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# CRISPR in Cancer Biology and Bovine Viral Diarrhea Therapy: Precision Genome Editing at the Frontier of Oncology and Viral Pathogenesis

**Jindong Gao[1], Mengdi Zhang[1], Linjia Gao[1], Lei Kuang[2], Tuniyazi Maimaiti[1], Ling Zhao[1], Qinghua Shang[1], Changmin Hu[2]**

[1]College of Animal Science and Technology, Tarim University, Alar, 843300, P.R. China
[2]College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, P.R. China

*Corresponding author: Jindong-Gao@taru.edu.cn

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## Abstract

CRISPR has become a revolutionary tool for cancer biology and therapy with unprecedented precision for genome editing. Besides cancer, CRISPR is also under research for possible use in the treatment of viral diseases, such as Bovine Viral Diarrhea Virus (BVDV), a serious livestock problem. Through enabling gene modifications to be made to individual genes, CRISPR is used in identifying oncogenic drivers, characterizing tumor suppressor networks, and designing innovative therapeutics. This review focuses on the various uses of CRISPR for cancer studies with special attention to methods like gene knockout, gene activation, and base editing that each has a great potential for correcting oncogenic mutations and restoring tumor suppressor activity. Moreover, CRISPR's capacity to regulate viral replication, as that of BVDV, signifies its dual role in oncology as well as in viral pathogenesis. Interventions based on CRISPR such as chimeric antigen receptor (CAR) T cell therapy and synthetic lethality are essentially revolutionizing cancer therapy by improving immune responses and capitalizing on the specificity that is characteristic of cancer cells. However, off-target effects, tumor heterogeneity, and ethical dilemmas remain onerous challenges for clinical application. CRISPR delivery systems, despite playing a central role in advancing cancer therapies, also have prospects in optimizing the effectiveness of treatment against viral pathogens like BVDV. Advances in delivery systems using nanoparticles and viral vectors are mitigating against these challenges and improving efficacy and specificity of CRISPR reagent in vivo. In addition to this, continued progress in new technologies such as prime editing and base editing is predicted to improve precision and efficacy of CRISPR-based therapy. Since CRISPR technology is continuously developing, CRISPR's potential to treat both cancer and viral infections, like BVDV, simultaneously will be instrumental in precision medicine. This review highlights the revolutionary potential of CRISPR to revolutionize cancer treatment paradigms and brings hope for more effective and individualized therapy. With potential developments to emergent and potentiated CRISPR tools and reagents, it is predicted that they will play a center stage role in precision oncology and promote better patient results.

**Keywords:** CRISPR, precision oncology, cancer therapy, synthetic lethality, genome editing



## 1. Introduction

Precision oncology is a revolutionary strategy for treating cancer with a deeper understanding of tumor genetic makeup. Traditional treatment options, e.g., chemotherapy and radiation, deliver suboptimal treatment responses that increase unwanted effects. Precision oncology uses specific biomarkers for individualizing treatment protocols that maximize treatment effects and reduce toxicity. The development of CRISPR technology has provided a new level by providing unprecedented precision in gene editing that brings innovative modes of therapy (Deng, 2023). Apart

from oncology, CRISPR technology has found extensive application in viral disease treatment and research. An example of this is its application in the fight against viral pathogens like the Bovine Viral Diarrhea Virus (BVDV), a major viral cattle disease. BVDV, by virtue of its implications for cattle health and agriculture, presents a special challenge with regard to its replication and pathogenesis. CRISPR-mediated genome editing also promises new possibilities in manipulating viral replication and editing host immune responses, thus presenting a new strategy for treating BVDV (R. Yao et. Al., 2021) . This kind of cross-field potential of CRISPR, from cancer biology to viral pathogenesis, highlights its flexibility and wide-range therapeutic uses. Ever since it was first used in 2012, CRISPR has gained advancement in the last decade, going from basic research to clinical trials. In cancer biology, CRISPR is used to understand mechanisms of tumorigenesis, gene functions, and creating innovative modes by correcting oncogenic mutations or inhibiting tumor suppressor genes (Tian et al., 2019). Many clinical trials are ongoing to test the safety and efficacy of CRISPR-based therapies and try to adopt them as a part of standard clinical practice (Knott & Doudna, 2018). This mini-review explains a comprehensive understanding of recent advances in the potential and applications of CRISPR in the treatment of cancer. Here, we discuss different mechanisms involving CRISPR for precision genomic editing, e.g., gene knockout, and gene activation. We also consider ethical challenges that put hurdles to the clinical use of CRISPR in order to make trials safe and effective.

