CRISPR/Cas9 as a Treatment for Cancer

Tiffany Shaljian[1] and Dr. Chad Barber[1#]

[1]California Lutheran University
[#]Advisor

ABSTRACT

For decades, cancer treatments such as chemotherapy, radiotherapy, and surgery have been used to treat various types of cancer. These treatments have also taken a significant toll on the general health of the patient and do not always result in complete remission, which is why new methods of treatment are required. The many experimental methods of CRISPR/Cas9, a novel treatment using genetic engineering, have allowed researchers to discover its ability to successfully treat several types of cancers. With any discovery of treatment, continued research is required to determine the abilities of CRISPR/Cas9 among the other forms of cancer. However, the data collected thus far has shown that it is successful in being used as a cancer therapeutic, even among some of the more aggressive types of cancer. The use of CRISPR/Cas9 has shown proof of principle in the field of oncological research in treating a variety of cancers, with hopes for complete remission.

Introduction

In the last few decades, cancer treatments such as chemotherapy, radiotherapy, and surgery are being studied and modified due to their impermanent remission. In using CRISPR/Cas9, a novel treatment using genetic engineering, there may be potential for a lasting therapeutic effect in cancer patients. Researchers conduct experiments with CRISPR/Cas9 to attempt to edit the genome of the cancer cells to induce apoptosis or introduce apoptotic agents. CRISPR/Cas9 efficiency lies with the type of cancer cells that are being experimented with due to their tendency to respond differently to the treatments. Experiments will typically focus on a specific type of cancer, so their success with CRISPR/Cas9 cannot be applied to the remaining cancer types due to their varying cellular responses. In conclusion, this paper will synthesize the results of many studies performed using CRISPR/Cas9 as a cancer treatment to support if it could truly be effective as a cure.

CRISPR/Cas9 is short for Clustered Regularly Interspaced Short Palindromic Repeats, with Cas being the locating and cleaving protein associated with CRISPR. It has a variety of uses including gene therapy, identifying and targeting diseases using screening, as well as knockout and knockin capabilities (Pellagatti & Boultwood, 2022). The use of CRISPR/Cas9 for gene editing is performed using an enzyme to induce a double stranded break of DNA to correct, delete, or add genes. CRISPR/Cas9 can also be used as a gene delivery system, depending on the contents that are to be delivered. There are three categories of delivery using CRISPR: viral vectors, non-viral vectors, and physical delivery, each with their respective methods and success rates (Lino et al., 2018). Some diseases that CRISPR/Cas9 has shown success in curing include HIV, muscular dystrophy, autism, inherited eye disorders, and sickle cell anemia. In the review by Zhang et al. (2020), authors explain the technology, limitations, and potential uses for treating cancer. They begin by defining the two categories of CRISPR/Cas9, the single-plasmid and the dual-plasmid system. The single-plasmid system is a large system including Cas9, screening markers, promoters, and gRNA, guide RNA. The dual-plasmid system has higher efficiency including the promoter and gRNA. There are a multitude of uses for CRISPR/Cas9 among a diverse range of diseases, which makes it such a novel tool for cancer treatment. Its ability to be manipulated depending on the form of the disease that is being studied leaves the medical field with high hopes for a successful cancer therapy.

One of the most dangerous cancer diagnoses is regarding the pancreas. While pancreatic cancers are heavily studied and experimented for therapeutics, there are still no significant findings. Nearly all of the cancer therapeutics have been used against pancreatic cancers, including pancreatic ductal adenocarcinoma (PDAC) which is particularly aggressive, yet the cancer is resistant to treatment (Parassia et al., 2021). In addition, the mortality rate due to pancreatic cancer continues to increase with a 5-year survival rate of 5%, which is a driving force behind finding a successful therapeutic (Ilic & Ilic, 2016). Since traditional cancer therapeutic methods have been ineffective, researchers have begun to attempt using CRISPR/Cas9 as a method to treating PDAC. Multiple studies have been published, which are discussed later in this paper, reporting success with using the varying methods of CRISPR/Cas9 to target and treat PDAC.

The numerous methods of CRISPR/Cas9 are particularly beneficial to cancer research due to the variation among different types of cancers. Each cancer has its unique genetic makeup, which can make it resistant to certain treatments, resulting in a severe toll on the patient. Since CRISPR is a flexible method, it can target multiple genetic origins of the disease that it is being used for, making it an ideal therapeutic for cancer. Cancers can be in the form of tumors, as well as infecting the blood cells, lymph system, and the plasma of bones. As a result, treatments can be difficult and oftentimes uncooperative because they are spread throughout the entire body. This is why new therapeutic approaches are required in the field of oncology. CRISPR/Cas9 as a method of cancer treatment is proof of principle and continues to be used to study additional diseases and cancers for further treatment.

