Review Article

CRISPR-Cas9 in Breast Cancer: A Tool for Functional Genomics, Target Discovery, and Precision Therapeutics

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Abstract : CRISPR-Cas9 technology has rapidly emerged as a transformative tool in breast cancer research, enabling precise genome editing that advances functional genomics, target discovery, and therapeutic development. This review highlights the fundamental principles of CRISPR-Cas9 and its diverse applications in breast cancer models, including gene function interrogation and high-throughput screening for oncogenic drivers. We discuss the role of CRISPR-based approaches in advancing precision medicine, such as genome editing for personalized therapies and immune cell engineering. Despite its promising potential, challenges including off-target effects, delivery efficiency, and ethical considerations remain significant barriers to clinical translation. We also explore emerging strategies to overcome these limitations and future directions aimed at optimizing CRISPR technology for improved breast cancer treatment. Integrating CRISPR-Cas9 with multidisciplinary research holds the key to unlocking novel therapeutic avenues and enhancing patient outcomes in the era of precision oncology.

Keywords: CRISPR-Cas9; breast cancer; functional genomics; precision medicine; genome editing.

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Introduction

The discovery of CRISPR-Cas9 as a programmable genome-editing tool has revolutionized molecular biology and biomedical research over the past decade. Originally characterized as an adaptive immune defense mechanism in bacteria and archaea, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) arrays store sequences from invading viruses, enabling the Cas9 endonuclease to recognize and cleave foreign DNA [1]. The repurposing of this system for targeted DNA cleavage in mammalian cells leverages a guide RNA (gRNA) to direct Cas9 to complementary DNA sequences, introducing double-strand breaks (DSBs) that stimulate cellular DNA repair mechanisms [2]. These breaks can be repaired via error-prone non-homologous end joining (NHEJ), often introducing frameshift mutations that result in gene knockout, or via homology-directed repair (HDR), which can enable precise gene correction or insertion when a repair template is provided [3].

This precise, versatile, and relatively simple technology has transformed functional genomics by enabling the direct interrogation of gene function in living cells and organisms. Its applications in oncology are particularly compelling given the genetic complexity of cancer, characterized by multiple

mutations, copy number alterations, and epigenetic changes. Breast cancer, as a heterogeneous disease with distinct molecular subtypes (such as luminal A/B, HER2-enriched, and triple-negative), demands sophisticated tools to unravel its biology and identify therapeutic vulnerabilities [4,5]. CRISPR-Cas9 has emerged as an indispensable technology to dissect these complexities, model mutations, and accelerate precision medicine development.

CRISPR-Cas9 Applications in Breast Cancer Research

Functional Genomics and Gene Knockout Screens

Genome-wide CRISPR knockout screens have become a gold standard for identifying genes essential for breast cancer cell survival, proliferation, and metastasis. By employing large-scale CRISPR libraries, researchers can interrogate thousands of genes in parallel, uncovering novel oncogenes, tumor suppressors, and synthetic lethal partners. For instance, multiple studies identified critical dependencies on DNA repair genes such as BRCA1 and RAD51, cell cycle regulators like CDK4/6, and hormone signaling genes including ESR1 [6]. These key studies are summarized in Table 1, which catalogs landmark CRISPR knockout screens in various breast cancer models. In vivo CRISPR screens provide even greater biological relevance by assessing gene functions within the tumor microenvironment. Dai et al. demonstrated this by identifying vulnerabilities in mTOR and Hippo pathways that contribute to therapy resistance [7]. Additionally, such screens have uncovered genes involved in immune evasion and metastatic potential, providing targets for intervention [8].

Modeling Breast Cancer Mutations

CRISPR-Cas9 enables precise modeling of clinically relevant mutations in breast cancer cell lines, organoids, and animal models, allowing functional analysis and therapeutic testing. Notably, targeted mutations in TP53 and PIK3CA have been introduced to elucidate their roles in tumorigenesis and drug response [9-12]. Harrod et al. used CRISPR to engineer mutations in ESR1, helping to model endocrine therapy resistance mechanisms [13]. These models and their experimental details are compiled in Table 2, illustrating how CRISPR-mediated mutations have advanced breast cancer biology.