## 2. CRISPR Systems and Mechanisms Relevant to Cancer and Viral Pathogenesis

CRISPR-Cas9 has become an incredible tool of genetic engineering, especially in cancer biology, due to its accuracy and versatility concerns. Although Cas9 is by far the most widely used protein, other Cas proteins, e.g., Cas12a, have also been utilized to achieve multiplex gene editing, tackling the complex genetic landscape of cancers (Sun et al., 2020). The CRISPR-Cas9 mechanism starts with the induction of a predetermined double-strand, that breaks at precise genomic sites, activating non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in gene knockouts or precise edits. CRISPR-Cas9 has also emerged as an all-round gadget in cancer biology because of its specificity and versatility. Outside oncology, CRISPR is also being researched as a possible candidate for treating viral infections, including Bovine Viral Diarrhea Virus (BVDV), which inflicts colossal economic damage on livestock. The editability of viral genomes by CRISPR also raises new promise for inhibiting viral replication, similar to that of controlling cancer. But CRISPR component delivery remains a challenge, particularly in tumors and viral infections (Sioson et al., 2021). Novel delivery methods, such as nanoparticles, are set to enhance specificity and efficiency but remain hampered by challenges in off-target effects as well as efficiency of delivery. Further advancements in these fields will continue to broaden CRISPR therapeutic application in cancer as well as viral diseases such as BVDV. However, it disrupts oncogenic pathways or restores tumor-suppressing functionalities that furhter suppress the growth and survival of malignant tumor cells (Finn et al., 2018). Moreover, CRISPR/dCas9 variants, which do not cleave DNA but rather control gene expression at the transcriptional level, offer a safer alternative by minimizing off-target effects (Liu et al., 2018). Despite its huge potential, delivering CRISPR components to the target cells poses enormous hurdles, especially in the case of solid tumors with dense structures. While viral vectors are plagued by issues of immune responses and difficulties in cellular penetration, new nanoparticle systems and biomimetic carriers show great potential in elevating delivery efficiency (Huang et al., 2018). In the case of hematologic malignancies, systemic delivery of CRISPR components using nanoparticles has exhibited promising results; however, specificity and efficiency remain leading concerns (Shi et al., 2018) (Table 1).

**Table 1.** Mechanistic Insights of CRISPR in Cancer Applications presents a streamlined breakdown of the molecular events and technical strategies underpinning CRISPR-based cancer interventions.

| Mechanism | Brief Description | Application in Cancer | References |
|---|---|---|---|
| **CRISPR/Cas9 Target Recognition** | sgRNA directs Cas9 to specific DNA to induce DSBs. | Targets and edits cancer genes. | (Anirudh et al., 2021; Yang et al., 2021) |
| **Induction of DSBs** | Cas9 introduces site-specific DNA breaks. | Disrupts or repairs genes affecting cancer. | (Tesařík et al., 2018; Xia et al., 2018) |
| **DNA Repair: NHEJ vs HDR** | DSBs repaired by NHEJ (indels) or HDR (precise). | Used to disrupt oncogenes or correct mutations. | (Dai et al., 2021; Abdussadyk & Beisenova, 2023) |
| **Gene Knockout & Activation** | sgRNA can silence or activate genes. | Affects tumor growth and suppressor function. | (Dai et al., 2021; Zhao et al., 2024) |

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|---|---|---|---|
| **Delivery Methods** | Delivered via viral vectors, liposomes, etc. | Enhances tumor-targeted therapy. | (Zhen et al., 2020; Wang et al., 2018) |
| **Nanoparticle Delivery** | Uses nanocarriers for stability and targeting. | Improves precision and reduces side effects. | (Zhang et al., 2023; Wang et al., 2018) |
| **Tumor Microenvironment Modulation** | Edits immune/stromal cells to alter tumors. | Boosts immune response in tumor area. | (Zhao et al., 2024; Banerjee et al., 2021) |
| **CRISPR in Immunotherapy** | Enhances CAR-T cells for targeting. | Effective in leukemias and lymphomas. | (Xia et al., 2018; Ottaviano et al., 2022) |
| **Combination Therapies** | Used with chemo/photo-therapy. | Targets multiple tumor mechanisms. | (Zhang et al., 2023; Zhang et al., 2022) |
| **Ethical & Safety Concerns** | Concerns over off-target effects and safety. | Demands regulation and monitoring. | (Tesařík et al., 2018; Ottaviano et al., 2022) |
| **Emerging CRISPR Variants** | New editors enable precise, DSB-free edits. | Lower risk, better efficacy in therapy. | (Deng, 2023; Abdussadyk & Beisenova, 2023; Yang et al., 2021) |