Knockout Therapy of Cancer Genes Using CRISPR/Cas9

Initial studies using CRISPR/Cas9 have generated treatments for genetic disorders by editing the genome to remove mutations. However, recent studies have begun to utilize CRISPR/Cas9 as a method to treat cancer. In the experiments conducted by Aguirre et al. (2016), researchers used CRISPR/Cas9 to perform edits in the DNA of 33 cancer cell lines and observe the proliferation and survival responses of each. They began by identifying the genes that the cancer is dependent on. Next, they identified the respective amplifications of those genes in order to compare it to the rest of the genome. Then, they targeted those amplifications and used CRISPR/Cas9 and single guide RNAs, sgRNAs, to generate DNA damage. As a result, they found that a single cut at the DNA target site significantly decreased cell proliferation. Their findings demonstrate an increased number of sgRNA in cells, meaning DNA damage, caused a G_2 cell-cycle arrest. Based on their results, Aguirre et al. (2016) suggest two cellular responses to CRISPR/Cas9 treatment. One of the responses is the antiproliferative effect induced by CRISPR/Cas9 DNA cuts, increased with cuts induced by sgRNA, regardless of the target gene. The other response is the essential target gene (responsible for driving tumorigenesis) knockout, which caused decreased expression of proteins. Their results indicate that CRISPR/Cas9 was able to successfully reduce proliferation by targeting amplifications in the cancer cell genomes.

The blockade of programmed cell death protein (PD-1) and its ligand (PDL-1) using chimeric antigen receptor (CAR-T) has shown efficacy across a variety of cancers among patients. Authors Wang et al. (2021) suggest using CRISPR/Cas9 as a replacement in blocking PD-1. They performed a knockout in MPTK-CAR-T, mesothelin-specific CAR-T, cells, and found a 90% efficiency based on flow cytometry analysis. However, in their patient trials they found that PD-1 disruption did not trigger antitumor activity as successfully as the anti-PD-1 agent in mesothelin-targeted CAR-T. This could be due to the delivery method or the lack of persistence of MPTK-CAR-T cells long-term *in vivo*. Wang et al. (2021) believe that this could be due to the short-term viability of MPTK-CAR-T cells *in vivo*. In short, CRISPR/Cas9 edited CAR-T cells containing disrupted PD-1 did not demonstrate proliferation rates that mimic tumor growth. They were also able to conclude that these experiments did not result in safety issues for human patients. However, further research is required to understand the signaling between T cell receptor (TCR) and CAR to develop an efficient therapeutic in CAR-T cells.

Previous studies using CRISPR/Cas9 for PD-1 knockout T cell transfer have had a low effectiveness due to the brief lifetime of T cells when transferred into host cells. Yang et al. (2022) theorized that infusion of PD-1 knockout

T cells would be more effective in patients after receiving total body irradiation (TBI). They created a ribonucleoprotein complex from CRISPR/Cas9/gRNA delivered to the nuclei of T cells in order to knockout the PD-1 gene. Then, using flow cytometry they detected the rate of GFP, which detects protein expression, positive PD-1 knockout T cells at 80.8±9.23% and at 80.1±6.5% for the negative control. They repeated the PD-1 knockout in B16 melanoma cells with a 95% success rate determined by flow cytometry. The success rates from these experiments indicate that a potential therapeutic following the same protocol could be applied to humans in preclinical trials successfully. In summary, Yang et al. (2022) were able to successfully knockout PD-1 in T cells following TBI with statistically significant results. Their results strongly suggest that similar therapeutics, using CRISPR/Cas9 to knockout PD-1, would be effective in lymphopenic mice rather than immunocompetent mice for malignant melanoma. These findings are significant to the scientific community, especially for those studying cancer therapeutics because these methods could be repurposed for other types of cancer, including more aggressive ones. Methods such as these could be translated to preclinical trials for humans with malignant melanoma patients after they have been treated with radiation therapy or chemotherapy.