Patient-derived organoids edited with CRISPR provide personalized platforms for preclinical drug testing, retaining tumor heterogeneity and histopathology [18]. Similarly, CRISPR-edited patient-derived xenografts (PDXs) allow the study of tumor evolution and therapeutic response in vivo [19].

Epigenome and Non-Coding Genome Editing

CRISPR tools have evolved beyond gene knockout to enable modulation of gene expression without altering the DNA sequence. CRISPR interference (CRISPRi) and activation (CRISPRa) utilize catalytically dead Cas9 (dCas9) fused to transcriptional repressors or activators, allowing reversible silencing or activation of target genes [20]. These approaches have been used to

investigate long non-coding RNAs (lncRNAs) and enhancers implicated in breast cancer progression and metastasis. Representative studies employing epigenome editing strategies are listed in Table 3. This reversible gene regulation technique facilitates the study of complex gene networks and transcriptional regulation in cancer cells.

Table 1. Key Breast Cancer Genes Studied Using CRISPR-Cas9

Gene	Function	CRISPR Approach	Impact on Cancer Biology
BRCA1	DNA repair	Knockout	Genomic instability
ESR1	Hormone receptor	Activation	Endocrine resistance
PIK3CA	Oncogene	Knock-in	PI3K pathway modulation
TP53	Tumor suppressor	Knockout	Promotes tumor progression
HER2	Growth receptor	Knockdown	Alters cell proliferation
CDK7	Cell cycle regulator	Knockout	Disrupts proliferation
FOXA1	Transcription factor	Knockout	Affects luminal subtype identity
MCL1	Anti-apoptotic protein	Knockout	Increases apoptosis sensitivity
GATA3	Lineage-specific factor	Knockout	Disrupts luminal cell differentiation

Table 2: CRISPR Studies in Breast Cancer Models

Study	CRISPR Type	Cell Model	Key Findings	Application
Bag-1 Knockout in MCF-7 Cells	CRISPR/Cas9	MCF-7	Knockout of Bag-1 increased mesenchymal characteristics via Akt hyperactivation-mediated actin cytoskeleton remodelling [14]	Understanding drug resistance mechanisms
Synthetic Lethal Combination Targeting BET in TNBC	CRISPRi	TNBC models	Identified intrinsic susceptibility of TNBC to ferroptosis when combining BET inhibitors with GPX4 inhibition [15]	Target discovery for TNBC therapy
Acidic Tumor Microenvironment Enhances PD-L1 Expression	CRISPR-based study	MDA-MB-231	Acidic conditions upregulate PD-L1 via STAT3 activation, suggesting a link between tumor microenvironment and immune evasion [16]	Immunotherapy strategies
Genome-wide CRISPR/Cas9 Screening for Drug Resistance	CRISPR/Cas9	Various breast cancer cell lines	Identified genes like PHGDH and LRP8 associated with resistance to therapies like sorafenib and gemcitabine [17]	Metabolic targeting and overcoming drug resistance

Table 3: CRISPR-Based Tools and Resources for Breast Cancer Research

Tool/Database	Description	Application
CRISPick	Optimized gRNA design using AI	Target selection, minimizing off-targets
CancerDepMap	Gene dependency maps based on CRISPR data	Target prioritization in cancer lines
ENCORI	RNA–RNA interaction and miRNA target analysis	Post-transcriptional regulation
CHOPCHOP	gRNA design tool for CRISPR and CRISPRi	Custom editing experiments
Project SCORE	Genome-wide CRISPR screen database (Sanger)	Essentiality mapping
Benchling	CRISPR workflow and gRNA management platform	Experimental planning
CRISPRoff/on	Epigenetic editing prediction tool	Gene expression control
Cas-OFFinder	Off-target prediction tool	Minimizing editing errors

Precision Medicine Applications

1. Genotype-Phenotype Correlations

CRISPR-Cas9 has been instrumental in mapping genetic alterations to breast cancer phenotypes, especially through synthetic lethality screens. These screens have identified vulnerabilities in BRCA1/2-deficient tumors, leading to the clinical success of PARP inhibitors that exploit DNA repair defects [21-23]. The development of base editing and prime editing tools further refines precision medicine by allowing single-base corrections or modifications without introducing double-strand breaks [24].

