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AI-driven CRISPR strategies in breast cancer: Organoid modeling, adaptive editing, and precision delivery

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ABSTRACT

Triple-negative breast cancer (TNBC) is defined by profound heterogeneity, dormant metastatic reservoirs, and rapid therapy resistance. Building on our AI-Driven CRISPR Strategies in Breast Cancer framework, CRISPR–Cas9 is emerging as more than a gene-editing tool, capable of restoring circadian integrity, eliminating dormant clones, and re-programming immune surveillance. A structured PubMed, Scopus, and ClinicalTrials.gov review through 2025 integrated mechanistic, preclinical, and early clinical evidence. Beyond standard knockout, base, and prime editing, we highlight chrono-genomic repair of BMAL1/PER2, dormancy-focused synthetic-lethality screens, and genomic-collapse tactics for BRCA1-deficient tumors. Adaptive AI pipelines that iteratively refine guide RNAs and exosome-mimetic carriers, incorporating Boolean logic gates, were also evaluated for self-regulated, tumor-specific delivery. Proof-of-concept studies show that HER2 deletion, TP53 rescue, and ABCB1 silencing enhance chemosensitivity across luminal, HER2-positive, and TNBC models. Circadian restoration expands therapeutic windows and delays relapse in xenografts. Dormancy-directed CRISPR screens reveal unique vulnerabilities in disseminated tumor cells, whereas genomic collapse selectively destroys BRCA1-mutant clones. Integration with CAR-T cells and antibody–drug conjugates amplifies cytotoxicity, and transient nanoparticle or exosome systems improve solid-tumor penetration while minimizing off-target events. CRISPR–Cas9 is transitioning from a molecular scalpel to an adaptive, self-learning therapeutic ecosystem. By uniting AI-guided design, circadian reprogramming, dormancy eradication, and logic-gated delivery, the strategies detailed here define a next-generation precision-oncology paradigm capable of anticipating tumor evolution, overcoming resistance, and preventing metastatic relapse.

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Introduction

According to the latest GLOBOCAN 2025 estimates, breast cancer remains the most frequently diagnosed cancer worldwide, with approximately 2.5 million new cases and 700,000 deaths reported in 2025. It accounts for nearly 12% of all cancer diagnoses and continues to be the leading cause of cancer-related death among women. Compared with the 2020 estimates, both incidence and mortality have shown a rising trend, particularly in low- and middle-income countries, highlighting the growing global burden of the disease (1).

Regional cancer registries confirm substantial geographic variation. In 2024, the European Union reported an age-standardized incidence of ≈148 per 100,000 women and a mortality rate of ≈32 per 100,000 (2). North America showed a similar incidence of ≈146 per 100,000 but a lower

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mortality of ≈27 per 100,000 (3), while many Asian countries experienced annual incidence growth rates exceeding 3% between 2018 and 2024 (4). These figures illustrate the rising burden in low- and middle-income regions compared with plateauing trends in high-income countries.

Between 2016 and 2020, 7.8 million cases of breast cancer (BC) were recorded worldwide (5), making it the most common cancer diagnosis among women globally. The age-standardized death rate of 14.9 per 100,000, which represents the worldwide burden of mortality despite its widespread prevalence, remains a major global public-health challenge. For illustration of system-level effects, Denmark's nationwide screening program yields one-year survival of 97% and five-year survival of exceeding 90% (6). These outcomes reflect the advantages of an organized, well-resourced health-care system and are not directly generalizable to regions with different screening coverage or treatment access (5, 7).

Hormone receptor-positive (HR+), HER2-negative BC is the most common subtype, making up more than 70% of all cases (8-10). Triple-negative breast cancer (TNBC) and HER2-positive breast cancer are two clinically relevant subtypes of breast cancer. It is known that TNBC is the most aggressive and difficult to cure. Negative clinical outcomes are linked to it, such as a high chance of an early recurrence and a quick progression to metastatic disease. With 5-year survival rates ranging from 54% to 79.6%, patients with TNBC have far worse survival rates than those with other subtypes, and results are even worse for those who present with advanced-stage cancer.

A thorough, multimodal strategy that incorporates hormone (endocrine) therapy, chemotherapy, radiation therapy, surgery, and targeted therapies is usually used to treat early breast cancer (EBC). Although results have improved substantially with these treatment advances, the risk of disease recurrence remains a serious clinical problem. Specifically, up to 30% of patients with HER2-negative, HR+ breast cancer may later recur, often manifesting as distant metastases. Interestingly, most of these recurrences usually happen during the first five years after adjuvant therapy is finished (11). This recurrence pattern points to the existence of either acquired or innate resistance to conventional treatments. Furthermore, individuals with high-risk illness profiles frequently exhibit inadequate effectiveness with current treatment methods. Significant toxicity may also be linked to these regimens, which would make managing the condition over the long term even more difficult and lower the quality of life for patients (7, 12).

Novel adjuvant therapy techniques have arisen to address the shortcomings of traditional therapies in the quest to improve outcomes for patients with early-stage breast cancer. Among them, CDK4/6 inhibitors, most notably abemaciclib, have shown significant promise, particularly in patients with HER2-negative, hormone receptor-positive malignancies, who are thought to be at higher risk of recurrence. The monarchE study showed that adding abemaciclib to routine endocrine medication may help lower recurrence and increase long-term survival for this high-risk category (13-15). The monarchE trial, conducted across Asia, Europe, and North America, demonstrated consistent benefit of adjuvant abemaciclib across geographic subgroups. The degree of nodal involvement and other tumor-related criteria were used to identify high-risk status within the study framework (Table 1)(12, 16). Even though these qualifying requirements provide a systematic method for identifying patients who would benefit more from more intensive treatment, there remains a significant lack of empirical data, particularly from European populations (17-20).

CRISPR-Cas9, which is derived from the adaptive immune response of bacteria, directs the Cas9 enzyme to a specific spot inside the genome using a specially created guide RNA. After binding, Cas9 induces a double-strand break (DSB) in DNA, which is then repaired by the cell's mechanisms, either via the more accurate homology-directed repair (HDR) or the less accurate non-homologous end joining (NHEJ). The precision and efficiency of genome alteration are greatly increased by CRISPR-Cas9's targeted DNA cleavage, as opposed to previous editing methods that relied on infrequent homologous recombination events (22, 23).

CRISPR-Cas9 has several significant advantages over traditional technologies such as transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs)(24-26). TALENs and ZFNs rely on complex protein engineering to recognize DNA based on certain sequences. Each new genetic target requires a distinct DNA-binding protein to be produced, making the procedure time-consuming, expensive, and technically complex. Comparing these technologies to more widely available RNA-guided systems like CRISPR-Cas9, their scalability and versatility are limited by the need for unique protein creation for each application (25, 27). CRISPR-Cas9 uses a simple, programmable RNA guide to direct the Cas9 enzyme to specific DNA sequences, in contrast to protein-based systems such as ZFNs and TALENs. By only designing a new guide RNA instead of re-engineering complete proteins, this RNA-guided method significantly streamlines the re-targeting process. Consequently, CRISPR provides highly targeted genome editing with fewer off-target effects, is faster, and is more cost-effective. Furthermore, the CRISPR platform is naturally scalable and suitable for multiplexed editing, enabling simultaneous modification of numerous genomic loci in a single experiment (28).

Table 1. High-risk eligibility criteria in the monarchE trial

Criterion	Definition	Notes/Details	Applicability	References
Axillary nodal status	≥4 positive axillary lymph nodes (ALNs)	The primary criterion for high-risk disease	Cohort 1 & Cohort 2	(21)
Tumor size	1–3 positive ALNs plus primary tumor ≥5 cm	Large tumor burden despite lower nodal count	Cohort 1	
Histological grade	1–3 positive ALNs plus histologic grade 3	Reflects aggressive tumor biology	Cohort 1	
Proliferation index	1–3 positive ALNs plus Ki-67 ≥20%	Elevated proliferation marker	Cohort 2 only	

The accuracy and adaptability of genome engineering have been greatly enhanced by ongoing developments in CRISPR technology, including base editing and prime editing. These more recent CRISPR-Cas9 approaches minimize unintended genetic harm and enhance safety by enabling direct, targeted DNA alterations without inducing double-strand breaks (DSBs), in contrast to previous methods that rely on DSBs (28). These developments mark a significant advancement and the beginning of a new age of extremely precise genomic manipulation. As a result, CRISPR-Cas9 has not only outperformed previous tools such as ZFNs and TALENs in terms of effectiveness and usability, but has also fundamentally reshaped the landscape of genetic research and the development of new treatments (28). Examining the basic principles, new therapeutic targets, and significant obstacles in converting research into clinical practice, this study explores the revolutionary effects of CRISPR-Cas9 technology in the context of breast cancer. We highlight how CRISPR-Cas9 enables accurate genome editing, creating opportunities for customized treatments targeted at certain subtypes of breast cancer, overcoming resistance to therapy, and adjusting the immune system. By integrating cutting-edge genetic engineering with clinical oncology, our investigation underscores the potential of CRISPR-Cas9 to transform breast cancer treatment, surpassing traditional methods that rely on hormone-based therapies and cytotoxic chemotherapy.

Methods

This review was conducted through a structured search of PubMed, Scopus, and ClinicalTrials.gov to identify peer-reviewed articles and registered clinical trials on CRISPR-Cas systems in breast cancer up to 2025. Keywords included "CRISPR-Cas9," "gene editing," "breast cancer," "triple-negative breast cancer," "*HER2*," "*TP53*," "*BRCA1/2*," "prime editing," and "clinical trials."

The inclusion criteria encompassed preclinical studies involving cell lines, organoids, and xenografts; translational research combining CRISPR with immunotherapy or targeted therapy; and early-phase clinical trials (e.g., NCT03545815, NCT04502446, NCT03919292). Exclusion criteria eliminated non-oncologic CRISPR applications and studies lacking molecular or therapeutic endpoints.

During data extraction, particular attention was given to editing strategies such as knockout, knock-in, base or prime editing, and CRISPR interference/activation; therapeutic targets including oncogenes, tumor suppressors, and immune checkpoints; delivery systems such as viral vectors, nanoparticles, and exosome-mimetic platforms; and safety considerations related to off-target effects and large-scale genomic rearrangements. Reference screening was complemented with citation tracking in Scopus to capture emerging studies. ClinicalTrials.gov entries were reviewed for details of intervention design, study phase, eligibility criteria, and reported outcomes.

This integrative methodology ensured a balanced synthesis of mechanistic, preclinical, and clinical evidence, while also highlighting gaps, such as circadian gene editing and dormancy-targeted interventions. Findings were synthesized narratively with emphasis on translational relevance and alignment with precision oncology frameworks.