In peripheral T-cell lymphoma (PTCL), a DNA methylation inhibitor, or hypomethylating agent (HMA), responses are insufficiently understood. In addition, the activity of HMA in histological PTCL remains unexplained. Wong et al. (2022) performed experiments using CRISPR/Cas9 to determine genes whose loss of function could generate sensitivity responses to HMA. They knocked out Hut78 cells, due to their sensitivity to HMA, and their results support sensitivity to guadecitabine, an HMA, but the knockout does not inhibit the viability of Hut78 cells. Also, histone methyltransferase, SETD2, deletion caused HMA sensitivity, but tumor suppressor, TET2, did not cause any changes to HMA sensitivity. In addition, Wong et al. (2022) achieved an overall sensitivity response rate of 40%, with 10% complete responses, among 20 patients. In short, CRISPR/Cas9 screening allowed authors to predict sensitivity responses and administer guadecitabine accordingly. The CRISPR/Cas9 screens also demonstrated inhibitors at immune checkpoints as well as HMA inhibitors in combination with decitabine. However, guadecitabine is not in the developing treatment protocol for myeloid disease anymore, but the findings by Wong et al. (2022) provide a basis for future research to be conducted in lymphomas with mutated SETD2. If future findings further support the use of guadecitabine after CRISPR/Cas9 screening, the treatment protocol could be redefined.

Use of Vector Transfection of CRISPR/Cas9 to Deliver Apoptotic Agents

One of the most common sexually transmitted infections in the United States is human papillomavirus (HPV), which can progress to cervical cancer. Yoshiba et al. (2018) used CRISPR/Cas9 to identify and target the protein E6, which degrades p53, to cause mutations in the cervical cancer cells. The degradation of p53 will lead to tumor formation and growth, since p53 regulates cell proliferation. Next, they were able to identify and quantify the mutation rates among HPV positive cervical cancer cell lines: 82%, 77%, and 87% among HeLa/Cas9, HCS-2/Cas9, and SKG-1/Cas9 respectively. They then identified the expression of p53 amongst cells, both transduced and non-transduced, using a Western Blot, which detects specific proteins. The results indicate that CRISPR/Cas9 was able to successfully knockout E6. As a result of the knockout, there was an increase in p53 levels, and apoptotic events were noted (Yoshiba et al., 2018). Their results show a significant success rate in using CRISPR/Cas9 as a therapeutic in cervical cancer. CRISPR/Cas9 and AAV vectors demonstrated an effective treatment for patients whose HPV progressed into cervical cancer. In a dose-dependent manner, Yoshiba et al. (2018) inhibited E6 and allowed for p53 to suppress growth of cervical tumors. Cervical cancer treatment that utilizes CRISPR/Cas9 is an effective therapeutic and could become a preferred method of therapy for patients with cervical cancer.

Other Methods of Treatment Using CRISPR/Cas9

KRAS, a variant of RAS oncogenes, has one of the highest mutation rates in human cancer and is the most viable target for therapeutics. Cheng et al. (2022) found that RASON, a positive regulator of oncoproteins, acts as a regulator for RAS signaling and in pancreatic ductal adenocarcinoma (PDAC) causes significant overexpression of protein. In order to differentiate between RASON and LINC00673 function, authors performed a knockout using CRISPR/Cas9 on the RASON region in AsPC-1 and PANC-1 cell lines, which resulted in significant tumor growth inhibition and a decrease in cell proliferation. On the other hand, cells that had overexpression of LINC00673 or RASON-ORF demonstrated increased tumor growth (Cheng et al., 2022). Therefore, targeting RASON alone or with KRAS inhibitors, proliferation can be inhibited. Ultimately, Cheng et al. (2022) were able to achieve what other researchers believed to be impractical, which is to directly target and inhibit KRAS using the CRISPR/Cas9 system. They confirmed their results using pancreatic cancer sensitization to inhibitors after RASON knockout. In their discovery of RASON, Cheng et al. (2022) were able to provide the basis for the use of CRISPR/Cas9 in the treatment of KRAS mutated cancers. As a result of their findings, others can begin investigating this method as a therapeutic for treating cancers with KRAS mutations.

