses have on the tumor, which would gauge us on how many viruses may need to be engineered. One last detail that must be resolved is whether the replication-incompetent viruses will be able to kill cancerous cells. This is because viruses tend to kill cells by taking over the host and producing copies of itself. In other words, as far as we know, viruses cause programmed cell death in order to further spread itself through the body, but if it cannot replicate itself and cannot spread, the question still remains, will it complete its purpose which is to kill the cancerous cell? This key question can and will only be answered through numerous experiments with these non-replicating viruses and their effects on any cell. In comparison to cancer cells which have rapid growth, most cells with edited genomes cannot compete because they replicate slower, hence they are less fit.[21] Perhaps this means that the cells will not survive as long as the cancer cells, but perhaps they will survive long enough to target the cancerous tissue and start fighting. A clinician may be able to answer this question after various trials on the efficacy of cells with edited genomes.

Application of genome editing remains a challenge in cancer as well. Cancer is an extremely broad category, consisting of multiple different types and within each type, each cancer can be caused by multiple different mutations.[39] Because of the wide range of types of cancer and the variety that can be found in patients, each patient requires an individual assessment of the cancer, as different treatments or combinations may be required. Because of this, there is no one solution that can finally cure cancer, which is why these challenges and limitations must continue to be researched. TVEC, the only FDA approved form of virotherapy, has been successfully implemented as a form of treatment specifically for melanoma. However, like most drugs, TVEC has many side effects and downsides. TVEC is a herpes virus, which can cause symptoms such as rashes, inflammation from cell death, infections, hypoxia, fatigue, fever, and other flu like symptoms which were all observed in Neoadjuvant Intralesional Injection of Talimogene Laherparepvec With Concurrent Preoperative Radiation in Patients With Locally Advanced Soft Tissue Sarcomas, a study conducted by the University of Iowa and completed June of 2023.[40] We cannot control TVEC because of the way it was engineered. For example,

TVEC can target healthy cells, and when TVEC targets a healthy cell, the cell will kill itself, preventing the spread of the virus.[11] Better specificity will be required if we wish to safely and efficiently use TVEC to fight cancer.

Potential future directions

Many potential treatments using oncolytic virotherapy and genetic engineering against cancer are not ready for clinical trials and require further research. Most of the methods discussed in this paper focus on theoretical approaches towards usage of CRISPR in the cancer field. We do not fully understand how to effectively avoid off-targeting in the case of both virotherapy and genome editing. Therefore, off-targeting effects need to be studied in order to better understand both their clinical and molecular impact, to mitigate those effects, and in order to minimize or eliminate off-targeting of genome editing tools in disease. Off-targeting can be deterred by ensuring that the guide RNA is one-of-a-kind, in other words, the sequence in the gRNA is not repeated anywhere else besides the site of target, and there are some desired cut sites where this would not be possible due to similarity of sequences in different parts of the genome This can only be resolved through trial and error and continued efforts of ensuring the safety and application of CRISPR.

Even if we eliminate off-targeting of genome editing, there are potential side effects of virotherapy approaches, which can still be harmful or unpleasant for a patient. These problems can be mitigated by using a fabricated colony of both healthy and cancerous cells to test out the accuracy of virotherapy. For example, even though TVEC is only approved for melanoma[31], it is created to only reproduce in cancer cells, which can result in unknown side effects. Figuring out the effects of these excess viruses should remain a priority to mitigate side effects and ensure patient safety and comfort. Therefore, more clinical trials will be needed in order to understand those side effects and honestly convey them to patients and providers, and the results of these trials can then be used to inform pharmaceutical research in order to modify the treatment to minimize impact and risk of these side effects.

Effectiveness of the edited cells against cancer itself remains an open question in the field. Before these drugs become widely available and a common treatment against cancer, researchers must test and validate that they can effectively target and fight the cancer. Before entering into clinical trials, the most common mutations will need to be studied in the lab with this treatment, using cellular systems and animal models prior to clinical trial in order to ensure effectiveness and safety against the cancer. These experiments could expose cultured cancer cells to replication-incompetent viruses that are known to infect that particular cell type. For example, they could focus on several different cancer cell lines derived from liver cancer. The researchers could infect the panel of liver cancer cell lines with a panel of several different viruses, replication-incompetent versions of the viruses, and genetically modified versions of the viruses that, similar to TVEC[31], make the virus safer by making it less likely to spread. The