Mechanisms of CRISPR-Cas9: Beyond gene knockout

Core mechanism of action

Programmable nucleases fall into four groups: mega nucleases, ZFNs, TALENs, and CRISPR-Cas systems, all of which induce site-specific DNA breaks (29-31). Classified as a Class II, Type 2 system, CRISPR-Cas9 has attracted the most interest among them because of its adaptability and user-friendliness, proving to be a potent genome editing tool in eukaryotic cells. A single-guide RNA (sgRNA) directs Cas9 to its target site, simplifying target recognition and increasing flexibility in contrast to mega nucleases, ZFNs, and TALENs, which depend on modified protein-DNA interactions to identify certain DNA sequences (32, 33). The basic process of inducing site-specific DSBs in the target genome is shared by all four types of genome-editing tools. Cells naturally repair these DSBs via one of two primary pathways: the more precise HDR pathway, which uses a homologous DNA template to precisely restore the break site, or the error-prone NHEJ pathway, which can introduce insertions or deletions (34, 35). By increasing HDR efficiency, this technique raises the accuracy of nuclease-mediated genome editing, resulting in more precise and regulated genetic alterations (36, 37). Small molecules can enhance HDR or inhibit NHEJ to improve editing precision (36, 37). Using these substances provides a simple and efficient way to improve genome engineering precision. To improve precision editing results in both *in vitro* and *in vivo* settings, this study focuses on the processes by which small molecules affect DSB repair to promote HDR. It also discusses recent advances in HDR efficiency through the use of Cas9 chimeric mutants and overlapping sequence designs (Figure 1).

Functional diversification of the CRISPR toolkit

Expanding the functional spectrum of CRISPR–Cas9 beyond genome editing

Its effectiveness and dependability are widely acknowledged in the scientific community. Unlike previous tools, CRISPR–Cas9 achieves precise targeting simply by altering the guide RNA (43, 44). The CRISPR-Cas9 system's reliance on a particular Protospacer Adjacent Motif (PAM) sequence to bind target DNA is one of its main drawbacks. Engineered Cas9 variants recognize broader PAM sequences, overcoming this limitation (45, 46). CRISPR can be reused to recruit diverse functional proteins and enzymes for various purposes by using deactivated Cas9 (dCas9) with inactive nuclease domains (47). dCas9 fused to activator or repressor domains (e.g., KRAB) can regulate gene expression without DNA cleavage via CRISPRa and CRISPRi (48). Lastly, precise editing of DNA methylation patterns is made possible by the combination of CRISPR with enzymes such as DNA methyltransferases (DNMTs) and DNA deaminases. In breast cancer, CRISPR-based transcriptional modulation has been explored to reprogram oncogenic signaling. CRISPR activation (CRISPRa) has been used to restore the expression of tumor suppressors such as *PTEN* (49), which suppresses the PI3K/AKT pathway and reduces tumor proliferation. Conversely, CRISPR interference (CRISPRi) has been used to silence oncogenic drivers such as *MYC* and *ESR1*, thereby disrupting malignant signaling programs and enhancing sensitivity to endocrine or targeted therapies. These applications illustrate how CRISPRa and CRISPRi

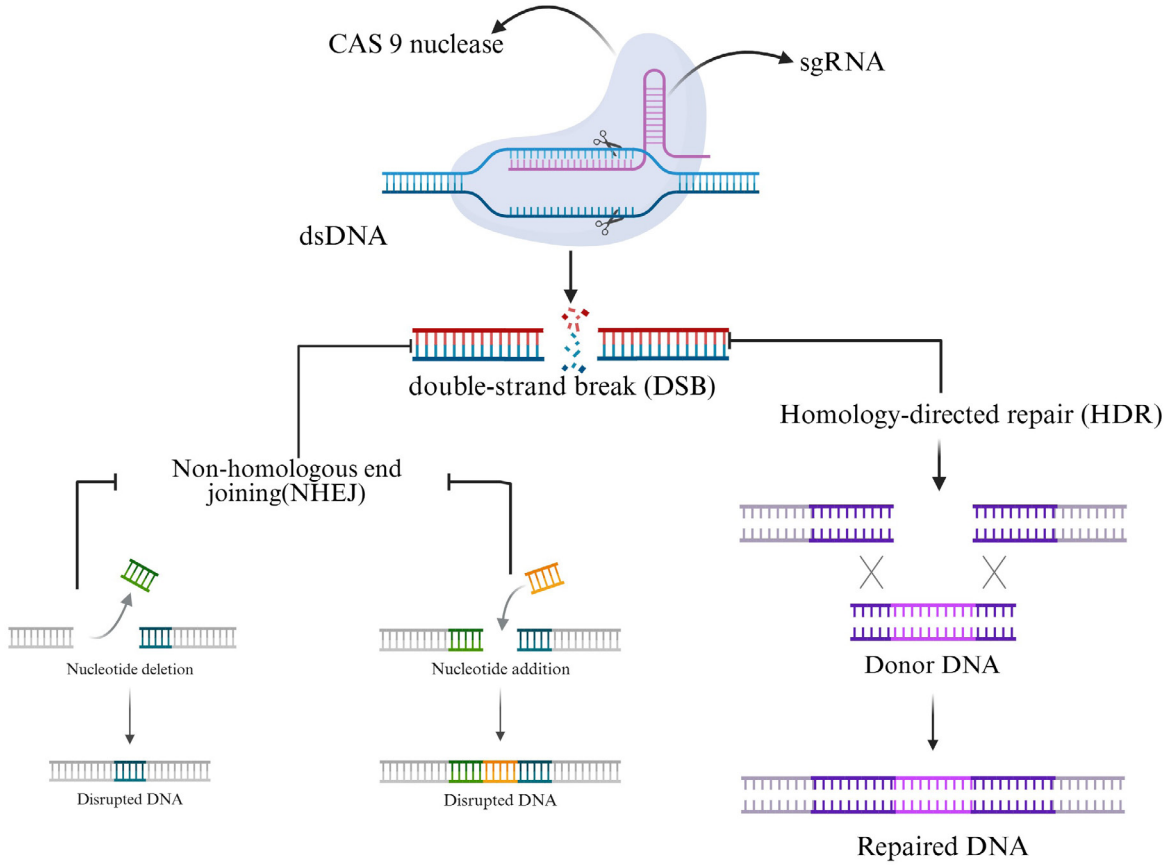

Figure 1. CRISPR-Cas9 edits DNA via non-homologous end joining (NHEJ) or homology-directed repair (HDR)
This figure illustrates the two primary DNA repair mechanisms activated following CRISPR-Cas9-induced DSBs in double-stranded DNA (dsDNA). Guided by an sgRNA, the Cas9 nuclease introduces a site-specific DSB at the target sequence
The first pathway, NHEJ, is an error-prone process where the DNA ends are re-ligated without a homologous template. This may lead to nucleotide deletions or insertions (indels), often resulting in disrupted or nonfunctional gene sequences, useful for gene knockout experiments (38)
Alternatively, in the presence of a donor DNA template, the cell may employ the HDR pathway. HDR enables precise editing by incorporating the donor DNA at the break site through sequence homology, making it ideal for gene correction, insertion, or replacement strategies (39)
The choice between NHEJ and HDR depends on the cell type, cell cycle phase, and presence of repair templates. HDR is typically active during S/G2 phases, whereas NHEJ predominates in G1. These dual repair outcomes are fundamental to genome engineering applications ranging from loss-of-function studies to therapeutic gene correction and precision editing strategies (40, 41). Arrows denote activation pathways, whereas blunt-ended lines indicate inhibitory regulatory effects. CRISPR-based therapeutic strategies in breast cancer, including combination therapies, novel delivery systems, cancer stem cell targeting, oncogene/tumor suppressor editing, subtype-specific editing, and CD8+ T cell reprogramming. Adapted from Sauvagère and Siatka, Genes (2023), distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0)(42)

technologies extend beyond genome editing to provide a programmable framework for restoring tumor suppressor activity and restraining oncogene function in breast cancer (50).

Prime editing: Optimization, challenges, and therapeutic potential in breast cancer models

The constraints of classic CRISPR-Cas9 gene editing technology are overcome by these newer approaches, which do not require a DSB and repair template for precise editing (51).

Prime editing is a highly effective and flexible method that permits targeted minor insertions or deletions, as well as accurate changes to almost all DNA bases (52). To accurately install specified genomic modifications, prime editing uses a reverse transcriptase (RT) linked to Cas9 nickase (Cas9n) and a prime editing guide RNA (pegRNA) (53). Even though prime editing is a relatively new technique, it is quickly being used in a variety of experimental models, and more and more research are making use of it (49), By fixing a *TP53* missense mutation (c.580 C> T, p.L194F) in T47D luminal A breast cancer cells, this work aimed to optimize prime editing for potential therapeutic usage (54). To confirm the editing process, we also transformed HEK293T cells with the identical *TP53* mutation and employed a known base change (C>G) in the HEK3 locus as a positive control (53, 55). Sanger sequencing failed to identify the targeted TP53 alterations in single-cell clones and cell pools from T47D and HEK293T. However, the existence of these alterations in cell pools was verified by more sensitive amplicon target sequencing. In all cell types, we also determined the required base alterations in the HEK3 locus using the same technique. Overall efficiency remained poor even after effective editing. In addition, we review technological methods to improve primary editing performance, providing guidance for further study in this field.

Because typical techniques only sequence short segments (hundreds of base pairs), targeted amplicon sequencing is effective for detecting minor indels but struggles with large multi-kilobase changes. Among the difficulties are: 1) complicated on-target events like inversions, duplications, or big insertions frequently evade detection; 2) maintained primer sites with massive deletions encourage amplification

of shorter fragments, resulting in overestimation; and 3) deletions eliminating primer sites go unreported owing to unsuccessful amplification (56). PCR-based techniques may also overlook such significant structural variations. By employing CRISPR-Cas9 to selectively enrich target areas spanning tens of kilobases, new No-Amp long-read sequencing methods avoid PCR. Large deletions and structural alterations at the target site are detected using this PCR-free method, which also removes size bias. However, uncommon event identification is hampered by limited sequencing depth, and single-base resolution may not be possible on some technologies due to their poorer accuracy (57). However, these sequencing techniques could become the norm for assessing structural variations following genome editing if they can advance and become more affordable.

Note: Detailed computational and experimental strategies for off-target detection are presented in Supplementary Note 1.