In order to create successful cancer therapeutics, cellular targets must be identified to further investigate their responses and apoptotic effects. Previous studies suggested a MEK inhibitor because the MEK pathway is a driver for tumor formation in pancreatic ductal adenocarcinoma (PDAC), but MEK inhibition failed in clinical trials in PDAC patients. Therefore, Szlachta et al. (2018) performed screens using CRISPR to identify the genes that affect PDAC cell survival while MEK is inhibited. Their analysis showed approximately 64% detection of sgRNAs, single-guide RNA, which directs Cas9 in cleaving DNA, in untreated tumors, suggesting that about 70% of cells that contain sgRNAs are contributors to tumor formation *in vivo*. Of the depleted sgRNAs are CENPE and RRM1, which are significant in tumor formation in PDAC. In conclusion, Szlachta et al. (2018) show that inhibition of MEK and CENPE will trigger cell death during mitosis because signaling by MEK is a requirement in overriding signals for apoptosis in the event of mitotic delay. Additionally, authors emphasize the use of CRISPR for cancer screening in order to predict drug responses since it remains a challenge for precise therapeutics. While this strategy is helpful for predicting drug responses in cancer, it can also be applied to other diseases as well.

Drug responses in specific cancer types can be difficult to predict and understand, due to their complex genetic makeup, which can also be the cause of their genetic nature. Basal breast cancers (BBCs) have the most severe prognosis out of the types of breast cancer due to their resistance to targeted therapies and high mitotic activity. One of its identified cell dependencies is an enzyme related to mitosis called MELK (the maternal embryonic leucine zipper kinase) that is currently being targeted for inhibition by a novel chemotherapy agent, OTS167. However, Lin et al. (2017) found that in mutating MELK, cancer cell growth was not observed. In addition, they found OTS167 targeted null mutations, meaning that the activity was achieved through an off-target termination, which is an unintended termination of an untargeted site. In their CRISPR system, Lin et al. (2017) found that they were able to deplete MELK expression. Additionally, they attempted to understand if MELK is truly a cell dependency for BBCs by measuring cell proliferation. One of their transduced cell lines form a type of cancer with an over-expression of MELK. It had a 16.9hr mean doubling time, while the MELK gRNA transduced line had 16.8hr mean doubling time, which raises the question whether MELK is a dependency for BBCs or other cancer types as well. Many other publications addressing MELK and its biological role have been conflicting with each other, and Lin et al. (2017) have found that by using CRISPR/Cas9, a more accurate model for MELK expression and inhibition can be created. Their findings conclude that MELK is not the direct cause for cancer progression, but can be combined with other kinases. Additional studies would need to be conducted to confirm if it is conjoining. In conclusion, the MELK inhibitors that are currently being used for patients with BBCs are doubtful to be efficient therapies, and further research is needed to develop an effective therapy.

One of the common myeloid malignancies (heterogenous malignancies) are myelodysplastic syndromes (MDS) which can cause higher susceptibility to infections, anemia, and other related blood disorders. U2AF1 and

SF3B1 are two of the many mutations found in splicing factor genes that cause abnormal pre-mRNA splicing of genes (Pellagatti and Boultwood, 2022). CRISPR/Cas9 and induced pluripotent stem cell (iPSC) technologies have been used to create new models of the mutated splicing factors that cause MDS. Pellagatti and Boultwood reference multiple experiments performed using CRISPR/Cas9 and iPSC to identify and missplice SF3B1 mutations to decrease the expression of genes responsible for the metabolism of heme and iron (Clough et al., 2022, Pellagatti & Boultwood, 2022). In addition, Clough et al. found that the driving force of ring sideroblast (RS) formation in SF3B1 is the excessive splicing and decreased expression of ABCB7, an iron transporter (Pellagatti & Boultwood, 2022). Pellagatti and Boultwood collectively reviewed the results of multiple studies using CRISPR and iPSC as a treatment for MDS and the specific mutations that are instrumental in the MDS phenotype. Furthermore, Pellagatti and Boultwood urge researchers to continue their studies with CRISPR and iPSC because the remaining genes that are responsible for contributing to MDS are yet to be determined.

Discussion

For decades, a cancer prognosis meant that a patient would undergo severe forms of treatment for months at a time, in order for them to hopefully reach remission. The treatments that are currently offered include radiotherapy, chemotherapy, and surgical intervention. While these treatments have been effective for some patients, they have not proven to be long lasting cures. In addition to their short-term durability, they take a significant toll on the general health of the patient. With the recent discovery of CRISPR/Cas9 as a cancer therapeutic, the field of oncology is optimistic for their patients.