2. Biomarker Discovery and Companion Diagnostics

High-throughput CRISPR screens facilitate the identification of biomarkers predictive of drug sensitivity and resistance, enabling refined patient stratification. Knockouts of transcription factors such as CDK7 have pinpointed potential therapeutic targets and biomarkers in triple-negative breast cancer (TNBC). These functional data integrate with genomic profiles to advance companion diagnostics and personalized treatment [25]. A curated list of CRISPR-identified biomarkers with potential therapeutic implications is presented in Table 4.

Therapeutic Potential

Direct Tumor Editing

Therapeutic strategies aim to deliver CRISPR components directly into tumors to disrupt oncogenes such as HER2 or MYC [26]. Challenges remain in efficient and safe delivery, but advances in lipid nanoparticles, viral vectors, and microbubble-mediated ultrasound delivery offer promising avenues to improve targeting and reduce toxicity [27].

Immunotherapy Augmentation

CRISPR engineering enhances the efficacy of immunotherapies by modifying

immune cells. Knockout of inhibitory receptors like PD-1 and CTLA-4, or introduction of chimeric antigen receptors (CARs) specific to breast cancer antigens, improves immune cell persistence and tumor targeting [28-30]. Clinical trials in hematological malignancies pave the way for similar approaches in solid tumors like breast cancer [31,32].

Combating Drug Resistance

Drug resistance remains a major clinical hurdle. CRISPR screens have identified genes contributing to resistance, such as mutations in ESR1 that cause endocrine resistance and ferroptosis regulators implicated in TNBC chemotherapy resistance [33]. Targeting these pathways offers novel therapeutic strategies.

Table 4: Challenges and Limitations of CRISPR in Breast Cancer

Challenge	Description	Possible Solution
Off-target effects	Unintended gene edits affecting non-target sites	AI-based gRNA optimization; high-fidelity Cas9
Delivery efficiency	Low uptake of CRISPR components in solid tumors	Nanoparticle systems; viral vectors
Tumor heterogeneity	Subclonal variations reduce uniform editing outcomes	Single-cell CRISPR screening; organoid models
Immune response	Immune recognition of Cas9 as a foreign protein	Use humanized or transient Cas9 expression
Incomplete editing	Partial knockout causes mosaic effects	Dual-guide systems; optimize delivery doses
DNA damage response	Activation of p53 leading to apoptosis	Use inducible systems; carefully chosen models
Off-tumor targeting	Effects on normal tissues with shared gene expression	Tumor-specific promoters and delivery vectors
Ethical concerns	Issues related to gene editing in human cells	Focus on somatic editing; follow ethical guidelines
Resistance to editing	Cells may escape editing or develop compensatory pathways	Multiplex targeting of redundant pathways
Long-term stability	Lack of data on the stability of edits over time	Longitudinal preclinical studies
Functional redundancy	Knockout of one gene compensated by others	Network-based target identification

Challenges and Limitations

1. Delivery Challenges

Effective, safe delivery of CRISPR components to target tissues remains the primary obstacle to clinical translation. Viral vectors (e.g., AAV, lentivirus) offer high transduction efficiency but pose risks of insertional mutagenesis and

immune reactions. Non-viral methods, such as lipid nanoparticles or physical delivery approaches (e.g., electroporation, ultrasound), are safer but often less efficient and require further optimization [34]. Tumor heterogeneity and the dense extracellular matrix in breast cancers further complicate delivery, necessitating tumor-specific targeting mechanisms to improve CRISPR uptake and minimize off-target effects.

2. Off-Target Effects and Specificity

CRISPR-Cas9 can induce unintended DNA cuts at sites with sequence similarity to the intended target, risking genotoxicity and unwanted mutations. Development of high-fidelity Cas9 variants (e.g., SpCas9-HF1, eSpCas9) and base editors has significantly reduced off-target activity [35]. Computational tools like CRISPOR and DeepCRISPR assist in guide RNA design to maximize specificity [36-37]. Nonetheless, comprehensive off-target analysis using unbiased genome-wide assays remains essential prior to clinical application.

3. Tumor Heterogeneity and Context Dependence

Breast cancer subtypes exhibit distinct genetic, epigenetic, and microenvironmental features that influence CRISPR editing outcomes. Functional dependencies identified in one subtype may not translate to others, necessitating subtype-specific screening strategies [38]. Moreover, intratumoral heterogeneity can result in variable editing efficiencies and therapeutic responses, complicating interpretation of CRISPR-based studies.