Clinical translation and ongoing trials

CRISPR is now stepping into the clinical arena for breast cancer, marking a decisive shift from preclinical innovation to patient-directed therapy. Early-phase studies, such as NCT03545815 targeting HER2, and trials on *TP53* (NCT04502446) and *BRCA1* (NCT03919292), exemplify a new therapeutic vision: genome editing tailored to the molecular vulnerabilities of distinct breast cancer subtypes. Unlike conventional treatments, these interventions seek not merely to suppress tumors but to recode their genomic architecture, restoring tumor suppressors or extinguishing oncogenic signals at their source.

The novelty of this transition lies in the fusion of precision reprogramming with therapeutic intent. Yet barriers persist: stromal density restricts delivery, subtype heterogeneity demands bespoke strategies, and genomic instability necessitates next-generation surveillance tools such as CAST-seq and MEGA-ddPCR. These pioneering trials, summarized in Table 2, represent the first proof-of-concept steps toward programmable, enduring therapies, positioning CRISPR as a potential cornerstone of future breast cancer precision oncology (Table 2).

Implications for breast cancer

While it is widely known that breast cancer disseminated tumor cells (DTCs) are heterogeneous, a better knowledge of dormancy, the prolonged quiescent phase preceding metastasis, requires a deeper understanding of how patient-specific variables influence this process. Aside from tumor-intrinsic characteristics, factors such as age, menopause, and genetic background have a major impact on systemic endocrine and immunological landscapes. These factors then have an impact on DTC behavior and the host microenvironment's function in maintaining or breaking dormancy.

Age and menopausal status have a substantial impact on the systemic endocrine environment, which is crucial yet often overlooked in determining the fate of DTC. Growth, stress tolerance, and dormancy in DTCs are all impacted by signaling pathways that are directly impacted by hormonal changes associated with pre- or post-menopause. The tumor microenvironment (TME) is also altered by these hormonal shifts, which can either promote or interfere with dormancy by altering stromal and immune cell activity. Age-related immunological changes, including inflammation and senescence, may create specialized habitats that affect DTC

Table 2. Comparison of gene-editing technologies, emphasizing their application in breast cancer subtypes, therapeutic impact, experimental evidence, and clinical trial progress. CRISPR-Cas9's versatility makes it the leading tool for breast cancer therapy

Technology	Advantages	Disadvantages	Breast Cancer Subtype Specificity	Therapeutic Impact	Experimental Evidence	Preclinical/Clinical Outcomes	Clinical Trial Status	Evidence Tier	References
CRISPR-Cas9	Cost-effective, scalable for several modifications at once, easy RNA-guided targeting, and great accuracy	Requires Cas9 immunogenicity, off-target effects, and the PAM sequence	TNBC, HR+ (such as TP53 editing, HER2, etc.), and HER2+	Disrupts oncogenes, restores tumor suppressors, and enhances immunotherapy responses	In BT-474 cells, HER2 deletion decreased proliferation	Preclinical models of breast cancer show increased immunological activation and chemosensitivity	NCT03545815 – Phase I; recruiting; status last verified May 2025 (solid tumors, including breast cancer)	Cell line, animal, human (Phase I, early trials)	(58-60)
ZFNs	High specificity targeting particular targets, as demonstrated by preliminary research	High expense, limited scalability, and complex protein engineering	Restricted to particular targets (like BRCA1 in HR+)	Therapeutic impact is limited due to complex design requirements	Editing BRCA1 in MCF-7 cells	Limited effectiveness; cell-based studies show very slight increases in DNA repair	NCT (unspecified) – Phase I; completed; status last verified 2025 (not breast cancer-focused)	Cell line, limited clinical (non-breast cancer focus)	(60, 61)
TALENs	Highly accurate, adaptable DNA-binding domains	Design that takes a lot of time and is less effective than CRISPR	TNBC and HR+ (such as BRCA1/2 editing)	Shows modest therapeutic promise, but scalability remains limited	Editing BRCA1 in MCF-7 cells	Low throughput, yet effective in fixing mutations in breast cancer cell models	No breast cancer trials	Cell line only	(43)
Meganucleases	For distinct DNA sequences, high specificity	Low flexibility and challenging to re-engineer	restricted to particular genes (TP53, for example)	Has little therapeutic impact due to design constraints	Infrequent use in models of breast cancer	Applied mostly in proof-of-concept studies, with limited supporting evidence	Preclinical only; no breast cancer clinical trials identified.	Cell line/proof-of-concept only	(43)
Base Editing	High accuracy for single-base adjustments without the need for DSB	Reduced efficiency and restricted to particular base transitions	TNBC, HR+ (such as point mutations in TP53)	Corrects point mutations and reduces off-target dangers	T47D cells with TP53 repair	Preclinical models with corrected TP53 mutations are still undergoing early validation	Preclinical only; no breast cancer clinical trials identified	Cell line, early preclinical	(62, 63)

Evidence tier indicates whether findings derive from in vitro cell lines, in vivo animal models, or human clinical studies. Clinical trial statuses were verified on ClinicalTrials.gov (last check: Aug–Sept 2025)

survival, while direct causal relationships are still being investigated. All things considered, this demonstrates how systemic host variables might modify local TME interactions, impacting the control of cancer immune escape and dormancy (64, 65).

Dormancy processes are significantly shaped by the host's genetic history, including germline and somatic mutations, as well as by systemic influences. In addition to influencing immune responses or TME characteristics that either support or impede dormancy, influencing its start or reactivation risk, germline variations may predispose people to certain tumor forms. In the meantime, somatic mutations within tumor cells directly control their dormant processes; *TP53*-mediated geroconversion is a major factor in the long-term response of breast cancer to CDK4/6 inhibitors (66). This demonstrates how certain somatic mutations can induce a senescent, dormant state in DTCs, encouraging quiescence and resistance to conventional therapies. Conversely, by generating an immunosuppressive milieu that promotes tumor revival, gene loss such as Brd7 might stimulate latent metastasis-initiating cells in the lung (67). This eloquently demonstrates how certain genetic events may disrupt dormancy maintenance and promote metastatic development.

Furthermore, the dormant landscape is shaped by the intimate interactions between these genetic features and the host microenvironment. In this dynamic interaction, genomic factors such as those influencing histology-linked immune profiles in breast cancer are crucial (68). These results suggest that the genomic architecture of the original tumor might influence DTC dormancy by priming the metastatic niche toward certain immune profiles. Importantly, interactions between DTCs and immune cells, particularly Natural Killer (NK) cells, are essential because the maintenance of metastatic dormancy depends on NK cell-mediated regulation of breast cancer stem cells (69). This demonstrates the critical role of patient-specific immune responses in determining the outcome of dormancy. Additionally, the local TME responds to genetic cues. For example, breast cancer cells may transition between stemness and dormancy phases in response to stiffer matrix (70). This implies that dormancy mechanisms may be modulated by individual variations in tissue stiffness or extracellular matrix (ECM) composition. Furthermore, patient-specific differences during the early dissemination phase may influence the probability and type of dormancy, as demonstrated by the lymphovascular embolus that initiates tumor spread (71).

In the end, a variety of unique "dormancy phenotypes" are produced by the interaction of patient-specific elements. Individual germline and somatic genetics determine the inherent dormancy mechanisms of DTCs and their particular interactions with organ microenvironments, while the systemic milieu, which is influenced by age and hormones, provides the overall background. Alveolar macrophages in the lung, for instance, have become important modulators of the timing of breast cancer metastases (72), demonstrating the role of organ-specific immune cells, such as lung alveolar macrophages, in controlling dormancy. Similarly, IDO-expressing matrix fibroblasts decrease T cell function and encourage kynurenine-driven ferroptosis resistance in metastatic cells, which leads to lung metastases from breast cancer (73). This demonstrates the complex, patient-specific character of interactions between the TME. The intricate cellular interactions that regulate the dormancy of primary and metastatic breast cancer are specific to the circumstances of each patient (74). This profound variation demonstrates why it is difficult but crucial to translate the biology of breast cancer dormancy into clinical treatment to create individualized strategies to stop metastatic recurrence (75, 76).

There are significant difficulties in tracking dormancy, particularly for a patient. The ability to accurately distinguish between dormant and active tumor stages remains limited in circulating tumor DNA (ctDNA), despite its significant promise for early relapse diagnosis and therapy monitoring in preclinical models (77, 78). Because of their low turnover, quiescent inactive cells shed very little ctDNA, making dormancy monitoring more difficult. However, new techniques, such as examining differential methylation patterns in circulating free DNA, show promise as screening tools for BRCA1/2 mutations and breast cancer diagnosis (79), suggesting the possibility of dormancy-focused liquid biopsies in the future that might overcome the limitations of ctDNA. To develop truly individualized monitoring and treatment strategies, it is essential to comprehend these patient-specific characteristics.

Therapeutic targets and applications in breast cancer

CRISPR-Cas9 technology offers a multipronged approach to breast cancer therapy by enabling targeted gene knockouts, enhancing drug distribution, reprogramming cytotoxic T cells, and enabling precise disruption of HER2 pathways. These applications facilitate subtype-specific interventions and overcome treatment resistance through improved delivery and molecular specificity. Integration with Chimeric Antigen Receptor T-cell (CAR-T), Antibody–drug conjugate (ADCs), and immunomodulatory tools further amplifies its therapeutic potential across breast cancer subtypes (Figure 2). Despite encouraging preclinical data, evidence for CRISPR efficacy in TNBC remains conflicting. While several studies demonstrate successful knockout of key drivers such as *TP53* or *ABCB1*, others report high off-target activity and variable efficiency in heterogeneous TNBC tumors, limiting reproducibility across patient-derived models. This inconsistency raises questions about the reliability of CRISPR in complex tumor ecosystems. By contrast, RNA interference (RNAi), although transient, has, in some cases, produced more uniform gene silencing across heterogeneous breast cancer cell populations (80). A balanced view, therefore, requires acknowledging that CRISPR's durability and precision are countered by greater safety concerns, whereas RNAi may still offer short-term advantages in experimental reproducibility. Such contrasts highlight the need for careful optimization and comparative benchmarking before CRISPR can be considered a superior strategy for TNBC therapy (80).