## 3. CRISPR for Functional Genomics in Cancer and Therapeutic Target Identification

CRISPR has become an indispensable tool in functional genomics, enabling genome-wide screens to identify oncogenic drivers and tumor suppressor networks. High-throughput CRISPR screens utilize pooled libraries of single guide RNAs (sgRNAs) to target genes across the genome, uncovering vulnerabilities in cancer cells that can be exploited for therapeutic interventions (Chen et al., 2015). CRISPR has proven to be a revolutionary technology in functional genomics and more specifically in cancer biology in that it allows for the high-throughput genome-wide screens to reveal the key oncogenic drivers and tumor suppressor networks. Its use covers from the discovery of these key pathways to the identification of potential therapeutic targets. With CRISPR screens, scientists can investigate gene interactions and determine vulnerabilities in cancer cells that can be targeted with therapy. For example, CRISPR screens have identified synthetic lethal interactions as a new potential therapeutic approach. This ability to detect and edit single genes is extremely promising in the development of personalized cancer treatments because it provides the ability to identify new targets that will enhance treatment specificity and effectiveness. CRISPR screens have also elucidated complex tumor suppressor networks, revealing essential genes that cooperate with known suppressors to maintain cancer cell viability. For example, screens using CRISPR-Cas9 in glioblastoma stem-like cells identified synthetic lethal relations between PKMYT1 and WEE1 and therefore presented new therapeutic targets (Toledo et al., 2015). In addition to this, CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) allow for specific gene modulation without compromising the structure of the gene. Both methods are particularly useful for exploring genes with context-dependent functionalities and provide increased insight into cancer progression and resistance to therapy regulation networks (Rousset et al., 2018).

## 4. CRISPR-Based Therapeutic Strategies in Cancer and Viral Diseases

CRISPR-mediated therapies are transforming cancer treatment through gene correction, knockout, and immune cell reprogramming. Using CRISPR to target specific genes may reactivate tumor suppressor genes, therefore, it might be used as a possible treatment for malignancies with single gene mutations (Lee et al., 2020). Knockout is used to inactivate oncogenes, e.g., MYC, KRAS, or PIK3CA, that cause tumor regression (Nüssing et al., 2020). The use of CRISPR-engineered T cells, especially CAR-T cells, are highly effective in treating refractory cancers. Reprogramming such T cells to overexpress CARs may trigger tumor antigens, strengthening immune responses to recognize and target malignant tumors (Stadtmauer et al., 2020). Hence, suppressing endogenous T cell receptors (TCRs) by CRISPR improves the safety profile (Legut et al., 2018). Synthetic lethality is also gaining attention, e.g., PARP inhibitors selectively target cancer cells with DNA repair, including cells with BRCA mutations. Therefore, CRISPR provides innovative treatment for all kind of cancers (Ashworth & Lord, 2018). CRISPR-based therapies have already revolutionized cancer therapy and have opened new avenues for gene correction, gene knockout, and immune cell reprogramming. Apart from its application in cancer therapy, the therapeutic capability of CRISPR is in viral diseases, one of which is Bovine Viral Diarrhea Virus (BVDV), a significant issue in veterinary medicine. Through targeting and manipulation of certain genes within the viral replication cycle, CRISCR might provide a new method for the control of viral infections, similar to its application in

cancer therapies (Yang et al., 2025). For example, CRISPR-based systems might suppress the replication of BVDV through editing viral genomes or host cell factors essential to the virus life cycle. CRISPR is being applied in cancer therapy to reactivate cancer suppressing genes or eliminate oncogenes such as MYC and KRAS to cause regression of the tumor. Moreover, CRISPR-edited T cells like CAR-T cells are showing amazingly promising indications of curing recurrent cancers. Immunological targeting of cancer antigens occurs by strengthened immunity responses by repopulating the T cells by the overexpression of CARs (Chandran et al., 2019). This double use of CRISPR in cancer treatment and in the treatment of viral diseases is a demonstration of what it can do and the ability to provide leading-edge therapeutic management of many diseases.

