WEP
Warwick
Evans
Publishing

Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 7
MISBP 2024

Application and Prospective of CRISPR-Cas in Cancer Diagnosis and Therapy

Xiaoshu Li*
East China University of Science and Technology, Shanghai, China
* Corresponding Author Email: lixiaoshu0517@163.com

Abstract. Cancer, a leading cause of mortality worldwide, poses a significant challenge to public health. The advent of CRISPR-Cas systems has introduced a novel and promising approach to overcoming the limitations of conventional cancer therapies. This review delves into the burgeoning role of CRISPR-Cas in the oncology field, highlighting its utility in both cancer diagnosis and therapy. In the diagnostic realm, CRISPR-based technologies, such as DETECTR and SHERLOCK, leverage the non-specific collateral cleavage activity of Cas12 and Cas13 proteins for highly sensitive detection of nucleic acid sequences, offering rapid and sensitive diagnostic capabilities. Therapeutically, CRISPR-Cas systems inactivate oncogenes and enhance treatment outcomes through engineered variants and innovative approaches. Variants such as nCas9 and dCas9 have been engineered to enhance specificity and transcriptional regulation, respectively. Challenges including the need for high specificity to avoid off-target effects, long-term safety assessments and ethical considerations persist, yet ongoing research aims to refine these technologies for enhanced efficacy and safety. Overall, CRISPR-Cas systems herald a new era in precision medicine for cancer patients.

Keywords: Cancer diagnosis; Cancer treatment; CRISPR-Cas; Genome editing.

1. Introduction

Cancer is a malignant neoplastic condition characterized by uncontrolled cellular proliferation with potential for invasive local growth and systemic metastasis, posing a significant threat to human health and life. According to the National Bureau of Statistics of China, since 2000, cancer has been the leading cause of mortality in urban areas, with a gradual increase in morbidity and mortality in rural areas [1]. An estimated 609,820 individuals in the United States are projected to succumb to cancer in 2023, equating to an average of 1,670 fatalities daily. In 2024, the number rises to 611,720 [2]. The increasing mortality associated with cancer underscores the urgent need for continuous advancement and improvement of oncological therapies. As the second leading cause of death globally, cancer highlights the critical importance of efforts in prevention, diagnosis, treatment, and management to address this growing public health concern [3].

In diagnostic aspect, current methodologies for cancer diagnosis exhibit limitations in specificity and sensitivity, compounded by significant inter-individual variability in biomarkers, which makes the capture of early signals challenging and can even lead to over-diagnosis or misdiagnosis [3]. In the context of cancer therapy, the timeliness of intervention during the early stages of cancer is crucial; hence, there is a necessity for a more sensitive and etiologically rooted diagnostic approach to facilitate the early detection of oncogenesis.

Currently, the main approaches to cancer treatment include chemotherapy, radiotherapy and immunotherapy. Chemotherapy are prone to multidrug resistance (MDR), which results in an inadequate suppression of tumor proliferation, metastasis, and recurrence[4]. Nanomedicine is capable of enhancing therapeutic efficacy; however, the potential toxicity of the introduced materials to the human body remains an issue that requires further investigation [5]. Radiotherapy is linked to adverse effects, encompassing cardiotoxicity, nephrotoxicity, myelosuppression, neurotoxicity, hepatotoxicity, which significantly impair the quality of life for oncology patients. Immunotherapy is posited to confer a substantial augmentation in survival outcomes and quality of life relative to

Content from this work may be used under the terms of CC BY-NC 4.0 licence (https://creativecommons.org/licenses/by-nc/4.0/).
Published by Warwick Evans Publishing.

conventional therapeutic approaches. However, there exists an exigent necessity for predictive biomarkers and strategies to attenuate the toxicity [6].