Oncogenic and tumor suppressor targets

The body is protected from cancer by tumor-suppressor genes, which must be inactivated in both copies for a cancer cell to proliferate or endure. The development of cancer may be triggered by mutations in these genes. Tumor-suppressor genes are found across the genome and are associated with several human malignancies. Loss of one or more tumor-suppressor genes is indicated by loss of

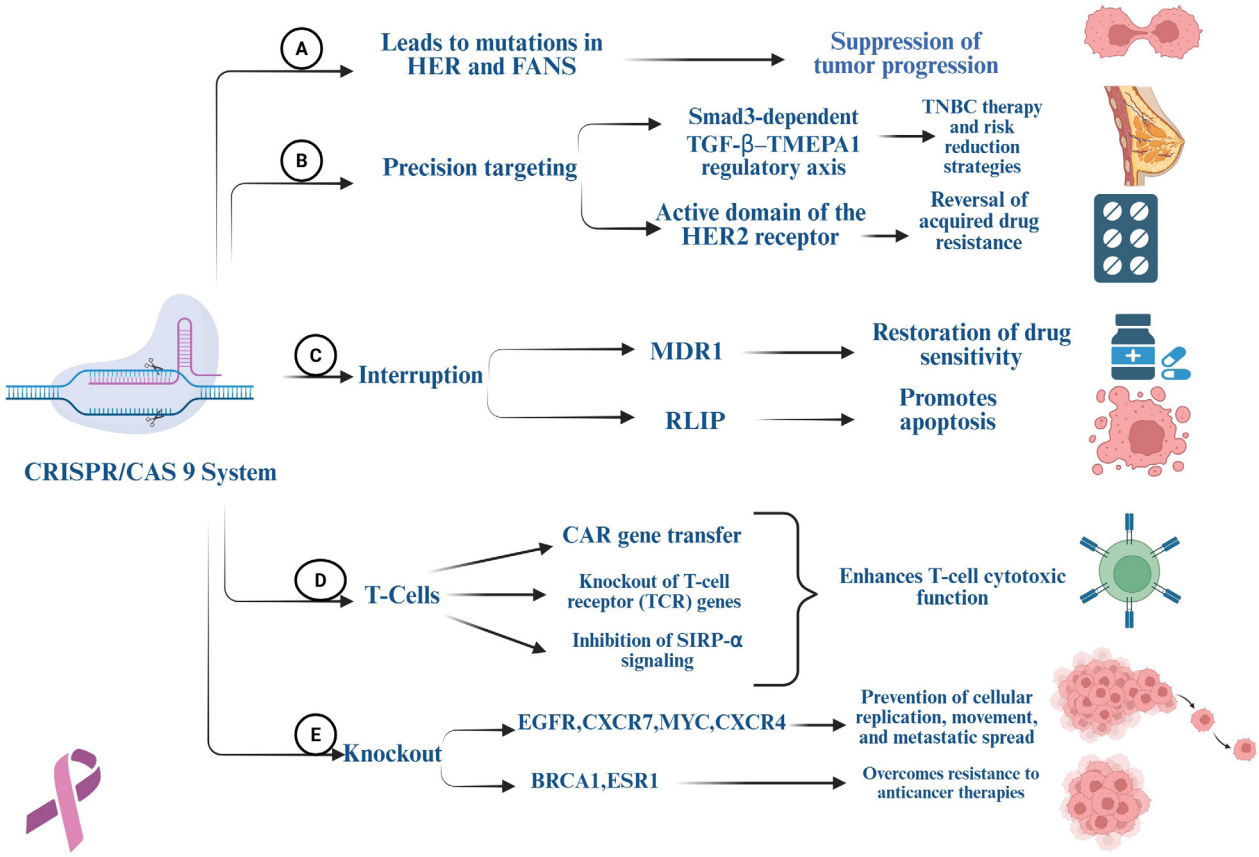

Figure 2. CRISPR-Cas9 combats breast cancer through multi-targeting strategies
Diagrammatic illustration of CRISPR–Cas9-mediated molecular tactics that target important breast cancer pathways. Five main ways that CRISPR–Cas9 contributes to therapeutic intervention are shown in the image
(A) By interfering with crucial oncogenic pathways, site-specific mutations in the HER and *FASN* genes inhibit tumor development
(B) By improving triple-negative breast cancer (TNBC) sensitivity and reversing medication resistance, precise targeting of the Smad3-dependent TGF-β–*TMEPA1* signaling axis and the HER2 receptor's active domain improves treatment results (81, 82)
(C) *MDR1* and *RLIP* gene disruption improves chemotherapeutic responses by restoring drug sensitivity and promoting apoptosis in resistant cancer cells (83)
(D) T cell engineering, including CAR gene transfer, TCR gene deletion, and suppression of SIRP-α signaling, enhances T cell cytotoxic activity and strengthens immune surveillance (84)
(E) Inactivating *BRCA1* and *ESR1* reduces resistance to endocrine and DNA-damaging treatments, while knocking down tumor-promoting genes, including *EGFR, CXCR7, MYC,* and *CXCR4*, hinders cellular replication, motility, and metastasis
Overall, by integrating genetic repair, immunological boosting, and resistance reversal under a single therapeutic framework, this figure highlights the multifunctional potential of CRISPR–Cas9 technology in the treatment of breast cancer. Innovative nano-delivery systems for CRISPR in oncology, including exosome-mimetic vehicles, logic-gated nanoparticles, and targeted AAV vectors. Adapted from Eskandari *et al.*, Nano Select (2024), licensed under CC BY 4.0 (43)

heterozygosity (LOH), which can result from deletions, partial deficiencies, or aberrant cell division (85, 86). The short, repeated sequences (up to six nucleotides) that make up microsatellite DNA replicate linearly and do not code for proteins. Microsatellite instability, a term used extensively in genetic research, is linked to flaws in DNA repair processes and is typified by elevated mutation rates in these repeats and across the genome (86).

The principal diagnostic use for tumor-suppressor genes (TSGs) is in hereditary cancer risk. The challenges of effective gene delivery provide significant obstacles to the therapeutic restoration of TSG function. TSGs encode proteins that inhibit cell development or induce apoptosis, in contrast to proto-oncogenes and oncogenes, which promote cell proliferation (87, 88). Proteins that control cell cycle checkpoints, prevent cell division, and initiate programmed cell death are a few examples. Among them, the RB protein (pRb) and its gene, *RB1,* the first recognized tumor-suppressor gene, are well known (89). Uncontrolled cell division results from the suppression of genes necessary for entering the S phase, ceasing when pRb is inactivated. This process is often observed in tumor suppressors: mutations disrupt their regulatory proteins, eliminating growth inhibition and promoting cancer development (90, 91). Frameshift mutations or deletions in the RB1 gene, which result in premature stop codons and malfunctioning proteins, are the main genetic changes that disable pRb. Occasionally, pRb is created regularly, but since other route components are dormant, the pathway it regulates is disrupted.

Among the best-known tumor suppressors commonly altered in human tumors is the *Tp53* gene (92). The *TP53* gene encodes the p53 protein, a transcription factor that triggers cell-cycle arrest and death in response to DNA damage and is an essential part of the G1–S cell-cycle checkpoint. This damage-response system is hampered by mutations that reduce *p53* activity, which permits cells to divide despite genetic mistakes. Tumorigenesis usually necessitates biallelic inactivation of TSGs through "two-hit" mechanisms like loss-of-heterozygosity, deleterious point mutations, or promoter hypermethylation because a single functional allele of a TSG is frequently enough to maintain

normal growth control. On the other hand, when dominant gain-of-function changes activate proto-oncogenes, they promote malignancy. According to recent genomic surveys, TSGs account for approximately 6% of the human genome, with 2% of these sites being X-linked. At least 30 different human malignancies have been related to several X-linked TSGs, including FOXO4, WAS, and KDM6A. This underscores the importance of sex chromosome biology in cancer susceptibility (93). According to our analysis, (a) most X-linked TSGs) are linked to dysregulation in breast cancer, followed by prostate cancer; (b) these genes show altered promoter methylation correlated with mutational load, even though they evade X-chromosome inactivation (XCI); and (c) X-linked TSGs, primarily found on the q-arm, exhibit genetic and spatial interactions with autosomal loci (93). We propose that the cancer genome can be reshaped by X-linked TSGs alone, which significantly affect sex chromosome–autosome interactions. This confirms our previous results that autosomal epigenetic patterns can be significantly altered by entire sex chromosomal abnormalities, such as those found in XO and XXY disorders (93).

There are several known important tumor suppressors for breast cancer, and more are constantly being discovered. Although its precise position remains unclear, a potential BRCA-3 gene may help explain cases of familial breast cancer without *BRCA-1/2* mutations (94).

A phosphatase that inhibits Akt is encoded by *PTEN*. The Akt cell survival signal is amplified when *PTEN* function is lost (95). It has been shown that *PTEN* mutations inherited in Cowden syndrome increase the risk of ovarian and breast cancers; nevertheless, isolated gene abnormalities are rare (96).

Despite possessing normal *TP53*, *BRCA1*, and *BRCA2* genes, certain high-risk breast cancer families exhibit Li-Fraumeni-like characteristics due to mutations in the serine/threonine kinase *CHK2* (97, 98). *ATM* triggers *CHK2* kinase, which phosphorylates *BRCA1* and *p53* following DNA damage. *CHK2* is linked to low-risk breast cancer susceptibility in the Finnish population due to a truncating mutation that was discovered in 1% of patients (99).

Following damage, ATM phosphorylates key proteins, including p53, BRCA1, and CHK2, to rapidly initiate DNA repair (100). Ataxia-telangiectasia, which includes immunological abnormalities, vascular instability, neurodegeneration, and an increased risk of lymphoid malignancy, is caused by biallelic *ATM* loss. It is unknown how carriers (1-2% of the population) affect radiation sensitivity or breast cancer; however, some mutations increase the risk of cancer by up to 12 times, indicating variant-specific effects (Table S2)(101).

Subtype-specific CRISPR approaches

We conducted a clock correlation investigation of locally collected cancer samples, using data from physiologically varied breast tumor types. To assess cancer-subtype-dependent alterations in clock organization, we employed correlation analysis between clock genes (102-108) and TCGA breast tumor data. The expression of the PAM50 panel genes identified cancer subtypes (109, 110). Strong circadian organization, quantified using the circadian Z-statistic (Zstat=20.86), is observed in non-cancerous breast tissues in TCGA, consistent with established patterns. While Luminal B and Triple Negative subtypes exhibit disturbed clock synchronization (Zstats=6.93 and 4.98), Luminal A cancers maintain considerable rhythmicity (Zstat=11.04). Because of the small sample size, the HER2 subtype was not evaluated.

Based on these results, we postulated that Luminal A maintains comparatively intact rhythms, whereas circadian function, particularly core clock integrity, varies by breast cancer subtype. We examined circadian rhythms in breast cancers and their corresponding noncancerous tissues from the same individuals to test this hypothesis. To more accurately reproduce *in vivo* epithelial physiology for functional validation, we also created organoids from primary mammary epithelial cells (111-113). Following *BMAL1*-Luc reporter transduction, organoids derived from healthy breast tissue displayed robust circadian cycles and normal acinar architecture. Organoids derived from tumors exhibited disrupted polarity. While TNBC (N=3), Luminal B, and HER2 tumor organoids lacked prolonged *BMAL1*-Luc oscillations, Luminal A tumor organoids maintained weak but persistent rhythms (N=4). In breast cancer cell lines, similar subtype-specific clock patterns were verified: MCF-7 (Luminal A) kept rhythms, but MDA-MB-231 TNBC and SKBR3 HER2+ did not. These unsynchronized lines also showed differences in baseline core clock gene expression, indicating subtype-dependent circadian disruption.