## 5. Challenges and Limitations of CRISPR in Cancer and Viral Therapies

Despite significant potential, CRISPR has unwanted off-target effects, e.g., tumor heterogeneity, and ethical concerns. Off-target mutations can cause unexpected genetic consequences, thus requiring high fidelity Cas9 variants and advanced screening to offsets (Kleinstiver et al., 2016). Tumor heterogeneity has challenges to such therapies due to diverse cellular populations that can react in different ways. Therefore, an in-depth understanding of tumor microenvironments and clonal evolution is important to overcome and achieve sustainable possibilities (Mondal et al., 2023). Despite its great potential, CRISPR technology is beset by several challenges, particularly in cancer and virus therapies. One of the primary concerns is off-target effects, wherein genetic changes outside the target sequence can be harmful. In cancer therapies, tumor heterogeneity also becomes a concern, wherein greater than one population of cells within the tumor can respond in unanticipated manners to treatment (Marusyk et al., 2009). Such unpredictability renders it difficult to produce consistent and predictable outcomes. Besides, the heterogeneity of viral genomes, like Bovine Viral Diarrhea Virus (BVDV), is problematic in terms of targeting viral sequences specifically without impairing host cells. Delivery modes are also a significant challenge because targeted and efficient delivery to target cells is required for both cancer and viral therapy. In addition, there are also ethical concerns, like the problem of germline editing, which raises questions about what the long-term consequences of CRISPR-based treatment will be. These issues need to be handled with sophisticated technology, greater knowledge of tumor biology and viral behavior, and careful weighing of the social and ethical consequences of gene editing. Ethical concerns regarding germline editing remain a significant barrier to CRISPR-based therapies. However, the regulatory guidelines and the public fears over genetic modifications underscore the transparent governance and proactive engagement among stakeholders (Brokowski & Adli, 2019).

## 6. Future Directions and Emerging Innovations

Future developments in CRISPR involve gene editing techniques, incorporating artificial intelligence (AI), and advancing applications in clinical trials. Prime editing and base editing techniques have proved to improve precision, allowing editing at the single nucleotide level without causing mutations (Stadtmauer et al., 2020). Also, the use of AI may help optimize RNA design, improve efficiency, and decipher intricate interactions in tumor heterogeneity (Schmidt et al., 2022). In addition, clinical-based treatments are being improved by new delivery systems that involve lipid nanoparticles and viral vectors, improving the specificity and stability of CRISPR reagents (Zhang et al., 2021). In the future, it is possible to transform both cancer and viral disease treatment by integrating CRISPR with emerging technologies. With an emphasis on CRISPR-based antiviral treatments, for example, there is promise in developing antiviral approaches such as targeting Bovine Viral Diarrhea Virus (BVDV). Utilizing CRISPR's capability to directly edit viral genomes, it has the potential to provide precision in managing BVDV replication and thereby mitigating the effects of this disease in animals. In addition to viral applications, continuing development of the editing function of CRISPR, as well as corresponding innovations in delivery vectors such as nanoparticles, will enhance its therapeutic applications, allowing greater precision and potency in treating inherited disease. Eventually, as technology with CRISPR advances even further, application with other novel technologies such as AI to rationally design therapeutics may likewise hasten more personalized cancer medicine with the capacity for more focal intervention and lesser toxicity. Hence, such developments have great potential to introduce a safe and efficient CRISPR-based therapy for genetic diseases and cancers (Figure 1).

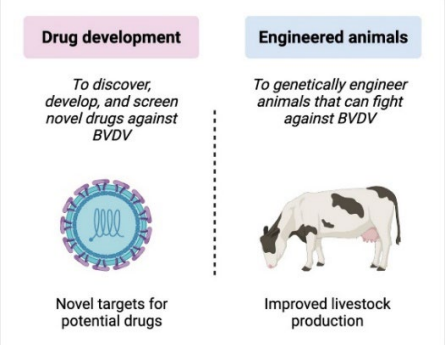


**Figure 1:** Strategies for overcoming BVDV.

## 7. Conclusion

CRISPR technology represents a revolutionary development in genome editing with specificity and tailored therapies. CRISPR presents new possibilities for cancer targeted therapy design through the ability to discover new oncogenic drivers and mechanisms of resistance. Apart from oncology, CRISPR holds the potential to revolutionize management of viral diseases, and more so that of infections such as Bovine Viral Diarrhea Virus (BVDV) that compromises animal health. With the advancement of CRISPR, refinements in delivery and precision editing will make it more useful for cancer therapy and viral control. Its continued development positions CRISPR as a leading edge of modern medicine as it is a revolutionary tool that can redefine the treatment of various diseases, from cancer to viruses.

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## Declaration of competing interest

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