HIGH SCHOOL EDITION
Journal of Student Research
Volume 14 Issue 1 (2025)

How Is Synthetic Lethality and CRISPR Able to Revolutionize the Field of Cancer Treatment?

Yixuan Liu

Shanghai Starriver Bilingual School, China

ABSTRACT

Synthetic lethality is an emerging form of cancer treatment that has the potential to alter the field of cancer therapy. Current therapies, unlike novel gene therapies, are more toxic to the human body as well as being harmful to healthy cells. Treating cancer cells with synthetic lethality can avoid damaging the healthy cells by only targeting the mutated ones. Synthetic lethality can be initiated by CRISPR, a gene editing tool that can significantly increase the efficiency of the process. Furthermore, CRISPR screenings can scan for potential gene partners that can be exploited to trigger synthetic lethality in cancer cells. There are a number of clinical trials that use this form of treatment to target cancer cells. However, there are still some obstacles that must be solved to utilize this mechanism to its fullest extent, such as off-target effects from gene editing and gene targeting efficiency. Future research concerning synthetic lethality points toward simplifying the process and improving its accuracy. Nonetheless, despite its drawbacks, using CRISPR to initiate synthetic lethality is a novel and promising form of therapy that can revolutionize the field of cancer treatment.

Introduction

Cancer is one of the most common causes of death in the world, accounting for more than ten million deaths each year (who.int, 2022). Cancer therapy is an incredibly difficult challenge because of the high rate of mutations and the rapid development of cancer's resistance to treatment (Worldwide Cancer Research, 2023). Additionally, the lack of tumor markers used for early diagnosis and the absence of reliable, simple screening techniques only contribute to the obstacles involved in treating cancer (Oberstein and Olive, 2013). Aside from the harms directly caused by cancer, patients undergoing treatment are also affected by their prescribed therapies. As one of the most common cancer treatments, chemotherapy damages the population of healthy cells during the process of killing mutated cells due to its toxicity (Corrie, 2008). Despite the gradual development and improvement of cancer treatments, such harms caused by current cancer therapies seriously affect the patient's health. Therefore, to decrease off-target treatment effects and improve cancer prognosis, novel treatment methods are needed.

One of the emerging cancer treatment methods is gene therapy, which offers a new way to battling and treating cancer. This form of treatment involves the use of genetic engineering to modify cancer cells, which has already proven to increase survival benefits of cancer patients (Cross and Burmester, 2006). Recent development in clustered regularly interspaced short palindromic repeats (CRISPR), a powerful gene editing tool which allows scientists to modify specific sections of the genome, may help increase the efficiency of this process by initiating a process called synthetic lethality. Synthetic lethality occurs when the simultaneous mutation of two or more genes results in cancer cell death, while the presence of only one mutated gene does not. In certain contexts, cancer cells may lack one gene, due to a sporadic mutation, and therefore relies on a second gene to exert its normal function. This compensating mechanism can be used in cancer therapy by targeting and knocking-out the other gene, preventing its function and effectively killing only the cancer cells that carry that particular mutation. Therefore, CRISPR can be used in cancer treatment by knocking out specific genes in cancer cells triggering synthetic lethality, which cause the cells to die while leaving healthy cells unharmed. Cancer gene therapy requires the use of a reliable vector to deliver new genetic material to targeted cancer cells (Douglas, 2003). Moreover, scientists can use large scale CRISPR screenings to

investigate a combination of gene knockouts which can result in cell death, and therefore offer synthetic lethality (Wang et al, 2020). Gene targeting by CRISPR can therefore be used to create individual therapies for different types of cancer that carry different mutations (Ding et al, 2020).

Treating cancer by initiating synthetic lethality via CRISPR is an emerging yet promising therapy, which can revolutionize the field of cancer treatment and increase the efficiency of cancer therapy and research. Novel and reliable synthetic lethality methods can be promoted by identifying new interactions between genes that could trigger synthetic lethality (Topatana, 2020). The current development of synthetic lethality drug targets available to be used to treat cancer still faces several challenges such as identifying specific biomarkers and knock-out accuracy. However, scientists have confirmed SL treatment as a crucial factor in the next generation of cancer therapy (Setton et al, 2021).