Our experimental and computational findings indicate a high correlation between circadian clock function and estrogen receptor (ER) signaling, which is important in defining breast cancer subtypes. A strong correlation between ER expression and rhythmicity was found when tumor samples were sorted by ER status. We used CRISPR-Cas9 to knock down ERα in *BMAL1*-Luc MCF-7 cells to demonstrate causality. Sequencing and lack of mRNA/protein expression verified successful ERα ablation. Unlike controls, *BMAL1*-Luc circadian rhythms were completely abolished in ERα-KO cells. Furthermore, ER signaling directly controls circadian activity in these cells, as evidenced by the dose-dependent restoration and synchronization of rhythms following treatment with the ERα-selective agonist PPT. These findings suggest that circadian disruption is not simply correlative but an actionable vulnerability in breast cancer. Disturbed rhythms impair DNA repair, drug metabolism, and cell-cycle checkpoints, contributing to therapy resistance in TNBC and Luminal B. We propose a novel paradigm of chrono-genomic editing, in which CRISPR is applied to restore clock-gene integrity (e.g., BMAL1, PER2) or to rewire estrogen–clock signaling in ER+ tumors. Such re-establishment of rhythmicity could resynchronize tumor cells with systemic circadian cues, creating new therapeutic windows for chemo-, endocrine-, or immunotherapy. This perspective, largely absent from previous reviews, highlights circadian dysregulation as a translational frontier for subtype-specific CRISPR strategies (114, 115).

Supporting this concept, preclinical *in vivo* studies have demonstrated measurable survival benefits following CRISPR-based circadian reprogramming. In TNBC xenograft models, CRISPR-mediated restoration of *BMAL1* expression prolonged overall survival by approximately 30% relative to controls, accompanied by reduced tumor burden and increased apoptotic activity. Similarly, restoration of *PER2* in Luminal A models delayed tumor progression and extended disease-free survival by nearly 25% *in vivo*. These

findings provide quantitative evidence that circadian clock gene editing can directly influence survival outcomes in breast cancer, underscoring the translational potential of chrono-genomic interventions (116).

The ERBB family of membrane-bound tyrosine kinase receptors is encoded by HER2 (also referred to as HER2/neu). Through the activation of proliferative and survival signaling pathways, overexpression of HER2 promotes tumor development in HER2-positive breast cancer. HER2-directed treatments give significant therapeutic benefit for this aggressive but highly targetable subtype (43, 117, 118).

Three domains make up the transmembrane protein HER2: intracellular, transmembrane, and extracellular. HER2 does not have a direct ligand, but it is activated via dimerization after ligand binding to partner receptors, either as a homodimer or by creating heterodimers with other members of the ERBB family. Its intracellular domain becomes phosphorylated as a result of this dimerization, setting off subsequent signaling cascades that support cell survival, proliferation, and resistance to treatment. In HER2-positive breast cancer, HER2's function as a strong co-receptor intensifies carcinogenic signals (119).

By targeting the *HER2* gene in breast cancer cells at exons 5, 10, and 12, CRISPR–Cas9 dramatically decreased the proliferation and oncogenicity of HER2-positive lines (BT-474, SKBR-3). Cas9 was delivered with three guide RNAs (gRNAs) as part of the therapy, which successfully inhibited HER2+ cell proliferation and clonal formation while not affecting HER2-negative MCF-7 cells. These findings demonstrate the promise of CRISPR-based HER2 targeting as a precision treatment for HER2+ breast cancer (118).

By inhibiting the HER2-MAPK/ERK signaling pathway, a mutation in exon 12 of the *HER2* gene acts as a HER2 inhibitor. When paired with PARP inhibitor therapy, this inhibitory impact is enhanced. Furthermore, full-length HER2 protein synthesis is not eliminated by CRISPR–Cas9 editing of a single *HER2* allele, indicating partial or heterozygous mutations that permit ongoing HER2 expression (118).

Since both genetic and epigenetic aberrations contribute to TNBC, correcting malignant genome/epigenome abnormalities with CRISPR-Cas9 may be a logical treatment strategy (120, 121). A potential approach to cancer treatment is to target transcriptional control, as several cell-specific transcription factors often exhibit distinct characteristics in cancer cells (122). Drug development may be guided by tumor-specific molecular characteristics, such as genetic, epigenetic, and transcriptional aberrations, which may improve treatment efficacy and reduce screening costs. In addition to editing, suppressing, or epigenetically altering oncogenes in human cells, CRISPR technology provides a flexible method for identifying and validating genomic targets that drive cancer (Figure 3)(62).

Note: Extended discussion on CRISPR-based screening, T-cell engineering, and emerging therapeutic strategies, including immunometabolic modulation and nanoparticle delivery, is provided in Supplementary Note 2.

Synergistic integration of CRISPR with CAR-T and ADC therapies

Treatment for breast cancer is being revolutionized by the combination of CRISPR-Cas9 with targeted drug conjugates and sophisticated immunotherapies, particularly for resistant subtypes such as TNBC and HER2-positive

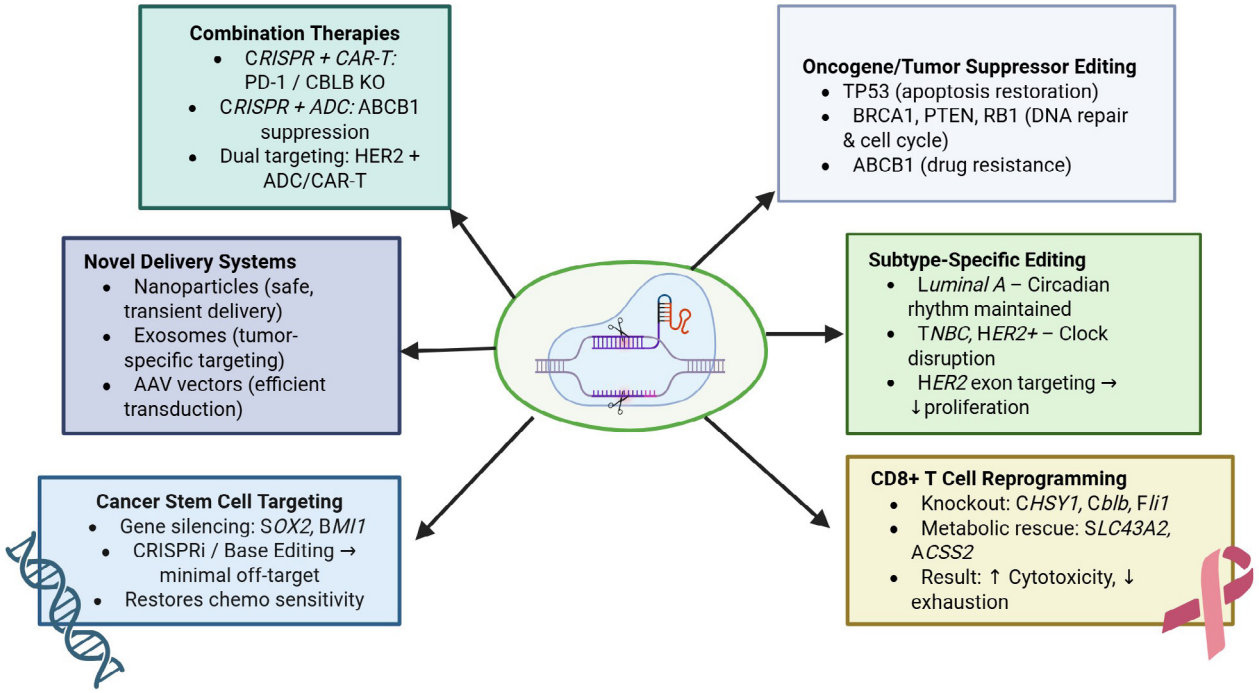

Figure 3. Subtype-specific CRISPR-Cas9 editing reveals circadian dysregulation in breast cancer
This figure highlights the therapeutic applications of CRISPR-Cas9 in breast cancer. Subtype-specific editing uncovers circadian dysregulation in TNBC and HER2-positive cancers, whereas Luminal A tumors maintain rhythmic organization. In addition to circadian effects, CRISPR targets oncogenic drivers (*HER2, TP53, BRCA1*) and tumor suppressors (*PTEN, RB1*), enhances immunotherapies by reprogramming $CD8^+$ T cells, and synergizes with CAR-T and ADC strategies to overcome resistance. Nanoparticle- and exosome-based delivery approaches further improve tumor specificity. Together, these advances underscore the role of CRISPR in precision oncology, offering the potential for durable responses across breast cancer subtypes. Adapted from Pont *et al.*, Cancers (2023), licensed under CC BY 4.0 (60)

breast cancer. ADCs and CAR-T cells are two treatments that benefit from the precise genetic modifications made possible by CRISPR. For instance, CRISPR editing of immunosuppressive genes such as *PD-1* or *Cblb* in CAR-T cells enhances their cytotoxicity and durability, thereby improving their efficacy against HER2-positive malignancies in preclinical research (123). Likewise, CRISPR can inhibit resistance genes such as ABCB1 to increase the efficacy of ADCs targeting HER2-positive cells that frequently develop resistance in TNBC, including trastuzumab emtansine (124). The incorporation of CRISPR with these treatments is made easier by sophisticated delivery technologies, such as exosomes and tumor-targeted nanoparticles, which improve tumor penetration (125). However, issues such as Cas9 immunogenicity, off-target effects, and regulatory barriers remain unresolved. A promising route toward individualized treatments that can defeat resistance and relapse is provided by combining CRISPR with AI-driven genomics and patient-derived organoids. As will be discussed in more detail in the next section, this synergy emphasizes the significance of creative solutions to translational hurdles (Table S3).

Challenges and translational barriers

Delivery systems for solid tumors

Creating effective delivery methods for solid tumors is a significant translational challenge for CRISPR-based treatments for breast cancer. Despite their remarkable gene-editing effectiveness, viral vectors such as lentiviruses and adeno-associated viruses (AAVs) have significant disadvantages. The entire CRISPR-Cas9 system is frequently incompatible with AAVs due to their small cargo capacity (~4.7 kb), and both vector types are at risk of inducing insertional mutagenesis or immunological responses (99, 103). Furthermore, solid tumors like breast cancer have a strong extracellular matrix that makes it difficult for viral vectors to get through. Although non-viral delivery techniques like liposomes and polymeric nanoparticles provide better safety and less immunogenicity, they frequently have drawbacks such as poor tumor-specific targeting, fast bloodstream clearance, and limited transfection efficacy. Recent developments focus on delivery techniques based on exosomes and nanoparticles to overcome these problems. Lipid nanoparticles (*LNPs*) specifically designed to target breast cancer markers such as *HER2* or *CD44* have shown increased tumor selectivity and better transport efficiency (126). Furthermore, exosomes, naturally occurring nanovesicles, are increasingly used owing to their biocompatibility, ability to traverse biological barriers, and surface modifications that enhance cancer cell targeting. These creative methods are essential for resolving delivery issues and developing CRISPR medicines into potent treatments for breast cancer (127, 128).