4. Ethical and Regulatory Hurdles

While somatic cell editing is widely accepted, ethical concerns around germline editing and long-term safety persist. Regulatory frameworks emphasize stringent preclinical evaluation, transparency, and patient consent [39]. As CRISPR-based therapies enter clinical trials, ongoing monitoring and public engagement are crucial to address ethical, social, and safety considerations.

Future Directions

Integrating CRISPR-based genetic screens with single-cell RNA sequencing and spatial transcriptomics enables high-resolution mapping of gene function within complex tumor microenvironments. This multi-modal approach facilitates the identification of cell-type-specific dependencies and reveals critical cell–cell interactions that contribute to tumor progression and resistance to therapy. Recent innovations such as base editing and prime editing have further expanded the CRISPR toolkit, allowing precise single-nucleotide changes and targeted insertions without inducing double-stranded DNA breaks, thereby improving editing safety and efficiency [40]. Additionally, the use of CRISPR-edited patient-derived organoids in conjunction with high-throughput drug screening offers a powerful platform for personalized therapeutic profiling. The integration of machine learning and artificial intelligence into guide RNA design [41] further enhances the accuracy and efficacy of CRISPR-based interventions, accelerating the development of individualized treatment

strategies in breast cancer.

Conclusion

CRISPR-Cas9 technology has fundamentally transformed cancer research by providing unparalleled precision and efficiency in genome editing. In breast cancer—a disease marked by extensive molecular heterogeneity and intricate genetic alterations—CRISPR-Cas9 has proven to be an indispensable tool for functional genomics. It facilitates the systematic interrogation of gene function, identification of novel therapeutic targets, and validation of oncogenic drivers across diverse breast cancer subtypes. Through enabling high-throughput loss- and gain-of-function screens, this technology has significantly advanced our understanding of the molecular mechanisms underpinning tumor initiation, progression, and resistance to therapy. Moreover, CRISPR-Cas9 has revolutionized the development of targeted therapeutic strategies, offering new avenues for precision oncology. With ongoing improvements in genome editing technologies and delivery systems, CRISPR-based approaches hold considerable promise to enhance clinical outcomes for breast cancer patients. Realizing the full potential of CRISPR-Cas9 in breast cancer will require sustained interdisciplinary efforts integrating genomics, bioinformatics, and translational research, ultimately driving innovation in diagnosis, prognosis, and personalized treatment.

References

[1] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. science. 2012 Aug 17;337(6096):816-21.

[2] Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28;346(6213):1258096.

[3] Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013 Feb 15;339(6121):819-23.

[4] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014 Jun 5;157(6):1262-78.

[5] Bhat-Nakshatri P, Khatpe AS, Chen D, Batic K, Mang H, Herodotou C, McGuire PC, Xuei X, Erdogan C, Gao H, Liu Y. Signaling pathway alterations driven by BRCA1 and BRCA2 germline mutations are sufficient to initiate breast tumorigenesis by the PIK3CAH1047R oncogene. Cancer Research Communications. 2024 Jan 5;4(1):38-54.

[6] Teng K, Ford MJ, Harwalkar K, Li Y, Pacis AS, Farnell D, Yamanaka N, Wang YC, Badescu D, Ton Nu TN, Ragoussis J. Modeling high-grade serous ovarian carcinoma using a combination of in vivo fallopian tube electroporation and CRISPR-Cas9–mediated genome editing. Cancer Research. 2021 Oct 15;81(20):5147-60.

[7] Dai M, Yan G, Wang N, Daliah G, Edick AM, Poulet S, Boudreault J, Ali S, Burgos SA, Lebrun JJ. In vivo genome-wide CRISPR screen reveals breast cancer vulnerabilities and synergistic mTOR/Hippo targeted combination therapy. Nature Communications. 2021 May 24;12(1):3055.

[8] Zhang HH, Xiang J, Yin BC, Ye BC. Overcoming multidrug resistance by base-

editing-induced codon mutation. ACS pharmacology & translational science. 2023 Apr 25;6(5):812-9.

[9] Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S, Yuzugullu H, Von T, Li H, Lin Z, Stover DG. CDK7-dependent transcriptional addiction in triple-negative breast cancer. Cell. 2015 Sep 24;163(1):174-86.