control would be each cell line growing without exposure to any virus, which will serve as a comparison for the cancer cell growth under non-treatment conditions. The team of researchers could then perform cell growth and cell division/cell state assays to assess how the cancer cell lines respond to each condition, and could perform experiments on the molecular responses in the cell types to each virus to better understand the mechanism of the response. For example, if all of the cell lines respond well to one of the conditions by showing decreased growth and cell cycle arrest, this might be a great candidate for further study. Then, the mechanistic study can give insight into how the virus is influencing cell health and could also help with understanding how a patient's body may respond to the treatment. The same procedure would be used later on in a mouse experiment, once a few treatment options are validated and approved in cell lines and the molecular mechanism behind the virus is adequately investigated to be safe and effective. That way, testing can move beyond a culture of cells and onto multicellular organisms, like mice to assess safety and side effects in an organismal setting. Similar steps would be repeated in this experiment, but the main difference would be the focus on how the virus affects the organism as a whole. The large hurdle in this step of trials would be to create a sufficient number of test subjects, in this case, use mice with different stages of liver cancer and make sure there are multiple at each stage to study. The experiment would consist of four control groups, where the mice will have liver cancer from stages 1-4. In these groups, the mice would live unexposed to the virus, while the experimental group would be exposed to different quantities of the virus. There would be four experimental groups as well, which will be organized based on the stage of liver cancer the mice are at. Each mouse would be exposed to different quantities of the virus to gauge the effectiveness of non-replicating viruses in a controlled environment. The key of this experiment is not only to further discover if the virus is effective in an organism, but to find the most efficient treatment method of the virus within the different stages of liver cancer and to see how the exposure to a foreign virus would affect the organism as a whole. The main question researchers should look at is how does the body react to the virus and will they bypass the immune system or will the immune system fight them off before they can kill the cancerous cells. Even though we are injecting viruses that should not attack healthy cells, they are still considered pathogens and foreign to our body, which must be understood before moving beyond these trials. The next question would be to determine whether the cancer comes back. Once these questions are answered, the most effective methods will be later used on larger organisms, and eventually on humans once they are deemed safe and effective for human trials.

In a hospital and clinic setting, each cancer is unique and has different mutations and affects different cell types, which means treatment will require a controlled environment for personalized treatment where the team of providers and researchers work together to develop a treatment plan based on the patient’s specific case, clinical presentation, and mutations. This controlled environment and personalized approach to medicine will help us learn about cancer more broadly and how it interacts with our cells. Studying the mutations that are observed in the clinic and for which this treatment is likely effective will help us better understand genetics and

healthy versus cancerous states of cellular behavior. Although promising, cancer remains one of the deadliest diseases and will most likely continue even after perfecting virotherapy. Each cancer case will vary and require consistent monitoring and continued use of current cancer treatments, such as chemotherapy or transplants to completely remove the cancer, may be required in coordination with emerging virotherapy treatments. Because each treatment may vary in effectiveness depending on the patient, virotherapy promises a new approach and option in fighting cancer. The field of study has not discovered exactly how the CRISPR/Cas9 system would function within a human body, but recent advancements have shown promising potential, specifically through the editing of mammalian cells.[41] Another key focus that remains undiscovered is how CRISPR may affect humans long term. For example, chemotherapy tends to harm people, especially children because it stops cells from dividing.[42] Even within adults, chemotherapy causes other problems such as fertility because of the reduced cell division. Therefore, it is safe to assume that CRISPR may result in similar or other long term problems that need to be discovered and understood before it can be considered a safe option for cancer. Even though CRISPR is known for its precision, unlike chemotherapy, researchers are not aware of the potential within the macro level of the human body. Such research is pivotal before the advancement and continued discovery of this new life saving technology.

Conclusion and Discussion

We have described that CRISPR/Cas9 genome editing has had a major impact on research advances, medical possibilities, and will continue to have a major impact in the clinical world. Oncolytic virotherapy, or the co-opting of viruses to fight cancer, is another cutting-edge advancement in cancer research. Genome editing paired with oncolytic virotherapy, have the potential to help improve quality of life and clinical outcomes for those suffering from cancer. The application of CRISPR shows great promise in fighting cancer. Through editing the viral genome, virotherapy becomes a viable treatment option because of its ability to avoid side effects present in other treatment options, such as chemotherapy. Utilization of CRISPR on viruses will likely bring virotherapy to the frontline of the battle against cancer.

The field is heading towards a promising future, but research must continue in order to achieve this dream. The main challenge remains in improving the effectiveness of virotherapy and how they can be safely administered to patients. Continued research and clinical trials will be required in order to ensure that virotherapy can efficiently fight cancer. Currently, even when cancer is fought off, it can reemerge as a result of leftover cancer cells or cancer-causing mutations. Virotherapy has the potential for complete eradication of cancer cells because the virus will seek to replicate in all cancer cells, eventually killing them all. Gene-targeted therapy with CRISPR has promise to target and fix the mutations that have already occurred, which can prevent future cases of cancer. The combination of the two will only increase the efficacy of

virotherapy and create safer treatment options for cancer patients and ensure that the cancer does not return.

Once CRISPR and virotherapy have effectively been integrated together and implemented into cancer treatment, its utilization and potential exists in other areas of health. Genetic engineering doesn't have to stop at cancer. It can help us fight other diseases and revolutionize the clinical field to improve human health. This isn't about winning the battle, it's about winning the war.

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