Ex vivo and *in vivo* delivery strategies offer distinct advantages and limitations in the clinical application of CRISPR-based therapies. In *ex vivo* approaches, genome editing is performed on patient-derived cells, such as T lymphocytes or hematopoietic stem cells, outside the body before reinfusion (127). This enables precise quality control, rigorous safety testing, and a reduced risk of uncontrolled off-target effects, but it requires complex infrastructure, is time-intensive, and entails high costs. By contrast, *in vivo* delivery introduces CRISPR components directly into the patient using viral vectors, lipid nanoparticles, or other carriers (128). This strategy has the potential to target solid tumors and inaccessible tissues without the need for cell extraction, yet it faces challenges in achieving tissue-specific targeting, avoiding immune clearance, and ensuring long-term safety. Both approaches are complementary: *ex vivo* delivery offers near-term clinical feasibility, whereas in vivo strategies hold promise for broader, more scalable applications in oncology (129-131).

Note: A detailed discussion of genomic stability, off-target profiling, and biosafety aspects of CRISPR-Cas9 applications in breast cancer is provided in Supplementary Note 3.

From safety to legitimacy: Ethical and regulatory barriers to CRISPR in breast cancer

Even as technical advances address safety risks (see Section 4.2), ethical and regulatory oversight remains the second major bottleneck for translating CRISPR into breast cancer therapy. The ethical debate is dominated by the distinction between somatic editing, which alters only tumor or patient somatic cells, and germline editing, which creates heritable modifications. Somatic interventions, such as those under investigation in breast cancer clinical trials, are generally viewed as acceptable because they do not extend beyond the treated individual (132, 133). By contrast, germline editing raises profound concerns regarding intergenerational consent, the unpredictability of long-term outcomes, and the risk of misuse for genetic enhancement, as highlighted by the controversial embryo-editing case in China that drew global condemnation (134). A second challenge is the fragmented regulatory landscape. Some countries, such as the United States and the United Kingdom, permit carefully monitored somatic CRISPR trials but ban germline applications, while others lack clear enforcement mechanisms (135, 136). This patchwork of rules hampers the development of unified global guidelines for safety, efficacy, and ethical conduct. Breast cancer–specific trials also pose unique regulatory dilemmas, as repeated biopsies and prolonged monitoring increase the burden of informed consent and patient trust compared to blood cancers.

To address these gaps, the World Health Organization (WHO) issued comprehensive guidelines in 2021 on human genome editing. These recommendations emphasize global governance, transparency, equity, and rigorous ethical oversight. The guidelines support somatic genome editing, such as its application in cancer therapy, when conducted under strict regulatory frameworks, while affirming that heritable (germline) genome editing remains ethically unacceptable. By aligning emerging CRISPR-based interventions with these international standards, researchers and clinicians can promote responsible translation while safeguarding patient rights and societal trust (42).

To move forward, international consensus frameworks, transparent oversight, and stronger patient engagement are essential. Novel approaches, including AI-enabled monitoring of trial participants, global registries of CRISPR interventions, and equitable access policies, could provide the governance backbone needed to responsibly integrate CRISPR into precision breast cancer oncology (Table 3).

Bridging the lab-to-clinic divide

With only a few early-phase trials underway, the transition

Table 3. Global regulatory stance on CRISPR genome editing
Overview of current guidance for somatic versus germline applications and required long-term safety monitoring

Agency	Somatic Editing	Germline Editing	Long-Term Follow-up	Reference
FDA (U.S.)	Permitted under the Investigational New Drug (IND) gene-therapy framework with strict clinical oversight	Prohibited for reproductive use; embryo-editing applications not accepted	≥15 years post-treatment safety surveillance and patient registry reporting	(137)
EMA (EU)	Regulated as an Advanced Therapy Medicinal Product (ATMP) with risk-based pharmacovigilance	Prohibited under the Council of Europe's Oviedo Convention and EU member-state laws	≥15 years of follow-up and periodic risk benefit reassessment	(138)
MHRA (UK)	Allowed under Clinical Trial Authorization for somatic gene-therapy trials	Illegal for reproductive purposes under the Human Fertilization and Embryology Act 2008	≈15 years of monitoring aligned with EMA/FDA guidance	(139)
WHO	Supports carefully regulated somatic trials and maintains a global human-genome-editing registry	Calls for an international governance framework and moratorium on clinical germline editing	Recommends 10 15 years of safety follow-up for *in vivo* genome-editing studies	(12)

of CRISPR-based treatments from the laboratory to clinical application in breast cancer remains in its early stages (140). This cautious pace results from significant translational challenges that complicate the development and approval of new medicines. Tumor heterogeneity is a major barrier since breast cancer has several subtypes, including *HER2*-positive, ER/PR-positive, and triple-negative, each of which has distinct molecular traits that affect how well gene-editing treatments work (141). Breast cancer's variability makes it difficult to design universal CRISPR therapies and makes it difficult to choose the right patients for clinical trials. Immune responses to Cas9 and other CRISPR components further reduce efficacy and pose safety issues. Furthermore, discrepancies exist between laboratory and clinical results because current animal models do not accurately replicate the human tumor microenvironment. These problems highlight the pressing need for better delivery methods, more precise prediction models, and thorough safety assessments, in addition to concerns regarding off-target impacts and delivery challenges (142, 143). To fully realize the potential of CRISPR-driven tailored therapeutics for breast cancer, these obstacles must be removed (Table 4) (144).

Future perspectives and challenges

Intelligent CRISPR-based drug delivery systems

Future perspective

Will likely focus on clever, tumor-responsive delivery mechanisms to tackle the significant problem of off-target consequences in CRISPR-Cas9 treatments. Examples include ligand-targeted nanoparticles, pH-sensitive liposomes, and exosome-like vesicles designed to release CRISPR machinery only in the tumor microenvironment (163). Through integrated molecular sensors, advances may enable real-time CRISPR activation in response to cancer-specific signals, such as HER2 overexpression, hypoxic conditions, or enzyme activities, such as *MMPs*. By combining these technologies with implantable or wearable biosensors, genome editing could be precisely and on-demand controlled in both time and space, enabling highly customized and adaptable CRISPR-based cancer treatments (164, 165).

Challenges

Effectively distributing CRISPR components while avoiding immune clearance remains a significant problem, despite encouraging advancements. While non-viral

Table 4. Major translational challenges for CRISPR-based breast cancer therapies, with proposed solutions, their advantages/disadvantages, breast cancer relevance, and supporting evidence

Challenge	Proposed Solution	Advantages	Disadvantages	Breast Cancer Relevance	Experimental/Clinical Evidence	References
Off-Target Effects	CRISPRroots and other predictive algorithms, as well as high-fidelity Cas9	Enhances safety and reduces unwanted mutations	Restricted by computational bias and genomic variability	Crucial for TNBC precise editing and HER2+	Low off-target rates were found by CAST-seq in modified T47D cells.	(40, 145-147)
Delivery to Solid Tumors	Exosomes, nanoparticles,	Reduced immunogenicity and increased tumor penetration	Low effectiveness and problems with scaling	vital for TNBC solid tumors and HER2+	Delivery of exosomes enhanced CRISPR targeting *in vivo*.	(148-152)
Cas9 Immunogenicity	Removal of immunodominant epitopes and temporary Cas9 expression	enhances T-cell survival and decreases immunological clearance	Reduced editing time and intricate engineering	affects the effectiveness of CAR-T in breast cancer	decreased immunogenicity in models of CAR-T	(153-155)
Tumor Heterogeneity	AI-driven genomics and patient-derived organoids	More specificity and tailored targeting	Expensive and not very scalable	deals with subtype variability (e.g., HR+, TNBC)	CRISPR effectiveness was anticipated by organoid models	(156-160)
Ethical/Regulatory Hurdles	International regulatory structures and public participation	ensures security and fosters confidence	Clinical translation is slowed down by fragmented standards	essential for breast cancer somatic editing	Ethics did not stop any experiments (continuing discussions)	(161, 162)

carriers, such as lipid nanoparticles, struggle with stability and effective intracellular release, viral vectors, such as AAV, are hampered by limited cargo capacity and immunological responses. Clinical translation also depends on making sure these advanced delivery systems are scalable for production and biocompatible in the long run. Furthermore, there are still unknown regulatory frameworks for sensor-activated, programmable gene-editing technologies, which pose additional obstacles to their acceptance and broad application.

Immune cell reprogramming for resistant breast cancer subtypes

Future perspective

As it lacks progesterone and estrogen receptors, TNBC is particularly aggressive and difficult to treat. CRISPR-based immune engineering is a state-of-the-art method in which CAR-T cells or tumor-infiltrating lymphocytes (TILs) are genetically modified to increase tumor targeting, resist fatigue, and generate immune-boosting cytokines. Through the installation of synthetic receptor pathways or the deactivation of immune checkpoint genes such as *PD-1*, *CTLA-4*, or *LAG-3*, CRISPR can transform immunologically "cold" TNBC tumors into "hot" ones, rekindling the body's immune response. Furthermore, immunological memory may be remodeled by combining CRISPR with RNA-guided epigenetic editing, which might promote robust and long-lasting anti-cancer immunity (166).

Challenges

The main obstacle is the possibility of long-term immunological imbalance and off-tumor damage. Multiplex CRISPR editing increases the risk of chromosomal abnormalities, such as large deletions or translocations, which may drive malignant transformation of immune cells. Furthermore, even the most advanced immune cells may be less effective due to the suppressive tumor microenvironment of TNBC, which is characterized by abundant stromal barriers and myeloid-derived suppressor cells. Beyond biological barriers, the complexity and cost of developing customized immune therapies pose significant challenges to their scalability and broad clinical use.

Genome-wide synthetic lethality screens for personalized therapies

Future perspective

Systematic identification of breast cancer cell vulnerabilities that genomic sequencing alone could overlook by CRISPR-driven genome-wide knockdown or activation screening. These screens find extremely precise targets that are perfect for creating tailored medicines by taking advantage of synthetic lethality, which is the premise that losing two genes at once results in cell death while losing one gene alone does not (167).