Current therapeutic strategies primarily target conditions resulting from genetic alterations. Additionally, the complex metabolic pathways found in cancer cells pose a significant challenge in the quest for targeted therapies. There is a clear need for the development of broadly applicable therapeutic approaches. Against this backdrop, the CRISPR-Cas system is gaining attention for its remarkable effectiveness and reliable performance [7]. Large-scale sequencing projects have unveiled a number of genetic variations, which are potential sites for gene therapy. CRISPR-Cas is a powerful gene-editing tool capable of precise manipulation of DNA sequences within cells. It facilitates the study of gene functions and holds promise for advancing therapies aimed at genetic disorders [8]. The core CRISPR-Cas mechanism involves two main components: the Cas protein, which cleaves the specific double-stranded DNA target, and the sgRNA, responsible for ensuring precise targeting by binding to the Cas protein. Following cleavage, the DNA repair process can proceed through two pathways: NHEJ or HDR. The HDR pathway, frequently employed in CRISPR-Cas systems, uses a matching segment from an intact DNA strand as a template to effectively mend the damaged DNA. This method holds considerable potential for clinical applications in the future. A growing number of clinical cases have illustrated the successful use of the CRISPR-Cas system in treating a range of medical conditions. These include single-gene disorders, infections, and malignant tumors [8].CRISPR-Cas system can precisely correct genetic mutations, thereby enabling the treatment of cancer at its fundamental source [9]. This review aims to examine present uses and future potential of the CRISPR-Cas system in cancer therapy, alongside addressing forthcoming challenges this technology might encounter.

2. CRISPR for therapy

The primary challenges currently encountered in cancer treatment are the drug resistance and high recurrence rates associated with conventional methods, which often lead to poor prognosis. With the advancement of genetic technology, directly modifying one or several parts of the cell genome, activating molecular targets for a long duration, and fundamentally correcting genetic mutations, has emerged as a treatment strategy with great potential [10].

Owing to the precision with which the CRISPR/Cas9 system can effectuate targeted gene knockouts, it has been extensively utilized to inactivate oncogenes, thereby facilitating investigations into the proliferation and developmental mechanisms of cancer cells, as well as the exploration of therapeutic strategies for cancer. Presently, Cas9 stands as the predominant CRISPR-associated endonuclease employed in cancer research, attributable to its capacity to target a substantial proportion, approximately half of the exonic regions within the human genome, as the most widely used Cas9 enzyme requires two G nucleotides as its PAM sequence [11].