Material and Methods

Online research screening for published articles, scientific journals, and research data were conducted in reliable databases such as PubMed and Google scholar. Trustworthy websites will also be included in the searches. Boolean Operators such as “and” were used to connect keywords including “CRISPR” and “synthetic lethality” to increase the relevancy of research findings. The relevance of these findings was evaluated before incorporating or referencing them in the paper. This will be accomplished by checking the publication date and how many times the articles have been cited.

Synthetic Lethality

Synthetic lethality occurs between two genes when the loss of both genes results in cell death, while the loss of one gene alone does not (Kaelin, 2005). In a typical scenario, cells have multiple pathways that ensure survival and correct cell functioning. This allow the cell to use an alternative pathway when the main one is not functioning as it should due to, for example, genetic mutations in any of the proteins required. In this context, cells can often rely on an alternative pathway for survival; however, if both pathways are disrupted at the same time, cells enter a process known as apoptosis or programmed cell death (Beijersbergen, 2017). In cancer cells, this concept is exploited because these survival genes are often mutated, allowing for an easier initiation of the process of synthetic lethality.

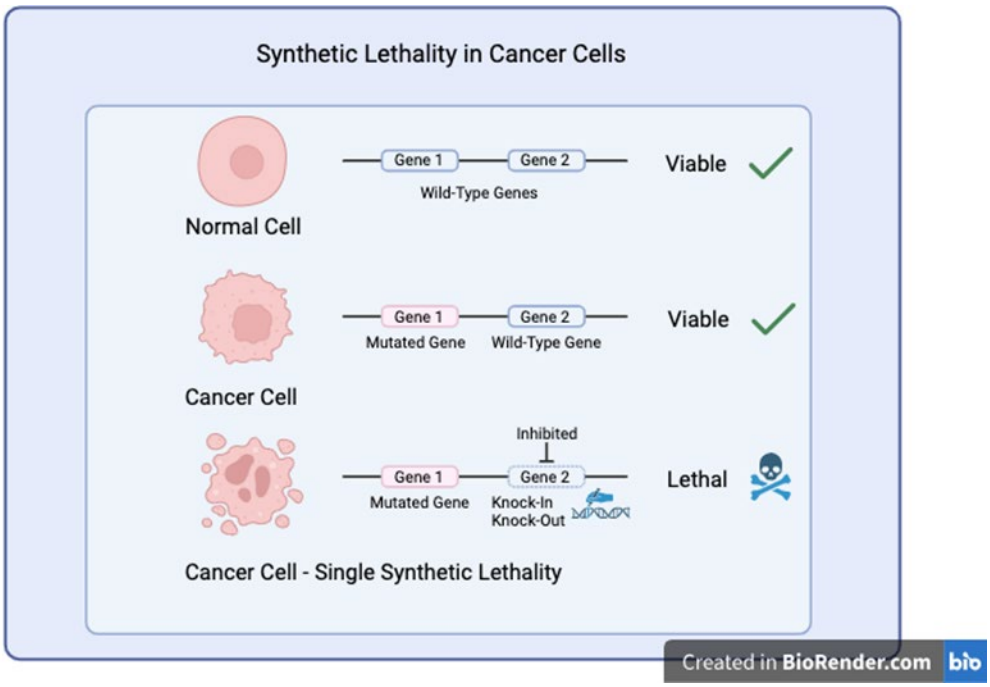

Figure 1. Synthetic Lethality in Cancer Cells. Synthetic lethality is initiated by removing a gene that the cancer cells rely on to survive. (Created in BioRender.com)

The idea of synthetic lethality was first introduced by an American geneticist Calvin Bridges in the 20th century. When studying Drosophila melanogaster, he noted that while the inheritance of one mutated gene does not result in its death, obtaining two specific genes results in a lethal interaction that ultimately kills the organism. The term's actual name is coined by Theodore Dobzhansky twenty years later when he was observing a similar interaction in Drosophila pseudoobscura (Nijman, 2010). Synthetic lethality remained an unstudied concept in genetics only until recently, when scientists have taken a closer inspection on these interactions between genes.