In using the multiple methods of CRISPR/Cas9, researchers have found that it can act as an effective therapeutic for cancer treatment. Since it is a relatively new technology, results continue to vary among different types of cancer. The reason behind the variation remains unknown, but many theorize that it could be due to the aggressive nature of some cancers compared to others. One study conducted an experiment which challenges the current clinical trials using CRISPR/Cas9 as a treatment for different types of cancer including breast cancer (Lin et al., 2017). However, in another experiment researchers found that CRISPR/Cas9 was able to suppress pancreatic ductal adenocarcinoma (PDAC) by repairing tumor-suppressor proteins (Azmi et al., 2020). Therefore, continued research is required to differentiate which cancers CRISPR/Cas9 is effective against.

Some research indicates that CRISPR/Cas9 can be significantly effective in tumor inhibition when combined with chemotherapy, which is important due to the numerous types of cancer. Each individual cancer will have its particular method of treatment that may or may not be different from another, the important aspect is that CRISPR/Cas9 will work successfully even if it is combined with another form of treatment. While it is preferable that CRISPR/Cas9 be used independently to preserve the overall health of the patient, combination therapy remains a viable option for particularly aggressive cancers. These aggressive cancers have been known to leave patients with a poor prognosis, so anything that can improve that prognosis is a step forward in oncological treatment.

In conclusion, CRISPR/Cas9 is a relatively new technology that has a variety of uses among a multitude of diseases. This allows it to be manipulated and molded for different forms of treatment, which is a significant advantage for oncological research. The novel technology has been shown to have significant success in treating multiple different types of cancers *in vitro*. These successes are being translated into pre-clinical and clinical trials in order to deliver these treatments to patients with cancer. With their continued success, CRISPR/Cas9 will be offered as a cancer therapeutic to all patients and could result in a lasting complete remission.

Future Directions

The future directions with CRISPR/Cas9 as a therapeutic for cancer have been identified as vector transfection, knockouts, and multiple other combined methods, which will allow them to move forward to pre-clinical trials. Many of the pre-clinical trials are currently in place, but what remains of them is durable and long-lasting success, so that CRISPR/Cas9 can begin being used equally to other cancer therapeutics such as radiotherapy, chemotherapy, and surgical intervention. Some studies have used CRISPR/Cas9 accompanied with the common cancer therapeutics for

longer lasting results. The hopes for the future of CRISPR/Cas9 would be to use it independently in order to preserve the general health and well-being of the patient. The benefits of CRISPR/Cas9 as a cancer treatment outweigh the current treatments for some cancers and will allow patients to have better qualities of life as a result.

Acknowledgements

I would like to thank Dr. Chad Barber for his guidance and feedback during the creation of this paper. He has provided incredible support throughout this process, for which I am grateful. I would also like to thank California Lutheran University for providing me with the opportunity to write this paper.

References

Aguirre, A. J., Meyers, R. M., Weir, B. A., Vazquez, F., Zhang, C. Z., Ben-David, U., Cook, A., Ha, G., Harrington, W. F., Doshi, M. B., Kost-Alimova, M., Gill, S., Xu, H., Ali, L. D., Jiang, G., Pantel, S., Lee, Y., Goodale, A., Cherniack, A. D., Oh, C., Kryukov, G., Cowley, G., Garraway, L., Stegmaier, K., Roberts, C., Golub, T., Meyerson, M., Root, D., Tsherniak, A., and Hahn, W. C. (2016). Genomic Copy Number Dictates a Gene-Independent Cell Response to CRISPR/Cas9 Targeting. *Cancer discovery*, 6(8), 914–929. https://doi.org/10.1158/2159-8290.CD-16-0154.

Azmi, A. S., Khan, H. Y., Muqbil, I., Aboukameel, A., Neggers, J. E., Daelemans, D., Mahipal, A., Dyson, G., Kamgar, M., Al-Hallak, M. N., Tesfaye, A., Kim, S., Shidham, V., M Mohammad, R., Philip, P. A. (2020). Preclinical Assessment with Clinical Validation of Selinexor with Gemcitabine and Nab-Paclitaxel for the Treatment of Pancreatic Ductal Adenocarcinoma. *Clinical cancer research: an official journal of the American Association for Cancer Research*, 26(6), 1338–1348. DOI: 10.1158/1078-0432.CCR-19-1728.

Cheng, R., Li, F., Zhang, M., Xia, X., Wu, J., Gao, X., Zhou, H., Zhang, Y., Shen, S., Kang, T., Liu, Z, Xiao, F., Yao, H., Xu, J., Yan, C., and Zhang, N. (2022). A novel protein RASON encoded by a lncRNA controls oncogenic RAS signaling in KRAS mutant cancers. *Cell Research*, Advance Online Publication. https://doi.org/10.1038/s41422-022-00726-7.