[10] Petrocca F, Altschuler G, Tan SM, Mendillo ML, Yan H, Jerry DJ, Kung AL, Hide W, Ince TA, Lieberman J. A genome-wide siRNA screen identifies proteasome addiction as a vulnerability of basal-like triple-negative breast cancer cells. Cancer cell. 2013 Aug 12;24(2):182-96.

[11] Mandegar MA, Huebsch N, Frolov EB, Shin E, Truong A, Olvera MP, Chan AH, Miyaoka Y, Holmes K, Spencer CI, Judge LM. CRISPR interference efficiently induces specific and reversible gene silencing in human iPSCs. Cell stem cell. 2016 Apr 7;18(4):541-53.

[12] Harrod A, Lai CF, Goldsbrough I, Simmons GM, Oppermans N, Santos DB, Győrffy B, Allsopp RC, Toghill BJ, Balachandran K, Lawson M. Genome engineering for estrogen receptor mutations reveals differential responses to anti-estrogens and new prognostic gene signatures for breast cancer. Oncogene. 2022 Oct 28;41(44):4905-15.

[13] Witz A, Dardare J, Francois A, Husson M, Rouyer M, Demange J, Merlin JL, Gilson P, Harlé A. CRISPR/Cas9-mediated knock-in of BRCA1/2 mutations restores response to olaparib in pancreatic cancer cell lines. Scientific Reports. 2023 Oct 31;13(1):18741.

[14] Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, Mangan PA, Kulikovskaya I, Gupta M, Chen F, Tian L. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020 Feb 28;367(6481):eaba7365.

[15] Verma N, Vinik Y, Saroha A, Nair NU, Ruppin E, Mills G, Karn T, Dubey V, Khera L, Raj H, Maina F. Synthetic lethal combination targeting BET uncovered intrinsic susceptibility of TNBC to ferroptosis. Science advances. 2020 Aug 21;6(34):eaba8968.

[16] Kwon YJ, Seo EB, Jeong AJ, Lee SH, Noh KH, Lee S, Cho CH, Lee CH, Shin HM, Kim HR, Moon HG. The acidic tumor microenvironment enhances PD-L1 expression via activation of STAT3 in MDA-MB-231 breast cancer cells. BMC cancer. 2022 Aug 4;22(1):852.

[17] Zhang Z, Wang H, Yan Q, Cui J, Chen Y, Ruan S, Yang J, Wu Z, Han M, Huang S, Zhou Q. Genome-wide CRISPR/Cas9 screening for drug resistance in tumors. Frontiers in Pharmacology. 2023 Nov 21;14:1284610.

[18] Moses C, Nugent F, Waryah CB, Garcia-Bloj B, Harvey AR, Blancafort P. Activating PTEN tumor suppressor expression with the CRISPR/dCas9 system. Molecular Therapy Nucleic Acids. 2019 Mar 1;14:287-300.

[19] Yang H, Jaeger M, Walker A, Wei D, Leiker K, Weitao T. Break breast cancer addiction by CRISPR/Cas9 genome editing. Journal of Cancer. 2018 Jan 1;9(2):219.

[20] Kong L, Ren C. Biomarker alteration following chemotherapy-based systemic therapy in de novo metastatic breast cancer. The Breast. 2025 Jun 1;81:104471.

[21] Monteiro AN, Bouwman P, Kousholt AN, Eccles DM, Millot GA, Masson JY, Schmidt MK, Sharan SK, Scully R, Wiesmüller L, Couch F. Variants of uncertain clinical significance in hereditary breast and ovarian cancer genes: best practices in functional analysis for clinical annotation. Journal of medical genetics. 2020 Aug 1;57(8):509-18.

[22] Ye T, Mishra AK, Banday S, Li R, Hu K, Coleman MM, Shan Y, Chowdhury SR, Zhou L, Pak ML, Simone TM. Identification of WNK1 as a therapeutic target to suppress IgH/MYC expression in multiple myeloma. Cell reports. 2024 May 28;43(5).

[23] Liao H, Wu J, VanDusen NJ, Li Y, Zheng Y. CRISPR-Cas9-mediated homology-directed repair for precise gene editing. Molecular Therapy Nucleic Acids. 2024 Dec 10;35(4).