Currently, synthetic lethality has already been applied in the development of targeted cancer therapies. The earliest clinical trial of using synthetic lethality to target cancer cells involves the use of poly ADP-ribose polymerase (PARP) inhibitors in the treatment of breast cancers with BRCA1 or BRCA2 mutations, which are tumor suppressor genes involved in the repair of DNA double-strand breaks through the homologous recombination repair pathway (Lord and Ashworth, 2017). These genes are frequently mutated in cancer cells, forcing them to rely on alternative DNA repair mechanisms, including PARP-dependent DNA repair mechanisms. Inhibiting PARP in BRCA-mutated cells leads to a buildup of DNA damage in the cells, ultimately resulting in cell death, demonstrating the therapeutic potential of exploiting synthetic lethality in cancer treatment.

Additionally, apart from BRCA1/2 mutations, synthetic lethality can also be used to treat pancreatic cancer, whose mutated genes are already identified and can be used to create synthetic lethality-based treatments. Cells showing "BRCAness", containing a homologous recombination repair (HRR) defect similar to BRCA1 or BRCA2 loss-of-function mutations, can also be treated in the same manner to create synthetic lethality (Murai, 2023). Due to certain genetic aspects of pancreatic cancer's BRCAness, similar approaches can be taken to treat this type of cancer. However, there are still difficulties in identifying specific mutations in "BRCAness", partially hindering the process of widely utilizing synthetic lethality. Nevertheless, understanding the relation between "BRCAness" of certain cancer cells and the effect of synthetic lethality on these cells is crucial for future development.

Since 2014, there has been an increasing trend in articles regarding synthetic lethality and in cancer treatment. The current development of synthetic lethality drug targets available to be used to treat cancer still faces several

challenges; however, scientists have confirmed synthetic lethality treatment is part of the next generation of cancer therapy (Setton et al, 2021). Comparatively, current next generation sequencing technologies (NGS) that identifies genetic mutation in cancer cells, which includes a combination of zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) and CRISPR, is able to aid the process of initiating synthetic lethality in cancer cells (Khan et al, 2016).

CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a technology used in gene-editing which contains a guide RNA and a CRISPR-associated protein 9 (Cas9) (Redman, 2016). CRISPR offers an efficient way for scientists to modify a specific section of a gene, significantly impacting the field of gene therapy. As mentioned earlier, CRISPR can also be effective in cancer treatment by introducing synthetic lethality to cancer cells and screening for possible combinations of synthetically related genes.