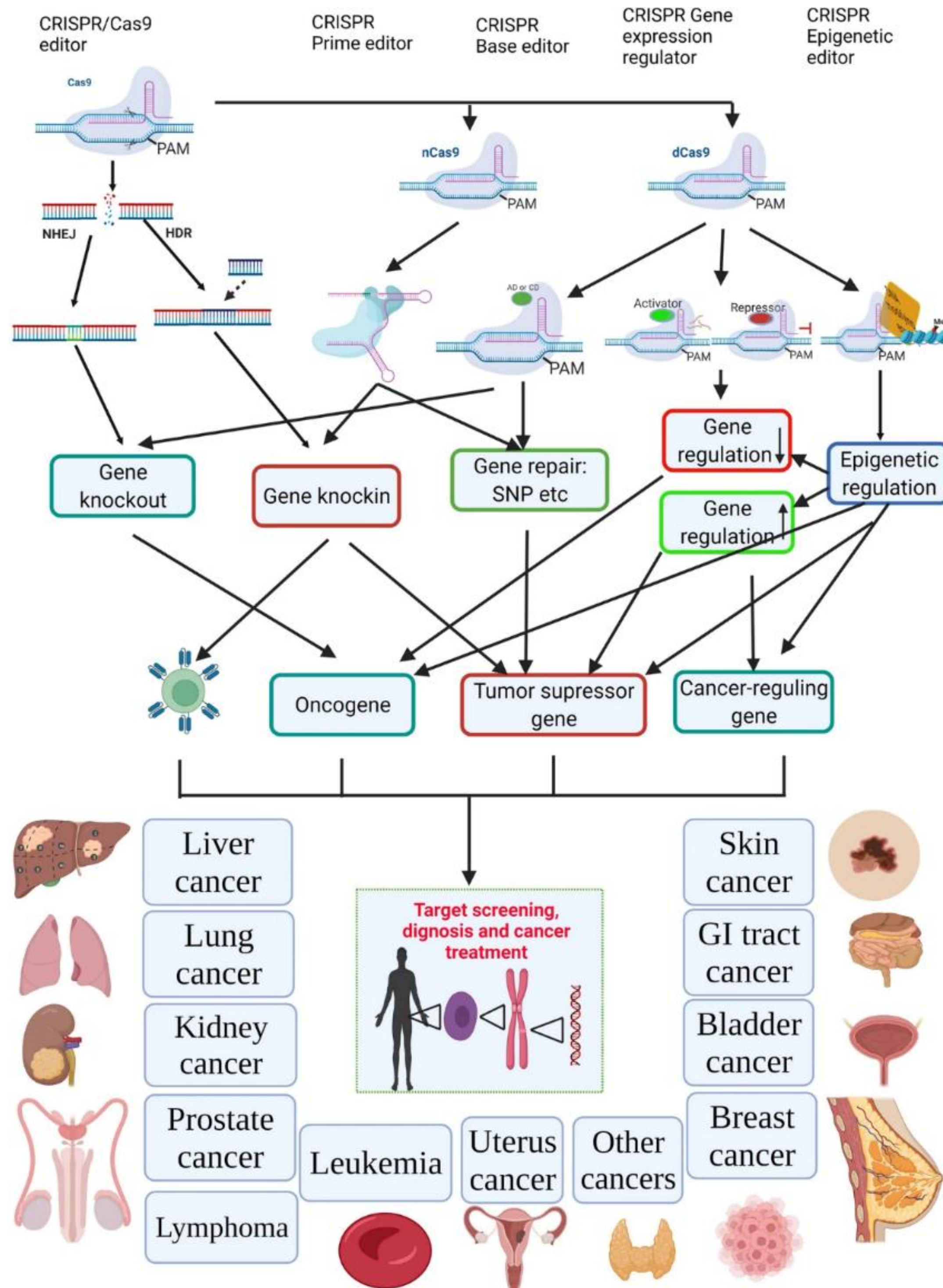

Figure 1. CRISPR/Cas9 based editors and their application in cancer therapy [22].

Five primary derivatives of the CRISPR/Cas9 system have been utilized in cancer biology and therapeutics, advancing the development of targeted cancer treatments (Fig. 1). The foundational CRISPR/Cas9 platform consists of the Cas9 nuclease and gRNA. Cas9 nuclease cleaves specific DNA sequences guided by gRNA, enabling gene knock-outs, knock-ins, or repairs. This system is extensively applied in gene function studies, disease modeling, and potential therapies. nCas9 is a Cas9 variant engineered to induce single-strand DNA breaks, enhancing editing specificity by reducing off-target effects. Paired gRNAs targeting opposite DNA strands at the same locus can efficiently introduce double-strand breaks. dCas9, a catalytically inactive Cas9 variant, retains its DNA-targeting capability but lacks DNA cleavage ability. dCas9 is commonly used for transcriptional regulation by fusing with transcription factors or effector domains to modulate gene expression, facilitating precise control over cellular functions [11].