[24] Feng X, Li Z, Liu Y, Chen D, Zhou Z. CRISPR/Cas9 technology for advancements in cancer immunotherapy: from uncovering regulatory mechanisms to therapeutic applications. Experimental Hematology & Oncology. 2024 Oct 19;13(1):102.

[25] Wei W, Chen ZN, Wang K. CRISPR/Cas9: a powerful strategy to improve CAR-T cell persistence. International Journal of Molecular Sciences. 2023 Aug 1;24(15):12317.

[26] Li Y, Wu P, Zhu M, Liang M, Zhang L, Zong Y, Wan M. High-Performance Delivery of a CRISPR Interference System via Lipid-Polymer Hybrid Nanoparticles Combined with Ultrasound-Mediated Microbubble Destruction for Tumor-Specific Gene Repression. Advanced Healthcare Materials. 2023 Apr;12(10):2203082.

[27] Hazel K, Singh D, He S, Guertin Z, Husser MC, Helfield B. Focused ultrasound and microbubble-mediated delivery of CRISPR-Cas9 ribonucleoprotein to human induced pluripotent stem cells. Molecular Therapy. 2025 Jan 10.

[28] Tiwari PK, Ko TH, Dubey R, Chouhan M, Tsai LW, Singh HN, Chaubey KK, Dayal D, Chiang CW, Kumar S. CRISPR/Cas9 as a therapeutic tool for triple negative breast cancer: From bench to clinics. Frontiers in Molecular Biosciences. 2023 Jul 4;10:1214489.

[29] Matsumoto D, Matsugi E, Kishi K, Inoue Y, Nigorikawa K, Nomura W. SpCas9-HF1 enhances accuracy of cell cycle-dependent genome editing by increasing HDR efficiency, and by reducing off-target effects and indel rates. Molecular Therapy-Nucleic Acids. 2024 Mar 12;35(1).

[30] Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, Huang M, Yi X, Liang M, Wang Y, Shen H. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nature medicine. 2020 May 1;26(5):732-40.

[31] Kilbas PO, Can ND, Kizilboga T, Ezberci F, Doganay HL, Arisan ED, Dinler Doganay G. CRISPR/Cas9-mediated Bag-1 knockout increased mesenchymal characteristics of MCF-7 cells via Akt hyperactivation-mediated actin cytoskeleton remodeling. PLoS One. 2022 Jan 7;17(1):e0261062.

[32] Wu Q, Sharma D. Autophagy and breast cancer: connected in growth, progression, and therapy. Cells. 2023 Apr 14;12(8):1156.

[33] Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science. 2016 Jan 1;351(6268):84-8.

[34] Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Joung JK. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016 Jan 28;529(7587):490-5.

[35] Haeussler M, Schönig K, Eckert H, Eschstruth A, Mianné J, Renaud JB, Schneider-Maunoury S, Shkumatava A, Teboul L, Kent J, Joly JS. Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. Genome biology. 2016 Dec;17:1-2.

[36] Lu Y, Godbout K, Lamothe G, Tremblay JP. CRISPR-Cas9 delivery strategies

with engineered extracellular vesicles. Molecular Therapy-Nucleic Acids. 2023 Dec 12;34.

[37] National Academies of Sciences, Medicine, National Academy of Medicine, Committee on Human Gene Editing, Scientific, Medical, Ethical Considerations. Human genome editing: science, ethics, and governance. National Academies Press; 2017 Jul 13.

[38] Ledford H. CRISPR, the disruptor. Nature. 2015 Jun 4;522(7554).

[39] Replogle JM, Norman TM, Xu A, Hussmann JA, Chen J, Cogan JZ, Meer EJ, Terry JM, Riordan DP, Srinivas N, Fiddes IT. Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing. Nature biotechnology. 2020 Aug;38(8):954-61.

[40] Kantor A, McClements ME, MacLaren RE. CRISPR-Cas9 DNA base-editing and prime-editing. International journal of molecular sciences. 2020 Aug 28;21(17):6240.

[41] Chuai G, Ma H, Yan J, Chen M, Hong N, Xue D, Zhou C, Zhu C, Chen K, Duan B, Gu F. DeepCRISPR: optimized CRISPR guide RNA design by deep learning. Genome biology. 2018 Dec;19:1-8.