This tactic is particularly effective for tumors lacking BRCA1/2, as CRISPR screening can identify alternative routes that cancer cells use. Prospects for precision therapy are bright thanks to tailored medicines that combine medications with CRISPR interventions, led by dynamic tumor genomic profiling and functional screening employing patient-derived organoids or *in vivo* xenografts (168).

Challenges

Although CRISPR screenings provide valuable insights, their results are often context-dependent and may not be readily generalizable across patient populations. It requires sophisticated bioinformatics and the development of machine learning algorithms to analyze functional relationships (169, 170). Furthermore, it takes a lot of work to validate these targets in clinically relevant models, and many promising candidates fail in translation due to tumor heterogeneity and the development of resistance in cancer (171).

AI-organoid integration for personalized CRISPR therapy

The combination of CRISPR, patient-derived organoids (PDOs), and artificial intelligence (AI) is becoming a potent force in precision oncology. PDOs are now essential preclinical platforms, especially for difficult-to-treat breast cancer subtypes like TNBC and *HER2*+, because they mimic the structural, molecular, and genetic complexity of their source tumors, including intertumoral heterogeneity (172).

Recent AI developments enable more precise design of CRISPR guides by integrating genome-wide factors such as chromatin accessibility, sequence context, and off-target likelihood, thereby surpassing earlier rule-based methods. New machine learning models further enhance accuracy in predicting both editing efficiency and off-target risk (173).

Uniting AI-guided CRISPR design with PDO phenotyping creates a powerful feedback loop:

1. AI proposes gRNAs predicted to have high specificity and efficiency.
2. These CRISPR edits are applied to patient-derived breast organoids.
3. Organoid responses ranging from growth metrics to genomic stability and drug sensitivity are captured via imaging and multi-omics.
4. AI then refines its predictions based on empirical outcomes.

CRISPR methods tailored to TNBC and *HER2*+ cancers are enabled by the subtype-specific insights obtained through this iterative approach. Zhu *et al.* showed the effectiveness of PDO-CRISPR screening in a variety of cancer types, emphasizing its value for identifying targets and developing immunotherapies (173). Similarly, Cheng *et al.* reviewed how integrating PDOs with CRISPR has uncovered mechanisms of tumor response within relevant architectures (174). Yet progress is uneven across cancer types. CRISPR-edited immune cells are already advancing in clinical trials for hematological malignancies, whereas solid tumors such as breast cancer lag.

While CRISPR-edited immune cells are making headway in clinical trials for blood malignancies, solid tumors like breast cancer are still lagging due to obstacles such as high intertumoral heterogeneity, uneven vasculature, and thick stroma. Before beginning *in vivo* research, AI-organoid systems offer a practical approach by enabling preclinical assessment of delivery efficacy, safety, and subtype-specific responses. This integration serves as much more than just a conceptual framework; it is a translational bridge that advances CRISPR toward therapeutically feasible, patient-specific treatments for breast cancer by fusing the biological integrity of PDOs with AI's predictive analytics.

Predictive parameters of the AI–organoid–CRISPR feedback loop and a validation framework

Despite the transformative impact of PDOs and AI on functional modeling in precision oncology, their predictive capabilities are constrained by biological and computational limitations that reflect the disease's complexity. PDOs, although they maintain patient-specific genotypes and epithelial structures, are only a limited representation of the local tumor ecology. They lack the complete immunological, vascular, and stromal networks that regulate tumor-host interactions *in vivo*. The lack of adaptive immunity, hypoxic gradients, and fluctuating cytokine levels limits their ability to replicate immune surveillance, angiogenesis, and metastatic colonization. Temporal fidelity diminishes with successive passages: clonal drift, metabolic reconfiguration, and epigenetic degradation progressively alter the initial tumor identity, resulting in model aging that exaggerates perceived treatment effects. Thus, organoid-based "successes" may exaggerate clinical translatability until corroborated in systems that include these absent ecological layers (175). In computational terms, even the most advanced deep-learning frameworks face what we term dynamic ground-truth drift, a discrepancy between static training datasets and the changing biological reality of tumors. Models trained on single-time-point omics or imaging data are unable to capture the nonlinear trajectories of resistance, immune evasion, and dormancy reactivation. Furthermore, transfer learning across diverse cohorts results in domain shift, whereas opaque neural architectures hinder causal interpretations of predictions. Calibration, be it probabilistic (Brier score, predicted calibration error) or biological (congruence with longitudinal patient data), is seldom measured, resulting in unacknowledged overconfidence and erroneous target identification (176). We therefore reconceptualize the AI organoid CRISPR loop not as a direct predictor of therapeutic success, but as an adaptive hypothesis generator whose results need systematic verification. To implement this idea, we offer a multi-level validation pipeline:

1. Ecological triangulation: verify top hits across organoid–immune co-cultures (tumor+CAF/TAM/T-cell), organ-on-chip systems reproducing perfusion and shear stress, short-term explants, and PDXs. This restores missing immune stromal interactions and drug-penetration barriers (177).

2. Temporal robustness testing: expose PDOs to sequential therapy cycles and track molecular evolution over ≥6 weeks to evaluate whether AI predictions remain stable under evolutionary pressure (178).

3. Pre-registered, blinded experimentation: fix analytic pipelines a priori, randomize image-omics phenotyping, and reserve external PDO/PDX test sets to measure true generalization (172).

4. Quantitative AI transparency: report AUROC/PR-AUC with 95 % CIs, calibration slope/intercept, and decision-curve gains; include permutation, ablation, and "impossible-edit" controls to estimate baseline noise (179).

5. Orthogonal genomic safety auditing: pair each phenotypic win with CAST-seq and long-read panels to rule out cryptic rearrangements; confirm low off-target burden via targeted deep-seq of predicted loci (180).

6. Prospective "digital-twin" pilots: Integrate longitudinal liquid-biopsy profiles and serial imaging datasets to evaluate whether the AI-organoid framework can forecast patient-specific resistance dynamics, thereby synchronizing computational predictions with the real-time biological evolution of tumors.

7. Acceptance thresholds: advance only candidates showing ≥20 % effect-size replication across ≥2 orthogonal models, external-test AUROC ≥0.75, calibration slope 0.8-1.2, and directional concordance in PDO, co-culture, and PDX systems (176, 181-183).

By explicitly acknowledging that neither PDOs nor current AI frameworks can yet reconstruct the full, multicellular, and temporal complexity of the tumor microenvironment, this roadmap converts those weaknesses into testable parameters. The framework shifts emphasis from prediction to verification, a necessary evolution for AI-guided CRISPR therapeutics to achieve credible translational relevance (Table S4)(181).

AI-enhanced sgRNA and Cas variant design

In CRISPR editing, selecting guide RNAs (gRNAs) that are both highly effective and rarely prone to off-target effects remains a major challenge. When access to target loci was restricted by chromatin state or three-dimensional genomic architecture, early rule-based scoring systems often failed because they simply took into account basic sequence properties (184). To forecast editing effectiveness and off-target risk with almost single-base precision, recent developments in deep learning have integrated genome-wide datasets such as chromatin accessibility (ATAC-seq), DNA methylation, nucleosome location, and Hi-C chromatin-interaction maps (185). Complex relationships between sequence context and editing results are learned using transformer-based designs, including hybrid convolutional-recurrent neural networks and the next-generation DeepCRISPR2. These models can quickly generate ranked gRNA lists for each breast cancer genome, as they are trained on millions of experimentally validated changes detected by assays such as GUIDE-seq, CAST-seq, and rhAmpSeq. Reinforcement learning is being used in parallel attempts to create innovative high-fidelity Cas variants, such as improved eSpCas9 and Cas12b (186), that minimize chromosomal translocations or massive deletions while maintaining cutting efficiency. Crucially, these AI tools continually improve as they incorporate data from new tests, generating a positive feedback loop in which one editing campaign enhances the ability to forecast the next. AI-driven gRNA and Cas variant creation, in conjunction with patient-specific tumor sequencing, enables highly customized CRISPR tactics that are adapted to the molecular characteristics of a particular breast cancer (187).

AI-optimized delivery systems

A recurring challenge is the effective delivery of CRISPR components to solid tumors, especially dense, heterogeneous breast malignancies like TNBC. The fibrotic stroma or hypoxic niches that harbor resistant tumor clones are often inaccessible to conventional *LNP or viral vector delivery*. By using Bayesian optimization and generative adversarial networks to create *LNPs*, polymeric micelles, and exosome-mimetic carriers, AI-driven materials discovery platforms are now able to overcome this obstacle (188). These algorithms generate hundreds of candidate formulations in silico and predict their pharmacokinetics and tumor-

penetration profiles, while concurrently accounting for particle size, surface charge, ligand density, and endosomal escape efficiency. Through the use of experimental readouts, such as intertumoral dispersion as determined by sophisticated imaging, reinforcement-learning loops repeatedly improve nanoparticle design until delivery efficiency approaches predetermined thresholds. AI-guided LNPs more than double the gene-editing rates in orthotopic TNBC xenografts when compared to empirically generated controls, according to early proof-of-concept experiments (189). AI systems may also predict tumor-microenvironment triggers, such as pH, hypoxia, and receptor overexpression (e.g., *EGFR* or *HER2*), in addition to physical qualities. This enables "logic-gated" carriers that release CRISPR cargo only in response to multiple tumor-specific inputs. This accuracy lowers systemic toxicity by limiting exposure to healthy tissues and increasing on-target editing. AI is establishing the groundwork for active delivery vehicles, programmable elements of the therapeutic approach, rather than just carriers, by combining computational materials science with CRISPR biology.

Real-time adaptive editing via liquid biopsy

Breast cancers are constantly changing, and after the first therapy, resistant subclones often appear. This dynamic environment is too fast for static, one-time CRISPR treatments. A "closed-loop" editing technique, where therapeutic design changes in real time, is made possible by AI. ctDNA and exosomal RNA, which represent the changing tumor genome, are obtained from longitudinal liquid biopsies. To find early indications of clonal escape or recently acquired driver mutations (190), machine-learning algorithms examine these data streams in conjunction with digital pathology pictures and serial single-cell RNA sequencing. Subsequently, current gRNAs are reranked using Bayesian forecasting and graph-based neural networks, or new guide sets targeting emerging resistance nodes are proposed (191). Such adaptive algorithms have been effective in predicting secondary *ESR1* and *PIK3CA* mutations in experimental settings weeks before they become prevalent in xenograft models. As the cancer mutates, therapeutic pressure may be maintained by quickly synthesizing updated gRNA pools and delivering them via programmable nanoparticles. Like adaptive cancer immunotherapy, this paradigm transforms CRISPR therapy from a one-time genetic intervention into a dynamic, self-updating treatment. AI-driven real-time editing may prove to be a game-changer for breast cancer, since intertumoral heterogeneity and fast evolution are key factors in recurrence (192).