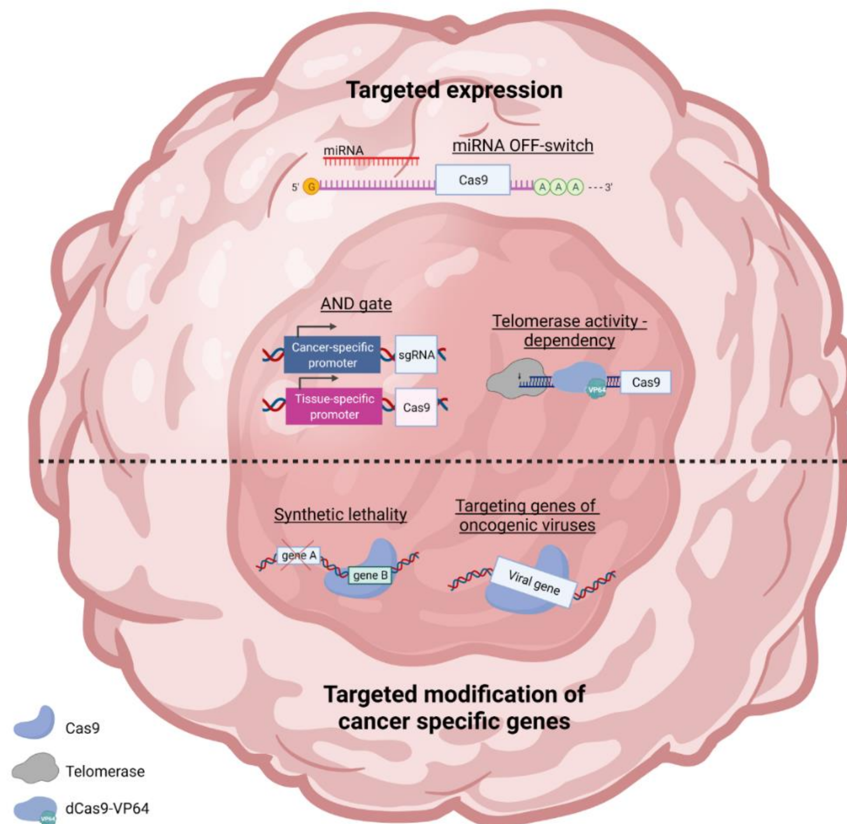

Figure 2. Targeted expression and modification using CRISPR/Cas9 gene-editing technology in cancer therapy [12].

Several approaches exist for finely tuning the CRISPR/Cas9 system to enhance the accuracy and effectiveness of cancer treatments. As figure 2 shows, miRNA strategy leverages specific miRNAs to regulate the expression of the Cas9 protein. The miRNA OFF-switch is capable of degrading Cas9 mRNA, thereby preventing the Cas9 protein from inadvertently attacking healthy cells. Conversely, when the switch is activated, it can induce the expression of the Cas9 protein, which is essential for gene editing in cancer cells. The regulation of Cas9 expression, either to inhibit or activate, is dependent on the differential presence of specific miRNAs in healthy versus cancerous cells. AND gate involves the activation of Cas9 protein expression based on two or more conditions. For instance, Cas9 might only be expressed if both a cancer-specific promoter and a tissue-specific promoter are activated simultaneously. This approach enhances the specificity of gene editing to minimize off-target effects on normal cells. Telomerase activity-dependency is a strategy contingent upon telomerase, an enzyme more active in rapidly dividing cells such as cancer cells. Expression of the Cas9 protein can be engineered to occur only in cells with high telomerase activity, thereby specifically targeting cancer cells. Synthetic lethality utilizes the CRISPR/Cas9 system to simultaneously disable a pair of genes, exploiting the redundancy of these genes in normal cells. In cancer cells, where one gene is already mutated, the inactivation of the second gene triggers cell death [12].

In a clinical trial conducted in 2014, the feasibility of in vivo gene editing was validated through the introduction of disease-resistant alleles via genome-directed editing [13].

Subsequent research has explored the therapeutic potential of CRISPR-Cas9 gene editing technology for transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD). Scientists targeted the erythroid-specific enhancer of BCL11A using CRISPR-Cas9 and conducted electroporation on CD34+ hematopoietic stem and progenitor cells obtained from healthy donors. Single-guide RNA molecules were employed for editing, resulting in the creation of CTX001 from these CD34+ cells.

Following the administration of CTX001, sustained engraftment of CRISPR-Cas9-edited HSPCs was observed. Over a year later, both patients displayed significant allele editing in their bone marrow and blood, along with elevated fetal hemoglobin levels in pancreatic β cells. This led to the cessation of transfusions in the TDT patient and the elimination of vaso-occlusive crises in the SCD patient. These initial findings highlight the potential of CRISPR-Cas9 gene editing approaches for further clinical exploration in the treatment of genetic disorders [14].

3. CRISPR for diagnosis

In recent years, in addition to the commonly used Cas9 proteins, a great deal of research has been done on Cas12 and Cas13.