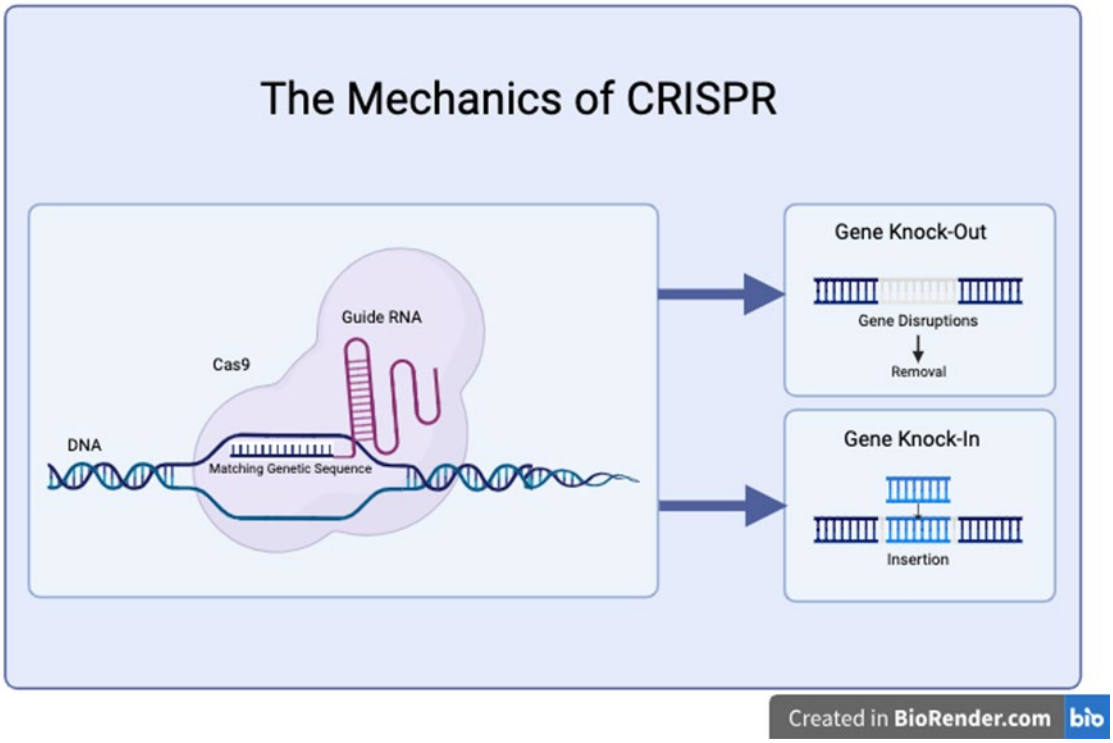

Figure 2. The Mechanics of CRISPR. CRISPR/Cas9 is a powerful tool that can initiate gene knock-outs or gene knock-ins efficiently. (Created in BioRender.com)

Before the introduction of CRISPR, gene-editing technology was time-consuming and often ineffective, most scientists used zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) to artificially modify the genome. ZFN is a protein that can be modified to bind to a specific section of DNA and create cleavage to target in gene editing (Carroll, 2011). Likewise, TALEN is a large protein with non-specific DNA targeting functions that can also cleave a wide arrange of DNA sections (Joung, 2012). However, these techniques have proven to be less effective than CRISPR technology. ZFN, for example, is known for its tendency to result in off target gene editing, posing significant risk for patients using this treatment (Gupta, 2014). Similarly, TALEN's large size means that one TALEN repeat is needed for each nucleotide that needs to be modified, resulting in lower efficiency and an elevated

cost (Bansal, 2023). However, CRISPR has proven to be a reliable and effective way to introduce changes to genes compared to its predecessors. Obtaining CRISPR modified cell lines is a relatively fast procedure, and the same applies for large scale CRISPR screenings, while still remaining at a fair cost (How CRISPR Is Changing Cancer Research and Treatment, 2020). All these CRISPR resulted in its widespread use and promising future in the field of cancer research.

CRISPR can be used to initiate synthetic lethality in cancer cells by knocking out specific genes related to the cell's survival. Gene knock-out can be induced by a deletion and gene knock-in can be induced by an insertion carried out by a guide RNA. CRISPR/Cas9 can also be used to homozygously inflict double-strand breaks in target regions (Ishibashi, 2020). These CRISPR-induced double-strand breaks result in disrupted gene function (Dalvie, 2021). Thus, the use of CRISPR is an effective strategy to trigger synthetic lethality. When CRISPR is used to knock out a particular gene in a cell, synthetic lethality may be initiated if that particular gene is the only one preventing the cell's death. Therefore, scientists need to identify the connection between different genes and that have compensating functions to identify potential synthetic lethal combinations. This can be achieved through CRISPR screening. CRISPR screening enables wide-scale analysis of gene function, providing quick understanding of the role of different genes, thus revealing their potential relationship to cell survival (Bock, 2022). CRISPR screening can therefore identify lethal gene partners that, when knocked out, can initiate synthetic lethality and result in cell death (Wei, 2020).