Clough, C.A., Pangallo, J., Sarchi, M., Ilagan, J.O., North, K., Bergantinos, R., Stolla, M.C., Naru, J., Nugent, P., Kim, E., Stirewalt, D.L., Subramaniam, A.R., Abdel- Wahab, O., Abkowitz, J.L., Bradley, R.K., Doulatov, S., 2022. Coordinated missplicing of TMEM14C and ABCB7 causes ring sideroblast formation in SF3B1-mutant myelodysplastic syndrome. *Blood*, 139, 2038–2049. DOI: 10.1182/blood.2021012652.

Ilic, M., & Ilic, I. (2016). Epidemiology of pancreatic cancer. *World journal of gastroenterology*, *22*(44), 9694–9705. DOI: 10.3748/wjg.v22.i44.9694.

Lin, A., Giuliano, C. J., Sayles, N. M., and Sheltzer, J. M. (2017). CRISPR/Cas9 mutagenesis invalidates a putative cancer dependency targeted in on-going clinical trials. *eLife*, 6, e24179. https://doi.org/10.7554/eLife.24179.

Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. *Drug delivery*, 25(1), 1234–1257. DOI: 10.1080/10717544.2018.1474964.

Parrasia, S., Zoratti, M., Szabò, I., & Biasutto, L. (2021). Targeting pancreatic ductal adenocarcinoma (PDAC). *Cell Physiol Biochem*, 55, 61-90. DOI: 10.33594/000000326.

Pellagatti, A., and Boultwood, J. (2022). Splicing factor mutations in the myelodysplastic syndromes: Role of key aberrantly spliced genes in disease pathophysiology and treatment. *Advances in biological regulation*, Advance online publication. DOI: 10.1016/j.jbior.2022.100920.

Szlachta, K., Kuscu, C., Tufan, T., Adair, S. J., Shang, S., Michaels, A. D., Mullen, M. G., Fischer, N. L., Yang, J., Liu, L., Trivedi, P., Stelow, E. B., Stukenberg, P. T., Parsons, J. T., Bauer, T. W., and Adli, M. (2018). CRISPR knockout screening identifies combinatorial drug targets in pancreatic cancer and Models Cellular Drug response. *Nature Communications*, 9 (1), 1-13. https://doi.org/10.1038/s41467-018-06676-2.

Wang, Z., Li, N., Feng, K., Chen, M., Zhang, Y., Liu, Y., Yang, Q., Nie, J., Tang, N., Zhang, X., Cheng, C., Shen, L., He, J., Ye, X., Cao, W., Wang, H., and Han, W. (2021). Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors. *Cell. & Mol. Immunology*, 18(9), 2188–2198. https://doi.org/10.1038/s41423-021-00749-x.

Wong, J., Gruber, E., Maher, B., Waltham, M., Sabouri-Thompson, Z., Jong, I., Luong, Q., Levy, S., Kumar, B., Brasacchio, D., Jia, W., So, J., Skinner, H., Lewis, A., Hogg, S. J., Vervoort, S., DiCorleto, C., Uhe, M., Gamgee, J., Opat, S., Gregory, G., Polekhina, G., Reynolds, J., Hawkes, E., Kailainathan, G., Gasiorowski, R., Kats, L., and Shortt, J. (2022). Integrated clinical and genomic evaluation of guadecitabine (SGI-110) in peripheral T-cell lymphoma. *Leukemia*, 36(6), 1654–1665. https://doi.org/10.1038/s41375-022-01571-8.

Yang, Z., Wu, H., Lin, Q., Wang, X., and Kang, S. (2022). Lymphopenic condition enhanced the antitumor immunity of PD-1-knockout T cells mediated by CRISPR/Cas9 system in malignant melanoma. *Immunology letters*, 250, 15–22. DOI: 10.1016/j.imlet.2022.09.004.

Yoshiba, T., Saga, Y., Urabe, M., Uchibor, R., Matsubara, S., Fujiwara, H., and Mizukami, H. (2019). CRISPR/Cas9 mediated cervical cancer treatment targeting human papillomavirus E6. *Oncology Letters*, 17, 2197-2206. DOI: 10.3892/ol.2018.9815.

Zhang, M., Eshraghian, E. A., Jammal, O. A., Zhang, Z., and Zhu, X. (2020). CRISPR technology: The engine that drives cancer therapy. *Biomedicine & Pharmacotherapy*, 113, 111007. DOI: 10.1016/j.biopha.2020.111007.