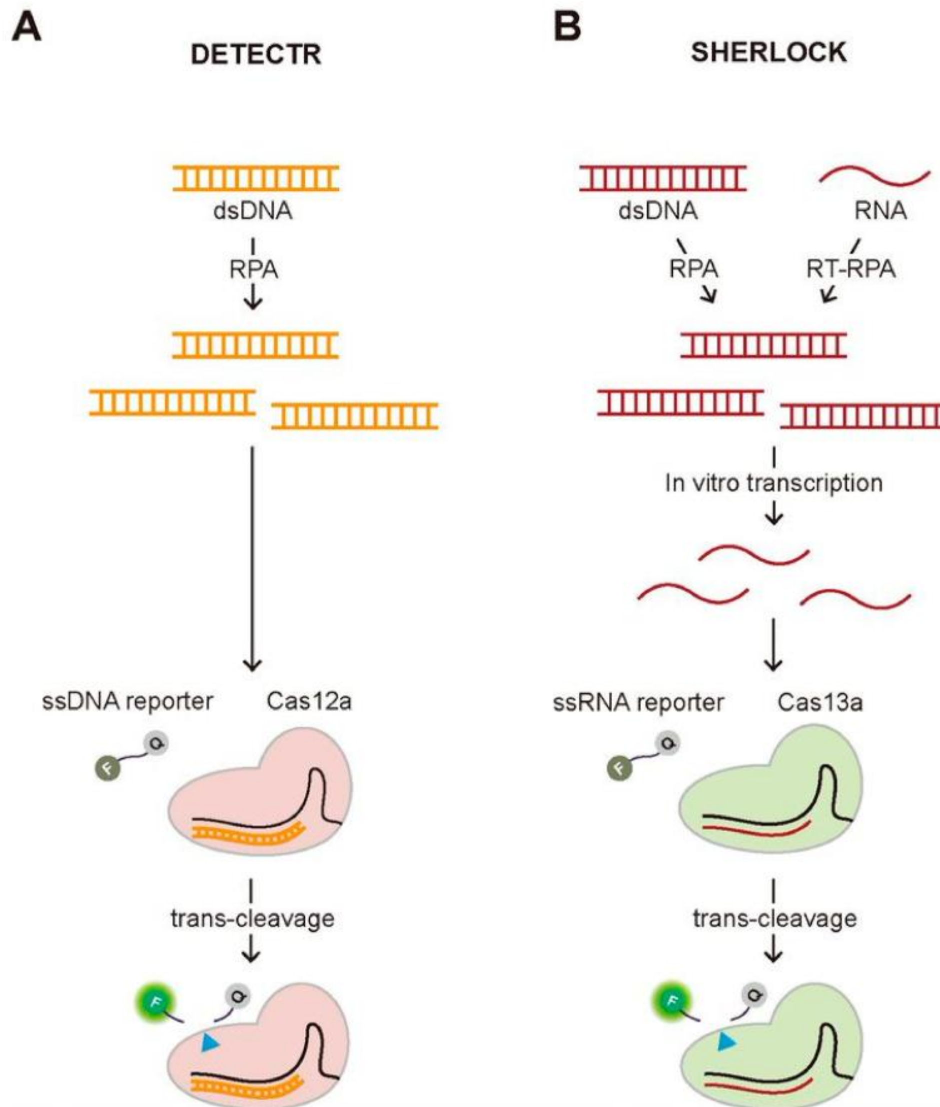

Figure 3. The mechanisms of action for two CRISPR-based diagnostic systems, DETECTR and SHERLOCK, which utilize CRISPR-Cas12a and CRISPR-Cas13a to detect nucleic acid sequences [6].