Cancer Treatment

Gene therapy in cancer treatment is not an emerging approach, nor is it novel in concept. The idea of combating cancer through a genetic perspective emerged after the American biochemist Paul Berg created the first recombinant DNA molecule in 1972. Recombinant DNA is a particular section of DNA that has been artificially modified in another organism. Berg achieved this by incorporating the DNA of the bacterium Escherichia coli into DNA polyoma tumor virus SV40, which holds the risk of causing cancer development after infection of an organism (Lukiw, 2023). The invention of this technique laid the groundwork of using gene therapy to treat cancer. However, it is not until 1986 that gene therapy was applied to cancer by researcher Dr. Steven A. Rosenberg. He pioneered the use of interleukin-2, a group of related protein the increase the growth and activity of T lymphocytes and B lymphocytes, in cancer immunotherapy, and later in gene therapy (Rosenberg, 1986). However, it should be noted that the introduction of gene therapy in cancer treatment should not be credited to any single individual due to the collective contributions of numerous scientists from its initial concept to its eventual utilization.

Novel advances of cancer gene therapy improve the efficiency and consistency of the treatment, increasing chances of survival and prolonging length of progression-free survival. There have already been several clinical cases that show the effectiveness of CRISPR-induced synthetic lethality in cancer treatment. The BRCA 1/2 mutations, as mentioned before, can be successfully targeted by creating synthetic lethality. A drug named Olaparib, first discovered by KuDOS Pharmaceuticals, developed by AstraZeneca, is a PARP inhibitor product that exploit the BRCA 1/2 gene's repair pathway to initiate synthetic lethality (Ragupathi, 2023). The clinical trials indicated that there was 70% less risk of disease progression or death in the 260 tested patients taking the drug with BRCA 1/2 mutations. The breast cancer patients taking the drug, had a median progression of a free survival period of 7 months, which is significantly higher than the chemotherapy patients whose median free survival progression period is of 4.2 months (Robson, 2017). The results of this clinical trial show that Olaparib is an effective treatment option for patients with BRCA 1/2 mutations. Since synthetic lethality is the key concept behind the function of this drug, it can be inferred that it may play a crucial role in the development of future drugs targeting mutations showing BRCAness.

Discussion

The implication of CRISPR-induced synthetic lethality is revolutionary in the field of cancer research by offering a new approach to targeting cancer directly. Synthetic lethality in cancer treatment is an emerging concept and further research needs to be done to implement synthetic lethality safely and effectively into cancer therapy. Aside from this, scientists have also proposed the idea of utilizing DNA damage response pathways to initiate synthetic lethality more efficiently, which holds much promise in future developments (Hu and Guo, 2020). However, the complication involved in initiating the process can yet be simplified in future developments, and there are still room left for improved accuracy. Thus, future research may be conducted revolving around these possible improvements to propagate its use in cancer therapy. However, synthetic lethality is still a powerful concept when exploited in treating cancer despite some of its drawbacks, containing the possibility of revolutionizing the field of cancer research through its advantage over other treatment methods, aided by CRISPR as vector.

The versatility of CRISPR screening also renders it a promising player in genetic research. It has been applied to various cancer genomic studies and will remain as a powerful tool in future studies. Yet, there are still some limitations of this technology, such as requirement for large cell numbers and the need of improved accuracy (He, 2021). But this does not hinder the wide-spread use CRISPR screening, especially in cancer research. CRISPR is a powerful tool that can improve research and treatment efficiency in various fields. In cancer therapy, this technology is especially significant due to its ability to knock-out specific genes quickly and to screen for synthetically related gene partners. CRISPR screening can effectively address one of the shortcomings of utilizing synthetic lethality, which is the difficulty in locating possible target genes to initiate the process. This technique, when compared to traditional methods, demonstrates higher efficiency and accuracy, thus able to quicken the search of lethal relations in gene partners.

Conclusion

Synthetic lethality and CRISPR holds much promise in cancer treatment because of their advantages over traditional methods such as chemotherapy. Not much research has yet been conducted regarding synthetic lethality, but there are already successful cases arising from the use of these techniques to treat cancer. More research is needed to ensure its safety and improve its efficiency. Future research must focus on increasing the accuracy of CRISPR screening as well as in decreasing the need for large cell numbers and finding more reliable vectors to initiate synthetic lethality in tumor cells. These improvements will ensure the widespread use of this form of therapy, and help cancer patients by offering less excruciating treatment while increasing their survival rate.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

World Health Organization: WHO. *Cancer*. 3 Feb. 2022, www.who.int/news-room/fact-sheets/detail/cancer.