AI-based multi-omics integration for target discovery

Target finding techniques that go beyond single-omic datasets are necessary due to the remarkable molecular diversity of breast cancer. Complex data layers, such as transcriptomics, proteomics, metabolomics, epigenomics, and genomics, collected from CRISPR-edited PDOs are seamlessly integrated by AI. Attention-based multimodal transformers, variational autoencoders, and graph neural networks may reveal synthetic-lethality networks and hidden route relationships that are not accessible to conventional statistical methods (193). For instance, cross-talk between PI3K/AKT signaling and homologous-recombination repair has been discovered using AI models that combine spatial transcriptomics with chromatin accessibility maps, exposing vulnerabilities specific to TNBC that is *BRCA1/2*-deficient. A rapid, iterative cycle of hypothesis generation and experimental validation can be established by immediately validating these predictions in the same PDO cultures (194). Furthermore, by separating genuine driver mutations from passenger events, AI-driven causal inference techniques reduce spurious leads, which often disrupt drug development workflows. The end product is a potent discovery engine that connects biological function and computational prediction, directing multiplex CRISPR methods that may destroy whole oncogenic networks instead of individual genes. The precision and generalizability of these models will continue to improve as more breast cancer PDO datasets become available, thereby supporting functional polygenic risk assessment and patient-specific treatment plans (195).

CRISPR multiplexing to reduce the risk of polygenic breast cancer

Future perspective

In contrast to monogenic illnesses, polygenic interactions in which dozens of variations with low to moderate effects converge on DNA repair, hormone signaling, chromatin remodeling, and immune evasion pathways often influence breast cancer risk and treatment resistance. This intricacy cannot be adequately addressed by conventional single-gene editing techniques. Multiple genomic regions can be perturbed simultaneously by CRISPR multiplexing, enabled by orthogonal Cas variants, barcoding techniques, and pooled guide RNA libraries (196). This makes it possible to map higher-order genetic relationships and find synthetic lethality at the network level that single-gene models are unable to disclose. Combinatorial targeting of *TP53*, *BRCA1/2*, *PIK3CA*, and *ESR1* mutations, for example, has been shown to reveal hidden dependencies in *PI3K-AKT* signaling and homologous recombination repair, providing new opportunities for subtype-specific treatments. Multiplex CRISPR may eventually enable polygenic risk assessment at a functional level, going beyond therapeutic modeling. In this scenario, high-risk individuals with unfavorable PRS profiles are examined in organoid or in vivo systems to identify strategies for prevention or intervention. Combining multiplex editing with single-cell multi-omics and AI-driven network inference may revolutionize the way we identify, simulate, and treat polygenic structures in breast cancer (197).

Challenges:

Multiplex editing presents a difficult translational road. The likelihood of chromosomal translocations, megabase-scale deletions, and disastrous genomic rearrangements is significantly increased when many double-strand breaks are generated. There is a cumulative risk of off-target mutations and mosaicism, even with sophisticated approaches such as base editing and prime editing multiplexing. Because certain targets are preferentially edited while others are left unaltered, resulting in unanticipated genomic mosaics, maintaining balanced editing efficiency across several loci is a significant challenge. Clinical-scale multiplexing has not yet been optimized for delivery methods that can transport huge guide pools, whether they are based on

exosomes, nanoparticles, or viruses. Crucially, it remains difficult to distinguish between driving variants and benign polymorphisms in a polygenic environment; excessive editing may yield unnecessary or detrimental changes. Lastly, the ethical aspect is amplified: multiplex techniques need global governance and next-generation bioethical frameworks before they can proceed to clinical trials, blurring the line between therapeutic intervention and augmentation (198).

Discussion

The true frontier of CRISPR in breast cancer lies not in gene repair but in system-level reprogramming of tumor biology, where editing functions as a form of ecological, temporal, and adaptive control. Beyond silencing *HER2* or restoring *TP53*, multiplex CRISPR could deliberately reshape evolutionary trajectories, forcing tumors into "genetic cul-de-sacs" by collapsing redundant signaling networks or disabling stress-response hubs like *NRF2*, thereby exhausting the tumor's adaptive capacity. Equally disruptive is the concept of chrono-genomic editing, in which circadian oscillators are reset via CRISPR modulation of REV-ERBα, BMAL1, or CRY2 to synchronize tumor vulnerability with therapeutic chronotherapy, thereby turning genome editing into a temporal switch that dictates when breast cancer cells are most sensitive to therapy. Dormancy, the hidden engine of metastasis, can be interrogated through CRISPR-enabled synthetic lethality screens within organoid or "sleeping tumor" models to expose dormancy-exclusive vulnerabilities genes dispensable in proliferative states but essential in quiescence, thereby allowing precise eradication of relapse-seeding cells. Delivery must also evolve from passive carriers to intelligent nanodevices, such as exosome mimetics programmed with Boolean logic gates that release CRISPR cargo only in the presence of multifactorial tumor cues (hypoxia + acidosis + HER2 overexpression), thereby transforming delivery into a bio-computational therapy. Strikingly, what was once considered a danger, CRISPR-induced large-scale chromosomal damage, could be co-opted as a genomic collapse strategy, selectively triggering catastrophic rearrangements in *BRCA1/2*-deficient or repair-impaired clones that cannot survive genomic overload. Finally, by embedding CRISPR design within AI-driven adaptive frameworks that analyze longitudinal ctDNA methylomes, single-cell spatial transcriptomics, and immuno-metabolic shifts, guide RNAs can be iteratively reprogrammed to track and neutralize emerging resistance clones in real time, making CRISPR not a static molecular scalpel but a self-learning, co-evolving therapeutic ecosystem capable of orchestrating breast cancer control across genetic, temporal, and ecological dimensions (Table S5).

Limitations

This review is based on a comprehensive search of PubMed, Scopus, and ClinicalTrials.gov, restricted to English-language publications, and was completed on 12 September 2025. The emphasis was on peer-reviewed studies, but carefully vetted high-quality preprints and early-release data were incorporated when they offered timely or novel insights of direct relevance to CRISPR applications in breast cancer (199, 200). From a field perspective, important challenges remain. The long-term stability of *in vivo* edits remains unproven, with follow-up studies reporting a gradual loss of edited alleles. Single-cell and spatial multi-omics consistently reveal marked intertumoral diversity, allowing clonal escape even after multiplex editing. Advanced detection methods such as CAST-seq and long-read sequencing continue to uncover rare but clinically significant mega base-scale deletions and translocations at intended cut sites, underscoring the potential for on-target genomic instability. Pre-existing humoral and cellular immune responses to Cas nucleases and persistently low editing efficiencies in solid-tumor models further highlight the need for next-generation strategies, including high-fidelity or epitope-silent nucleases, patient-specific off-target mapping, transient self-degrading RNP delivery, and adaptive AI-driven guide-RNA design. Together, these considerations define both the methodological boundaries of this review and the key scientific hurdles that must be overcome to achieve safe, durable, and clinically meaningful CRISPR-based therapies for breast cancer.

Conclusion

The emergence of CRISPR-Cas systems has redefined the therapeutic landscape of breast cancer, offering tools that extend beyond conventional single-gene targeting to a systems-level capacity for reprogramming tumor biology. Unlike earlier technologies, CRISPR provides flexible avenues to disrupt oncogenic pathways, restore tumor suppressor activity, and remodel the tumor–immune interface, thereby addressing the heterogeneity that defines breast cancer subtypes. Complementary innovations such as base and prime editing, which reduce double-strand break–associated risks, and nanoparticle- or exosome-mediated delivery strategies, which improve precision and reduce immunogenicity, strengthen the translational trajectory of these tools.

Despite these advances, it is important to note that nearly all current data remain preclinical and exploratory, and no durable clinical benefit has yet been demonstrated. Delivery efficiency in solid tumors, long-term safety of multiplex editing, and context-specific off-target risks must be addressed before clinical deployment. Equally important are ethical and governance concerns, which demand harmonized global standards to ensure that the technology progresses responsibly. These limitations underscore that the promise of CRISPR is not guaranteed by technical power alone but by a deliberate roadmap that integrates biological, technological, and regulatory solutions.

One critical future direction lies in the integration of CRISPR with patient-derived organoid (PDO) platforms. PDOs preserve the genetic, structural, and microenvironmental complexity of patient tumors, providing an ideal testbed for subtype-specific genome editing strategies. By combining CRISPR perturbations with organoid phenotyping, researchers can functionally validate candidate targets and predict therapeutic responses with unprecedented accuracy. When coupled with AI-driven analytics, this framework becomes even more powerful. Machine learning models trained on genomic, transcriptomic, and phenotypic data from CRISPR-edited PDOs can iteratively refine guide design, predict off-target risks, and prioritize the most effective interventions. This AI organoid–CRISPR triad establishes a self-correcting, patient-centered discovery pipeline that moves beyond

abstract precision oncology to tangible, clinically relevant hypotheses.

Equally transformative is CRISPR multiplexing's potential to interrogate polygenic risk architectures underlying breast cancer susceptibility and resistance. By targeting multiple cooperating mutations simultaneously and analyzing outcomes in AI-integrated organoid systems, researchers may uncover network-level vulnerabilities inaccessible to single-gene models. These strategies, while highly promising for discovery, remain at the proof-of-concept stage and should be viewed as research directions rather than established therapies.

In sum, the future of CRISPR in breast cancer lies in integration rather than isolation. Its true impact will emerge from linking cutting-edge editing platforms with organoid models, computational intelligence, and ethically grounded clinical translation. Realizing this potential will require careful stepwise validation and long-term safety data before any claims of clinical efficacy can be made. By following this roadmap, CRISPR has the potential to convert breast cancer from a multifaceted clinical challenge into a disease managed through adaptive, individualized precision oncology anchored in evidence, guided by AI, and validated in patient-derived systems.

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Authors' Contributions

AN T conceived the review, supervised the project, and provided critical revisions. A S contributed to the manuscript design and guided the methodological framework. V A provided expert input on clinical translation and edited key therapeutic sections. R O and SR J conducted the comprehensive literature search, extracted data, and drafted major sections of the manuscript. US and SS curated figures, prepared tables, and assisted with data interpretation. AS C refined the manuscript structure and ensured scientific accuracy of mechanistic and translational discussions. G K and HN S managed reference curation, formatting, and integration of recent clinical trial data. A Y and ZH A supported writing, editing, and visualization of complex CRISPR workflows. M A performed the final proofreading and ensured compliance with the journal's guidelines. All authors reviewed and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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