As Fig.3 shows, the DETECTR system is designed for detecting double-stranded DNA (dsDNA). Upon the sequence-specific cleavage of double-stranded DNA (dsDNA) by CRISPR-Cas12a proteins, a robust nonspecific trans-cleavage activity is induced, targeting single-stranded DNA (ssDNA) molecules. This phenomenon is characterized by the enzyme's ability to cleave ssDNA sequences in a manner that is not dependent on the specific recognition sequence, thereby broadening the scope of its nuclease activity [15,16]. The Cas12a endonuclease is directed to double-stranded DNA (dsDNA) targets using a complementary crRNA, which initiates collateral cleavage of adjacent short single-

stranded DNA (ssDNA) reporters linked to a fluorophore and quencher. Upon target recognition and reporter cleavage, the quencher is spatially separated from the fluorophore, resulting in detectable fluorescence [17]. The SHERLOCK system is designed for detecting both dsDNA and RNA. For RNA detection, it employs CRISPR-Cas13a guided by a gRNA to target and cleave specific single-stranded RNA (ssRNA) sequences. In both scenarios, Cas12a and Cas13a utilize their trans-cleavage activity to cleave separate ssDNA or ssRNA reporter molecules. This cleavage releases a fluorescent or colorimetric signal, indicating the presence of the targeted nucleic acid [18].

Recently, both Cas12 and Cas13 have demonstrated significant potential in the field of cancer diagnostics. These two proteins share the following advantages. The Cas12 and Cas13 systems can leverage their non-specific collateral cleavage activity for highly sensitive detection of target DNA or RNA sequences. For instance, Cas12a has achieved attomolar sensitivity in detecting specific targets, which is more sensitive than traditional digital PCR technologies. Also, diagnostic tools based on the Cas12 and Cas13 systems can provide results in a short timeframe. For example, the SHERLOCK system utilizing Cas13 can yield results within two hours, which is highly beneficial for clinical scenarios requiring prompt diagnosis. Unlike traditional methods that require nucleic acid amplification, some Cas12 and Cas13-based detection can directly analyze nucleic acids from samples without amplification, reducing errors and contamination that can occur during amplification. In addition to DNA detection, Cas13a can also detect mRNA, miRNA, and even exosomal protein markers, offering new possibilities for early cancer diagnosis and therapeutic monitoring [11,18,19].

4. Discussion

The conclusions drawn from various studies on the application and prospective use of CRISPR-Cas in cancer diagnosis and therapy collectively paint a picture of immense potential intertwined with significant challenges. CRISPR-Cas9, heralded as a simple, cost-effective, and precise genome editing tool, holds promise in revolutionizing cancer-related research and treatment. From enhancing CAR-T-cell therapy to facilitating the study of genetic and epigenetic alterations involved in tumorigenesis, CRISPR-Cas technology offers new avenues for understanding and combating cancer. However, the journey towards realizing the full potential of CRISPR-Cas in cancer diagnosis and therapy is fraught with obstacles. High specificity remains a central challenge, necessitating sophisticated strategies for precise targeting and delivery. Concerns surrounding off-target effects, long-term safety, and ethical implications underscore the need for further research and refinement. Nevertheless, ongoing efforts to improve the potency and specificity of CRISPR-Cas, coupled with advancements in delivery methods and ethical considerations, offer hope for overcoming these challenges. As CRISPR-Cas technology continues to evolve, its role in cancer research and therapy is poised to expand, potentially transforming the landscape of oncology. In conclusion, while hurdles remain, the collective insights gleaned from these conclusions underscore the transformative potential of CRISPR-Cas in cancer diagnosis and therapy. With continued research and innovation, CRISPR-Cas stands poised to become a cornerstone of precision medicine, offering new hope for patients battling cancer worldwide [3,7,11,12].

References

[1] M. Cao, H. Li, D. Sun, S. He, X. Yan, F. Yang, S. Zhang, C. Xia, L. Lei, J. Peng, W. Chen, Current cancer burden in China: epidemiology, etiology, and prevention, Cancer Biology & Medicine 19 (2022) 1121–1138.

[2] R.L. Siegel, A.N. Giaquinto, A. Jemal, Cancer statistics, 2024, CA Cancer J Clin 74 (2024) 12–49.

[3] G. Behrouzian Fard, M.H. Ahmadi, M. Gholamin, R. Amirfakhrian, E. Saberi Teimourian, M.A. Karimi, M. Hosseini Bafghi, CRISPR-Cas9 technology: As an efficient genome modification tool in the cancer diagnosis and treatment, Biotechnology and Bioengineering 121 (2024) 472–488.