Worldwide Cancer Research. “Why Haven’t We Cured Cancer Yet?” *Worldwide Cancer Research*, 16 Nov. 2023, www.worldwidecancerresearch.org/news-opinion/2021/march/why-havent-we-cured-cancer-yet.

Oberstein, Paul E., and Kenneth P. Olive. “Pancreatic Cancer: Why Is It so Hard to Treat?” *Therapeutic Advances in Gastroenterology*, vol. 6, no. 4, Mar. 2013, pp. 321–37, doi:10.1177/1756283x13478680.

Corrie, Pippa G. "Cytotoxic Chemotherapy: Clinical Aspects." *Medicine*, vol. 36, no. 1, Jan. 2008, pp. 24–28, doi:10.1016/j.mpmed.2007.10.012.

Cross, D., and J. K. Burmester. "Gene Therapy for Cancer Treatment: Past, Present and Future." *Clinical Medicine & Research*, vol. 4, no. 3, Sept. 2006, pp. 218–27, doi:10.3121/cmr.4.3.218.

Douglas, Joanne T. "Cancer Gene Therapy." *Technology in Cancer Research & Treatment*, vol. 2, no. 1, Feb. 2003, pp. 51–63, doi:10.1177/153303460300200107.

Wang, Dongrui, et al. "CRISPR Screening of CAR T Cells and Cancer Stem Cells Reveals Critical Dependencies for Cell-Based Therapies." *Cancer Discovery*, vol. 11, no. 5, Dec. 2020, pp. 1192–211, doi:10.1158/2159-8290.cd-20-1243.

Ding, Shuning, et al. "Single-cell RNA Sequencing in Breast Cancer: Understanding Tumor Heterogeneity and Paving Roads to Individualized Therapy." *Cancer Communications*, vol. 40, no. 8, July 2020, pp. 329–44, doi:10.1002/cac2.12078.

Topatana, Win, et al. "Advances in Synthetic Lethality for Cancer Therapy: Cellular Mechanism and Clinical Translation." *Journal of Hematology & Oncology*, vol. 13, no. 1, Sept. 2020, doi:10.1186/s13045-020-00956-5.

Setton, Jeremy, et al. "Synthetic Lethality in Cancer Therapeutics: The Next Generation." *Cancer Discovery*, vol. 11, no. 7, Apr. 2021, pp. 1626–35, doi:10.1158/2159-8290.cd-20-1503.

Kaelin, William G. "The Concept of Synthetic Lethality in the Context of Anticancer Therapy." *Nature Reviews. Cancer*, vol. 5, no. 9, Aug. 2005, pp. 689–98, doi:10.1038/nrc1691.

Beijersbergen, Roderick L., et al. "Synthetic Lethality in Cancer Therapeutics." *Annual Review of Cancer Biology*, vol. 1, no. 1, Mar. 2017, pp. 141–61, doi:10.1146/annurev-cancerbio-042016-073434.

Nijman, Sebastian M. B. "Synthetic Lethality: General Principles, Utility and Detection Using Genetic Screens in Human Cells." *FEBS Letters*, vol. 585, no. 1, Nov. 2010, pp. 1–6, doi:10.1016/j.febslet.2010.11.024.

Murai, Junko, and Yves Pommier. "BRCAness, Homologous Recombination Deficiencies, and Synthetic Lethality." *Cancer Research*, vol. 83, no. 8, Apr. 2023, pp. 1173–74, doi:10.1158/0008-5472.can-23-0628.

Khan, Faheem Ahmed, et al. "CRISPR/Cas9 Therapeutics: A Cure for Cancer and Other Genetic Diseases." *Oncotarget*, vol. 7, no. 32, May 2016, pp. 52541–52, doi:10.18632/oncotarget.9646.

Redman, Melody, et al. "What Is CRISPR/Cas9?" *Archives of Disease in Childhood Education & Practice*, vol. 101, no. 4, Apr. 2016, pp. 213–15, doi:10.1136/archdischild-2016-310459.