[4] C.-Y. Zhao, R. Cheng, Z. Yang, Z.-M. Tian, Nanotechnology for Cancer Therapy Based on Chemotherapy, Molecules 23 (2018) 826.

[5] G. Wei, Y. Wang, G. Yang, Y. Wang, R. Ju, Recent progress in nanomedicine for enhanced cancer chemotherapy, Theranostics 11 (2021) 6370–6392.

[6] K. Esfahani, L. Roudaia, N. Buhlaiga, S.V. Del Rincon, N. Papneja, W.H. Miller, A Review of Cancer Immunotherapy: From the Past, to the Present, to the Future, Current Oncology 27 (2020) 87–97.

[7] A. Katti, B.J. Diaz, C.M. Caragine, N.E. Sanjana, L.E. Dow, CRISPR in cancer biology and therapy, Nat Rev Cancer 22 (2022) 259–279.

[8] Y. Xu, Z. Li, CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy, Comput Struct Biotechnol J 18 (2020) 2401–2415.

[9] H.-X. Wang, M. Li, C.M. Lee, S. Chakraborty, H.-W. Kim, G. Bao, K.W. Leong, CRISPR/Cas9-Based Genome Editing for Disease Modeling and Therapy: Challenges and Opportunities for Nonviral Delivery, Chem. Rev. 117 (2017) 9874–9906.

[10] L. Yi, J. Li, CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges, Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 1866 (2016) 197–207.

[11] X. Yang, B. Zhang, A review on CRISPR/Cas: a versatile tool for cancer screening, diagnosis, and clinic treatment, Funct Integr Genomics 23 (2023) 182.

[12] K. Balon, A. Sheriff, J. Jacków, Ł. Łaczmański, Targeting Cancer with CRISPR/Cas9-Based Therapy, Int J Mol Sci 23 (2022) 573.

[13] Tebas Pablo, Stein David, Tang Winson W., Frank Ian, Wang Shelley Q., Lee Gary, Spratt S. Kaye, Surosky Richard T., Giedlin Martin A., Nichol Geoff, Holmes Michael C., Gregory Philip D., Ando Dale G., Kalos Michael, Collman Ronald G., Binder-Scholl Gwendolyn, Plesa Gabriela, Hwang Wei-Ting, Levine Bruce L., June Carl H., Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV, New England Journal of Medicine 370 (2014) 901–910.

[14] Frangoul Haydar, Altshuler David, Cappellini M. Domenica, Chen Yi-Shan, Domm Jennifer, Eustace Brenda K., Foell Juergen, de la Fuente Josu, Grupp Stephan, Handgretinger Rupert, Ho Tony W., Kattamis Antonis, Kernytsky Andrew, Lekstrom-Himes Julie, Li Amanda M., Locatelli Franco, Mapara Markus Y., de Montalembert Mariane, Rondelli Damiano, Sharma Akshay, Sheth Sujit, Soni Sandeep, Steinberg Martin H., Wall Donna, Yen Angela, Corbacioglu Selim, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia, New England Journal of Medicine 384 (2021) 252–260.

[15] J.P.K. Bravo, T. Hallmark, B. Naegle, C.L. Beisel, R.N. Jackson, D.W. Taylor, RNA targeting unleashes indiscriminate nuclease activity of CRISPR–Cas12a2, Nature 613 (2023) 582–587.

[16] O. Piepenburg, C.H. Williams, D.L. Stemple, N.A. Armes, DNA Detection Using Recombination Proteins, PLoS Biol 4 (2006) e204.

[17] M.M. Kaminski, O.O. Abudayyeh, J.S. Gootenberg, F. Zhang, J.J. Collins, CRISPR-based diagnostics, Nat Biomed Eng 5 (2021) 643–656.

[18] S. Kim, S. Ji, H.R. Koh, CRISPR as a Diagnostic Tool, Biomolecules 11 (2021) 1162.

[19] M. Wang, M. Chen, X. Wu, X. Huang, B. Yu, CRISPR applications in cancer diagnosis and treatment, Cell Mol Biol Lett 28 (2023) 73.