Gupta, Rajat M., and Kiran Musunuru. "Expanding the Genetic Editing Tool Kit: ZFNs, TALENs, and CRISPR-Cas9." *Journal of Clinical Investigation*, vol. 124, no. 10, Oct. 2014, pp. 4154–61, doi:10.1172/jci72992.

Bansal, Roohi. "Limitations of ZFNs and TALENs (Part 18- CRISPR in Gene Editing and Beyond)." *Medium*, 10 May 2023, medium.com/biotechnology-by-tsb/limitations-of-zfns-and-talens-part-18-crispr-in-gene-editing-and-beyond-17593e183b8f.

"How CRISPR Is Changing Cancer Research and Treatment." *Cancer.gov*, 27 July 2020, www.cancer.gov/news-events/cancer-currents-blog/2020/crispr-cancer-research-treatment.

Ishibashi, Airi, et al. "A Simple Method Using CRISPR-Cas9 to Knock-out Genes in Murine Cancerous Cell Lines." *Scientific Reports*, vol. 10, no. 1, Dec. 2020, doi:10.1038/s41598-020-79303-0.

Dalvie, Neil C., et al. "Simplified Gene Knockout by CRISPR-Cas9-Induced Homologous Recombination." *ACS Synthetic Biology*, vol. 11, no. 1, Dec. 2021, pp. 497–501, doi:10.1021/acssynbio.1c00194.

Wei, Xiaolong, et al. "Targeted CRISPR Screening Identifies PRMT5 as Synthetic Lethality Combinatorial Target With Gemcitabine in Pancreatic Cancer Cells." *Proceedings of the National Academy of Sciences of the United States of America*, vol. 117, no. 45, 2020, pp. 28068–79. *JSTOR*, www.jstor.org/stable/26970706.

Bock, Christoph, et al. "High-content CRISPR Screening." *Nature Reviews Methods Primers*, vol. 2, no. 1, Feb. 2022, doi:10.1038/s43586-021-00093-4.

He, Chenglong, et al. "CRISPR Screen in Cancer: Status Quo and Future Perspectives." *PubMed Central (PMC)*, 2021, www.ncbi.nlm.nih.gov/pmc/articles/PMC8085856.

Carroll, Dana. “Genome Engineering With Zinc-Finger Nucleases.” *Genetics*, vol. 188, no. 4, Aug. 2011, pp. 773–82, doi:10.1534/genetics.111.131433.

Joung, J. Keith, and Jeffry D. Sander. “TALENs: A Widely Applicable Technology for Targeted Genome Editing.” *Nature Reviews Molecular Cell Biology*, vol. 14, no. 1, Nov. 2012, pp. 49–55, doi:10.1038/nrm3486.

Ragupathi, Ashwin, et al. “Targeting the BRCA1/2 Deficient Cancer With PARP Inhibitors: Clinical Outcomes and Mechanistic Insights.” *Frontiers in Cell and Developmental Biology*, vol. 11, Mar. 2023, doi:10.3389/fcell.2023.1133472.

Robson, Mark, et al. “Olaparib for Metastatic Breast Cancer in Patients With a Germline BRCA Mutation.” *New England Journal of Medicine*, vol. 377, no. 6, Aug. 2017, pp. 523–33, doi:10.1056/nejmoa1706450.

Lukiw, Walter J. “Commentary: A Tribute to Dr. Paul Berg (1926–2023) American Biochemist, Nobel Laureate and Discoverer of Recombinant DNA Technology, Vaccine and Genetic Engineering.” *Frontiers in Cell and Developmental Biology*, vol. 11, May 2023, doi:10.3389/fcell.2023.1210530.

Rosenberg, Steven A., et al. “A New Approach to the Adoptive Immunotherapy of Cancer With Tumor-Infiltrating Lymphocytes.” *Science, New Series*, vol. 233, no. 4770, 1986, pp. 1318–21. *JSTOR*, www.jstor.org/stable/1697986.