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Next-generation gene editing strategies in cancer: Integrating CRISPR, PROTACs, and advanced molecular technologies

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ABSTRACT

Cancer is a significant therapeutic problem as tumors are heterogeneous, multidrug-resistant, and oncogenic drivers are undruggable. Genome editing and targeted protein degradation are emerging approaches that are transforming precision oncology by allowing genetic and proteomic interventions. Such technologies as CRISPR-based systems and proteolysis-targeting chimeras (PROTACs) are alternative methods of correcting genes and the selective removal of a protein. Their combination provides them with new possibilities regarding long-lasting and specific cancer treatment and discovery of new therapeutic targets. Although this has good news, there are delivery, off-target effects and safety challenges. The continued advancements in nanotechnology, artificial intelligence (AI), and personalized medicine will be likely to improve clinical translation. Generally, the integration of these technologies is an inducing trend in the treatment of cancer in the next generation.

1. Introduction

Cancer is a complex and heterogeneous disease that is marked by the uncontrolled proliferation of cells, genetic abnormalities, and disruption [3,4]. Although better treatment methods have been developed, including chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the effectiveness of treatment is frequently limited due to challenges such as tumor heterogeneity, multidrug resistance, immune evasion, and off-target toxicity [5]. Traditional methods often focus on a single molecular pathway, which may result in adaptive resistance mechanisms and a narrow scope of effect [6]. Moreover, several oncogenic drivers are considered intractable with conventional small molecules or antibodies, and substantial gaps remain in therapeutic options. All these complications underscore the need to develop innovative approaches that have the potential to directly target cancer at its genetic and molecular origins [7]. Recent discoveries in molecular biology and biotechnology have established genetic manipulation as an effective paradigm in treating cancer [8]. Initially, gene editing technologies, including CRISPR-Cas systems [1], TALENs, zinc-finger nucleases (ZFNs), and base editing, as well as prime editing, have the capacity to increase precision in the targeting of oncogenes and tumor suppressor genes with precision never before attained [9]. At the protein level, PROTACs (PROteolysis-TArgeting Chimeras) and other targeted protein degradation techniques open new possibilities by eliminating disease-causing proteins rather than merely inhibiting them. Together, these technologies allow researchers to precisely explore and regulate cancer biology, offering the ability to rectify mutations, rewire signaling networks, and selectively remove malignant drivers [2,10]. Together with innovative delivery technologies and synergistic therapeutic modalities - through immunotherapy, nanomedicine, and computational design - gene editing, and protein degradation technologies have established about a novel change to next-generation precision oncology. This review synthesizes recent high-impact findings on integrating CRIPSR, PROTACs, and advanced molecular technologies, focussing on mechanistic insights, clinical translation, and therapeutics limitations. It clearly evaluates efficacy, safety, and emerging trends, while highlighting key challenges and future directions for clinical application.

2. Conceptual framework for targeted interventions in oncology

New technologies in oncology have recently emerged through the creation of a wide range of technologies to combat cancer at various biological levels, such as DNA, RNA and protein [11]. Nonetheless, the classification based on the technology alone can hide the logic behind

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the therapy. To offer a more combined viewpoint, these strategies can be theorized in accordance with the biological layer of intervention and their translational preparedness [12]. Genome editing tools including CRISPR-Cas systems, base and prime editors seek to modify oncogenic mutations or regenerate tumor suppressor activity at the DNA level directly. These methods have proven to be highly effective in the preclinical setting, but their use in the clinical setting remains mostly confined to ex vivo methods because of delivery, specificity, and safety concerns in the long term [13].

On the RNA level, RNA-targeting technologies, such as RNA interference (RNAi), antisense oligonucleotides, and CRISPR-based RNA editors, provide a reversible and, possibly, safer method of regulating gene expression [14]. The recent progress in chemically modified oligonucleotides and lipid nanoparticles delivery technologies has facilitated early clinical translation, especially in the delivery of oncogenic transcripts and splice variants [15]. At the protein level, targeted protein degradation approaches like PROTACs and molecular glues represent a breakthrough in that they allow disease-driving proteins, even those that have been thought to be undruggable, to be degraded [16]. Notably, the maturity of translation is also differentiated by these approaches. Although other modalities like RNA-based therapeutics and some protein degraders are progressing to clinical development, others are at the preclinical or early translational phase. At every level, potential cellular barriers, such as efficiency of delivery, tumor heterogeneity, immune responses and resistance mechanisms, still play critical roles to determine the outcome of treatment [17]. This stratified structure does not only elucidate the interactions between emerging technologies, but also emphasizes how they will all provide solutions to underlying issues in cancer therapy, thus giving a more consistent and clinically meaningful approach.

3. Overview of gene editing technologies in cancer

3.1. Traditional vs. advanced molecular tools

The therapeutics of cancer have taken a significant turn, and the old cytotoxic approach has been substituted with a newer, focused molecular approach [18]. Traditional modalities, including chemotherapy and radiotherapy, are based on general cytotoxic effects affecting malignant and normal cells with little selectivity and serious side effects [19]. On the same note, the original generation of targeted therapeutic strategies, such as small molecule kinase inhibitors and Companion Diagnostics (CDx), were more specific, but often subject to resistance development, limited to a small number of druggable target types, and incomplete killing of the tumor [20]. Conversely, extremely complex molecular platforms are oriented toward controlling the genetic and proteomic basis of cancer [21]. CRISPR-Cas9, TALENs, and ZFNs are technology platforms with the capability to be designed to hit oncogenes and tumor suppressors, enabling researchers to knock out, repair, or reprogram vital human genetic determinants of malignancy [22]. Moreover, recent technologies, such as base editors and prime editors, provide an even higher level of precision, enabling the editing of a single nucleotide without causing a double-strand break and, therefore, a minimal level of genomic instability [23]. Protein-based technologies such as PROTACs, molecular glues, and LYTACs are used to precisely destabilize oncogenic proteins that have been considered undruggable, in addition to nucleic acid editing [24]. These innovative methods used together exceed inhibition to deliver total elimination or functional recovery of pathogenic drivers on the DNA, RNA, or protein level.

3.2. Advantages over conventional chemotherapy and targeted drugs

Compared to both conventional chemotherapeutics and even most targeted therapies, there are a variety of benefits of gene editing technologies (Table 1). To start with, they offer an unprecedented level of accuracy, which allows altering certain mutations or regulatory sequences without damaging normal tissues. This decreases off-target toxicity, which is a significant constraint of traditional therapies [25]. Second, previously inaccessible oncogenes and tumor suppressors can be edited with gene editing, and this has provided alternative therapies to mutations with no small-molecule inhibitors [26]. Third, genome editing can provide sustained effects or potentially cure genetic defects, as this method, unlike chemotherapy, does not require repeated treatment cycles [27]. Also, more complicated editing and degradation methods can be combined with immunotherapy, like making T cells immune to exhaustion by editing with CRISPR or immunotherapy modulating immune checkpoints with PROTACs. These instruments also present the benefits of the research; high-throughput CRISPR screens can be used to determine new cancer vulnerabilities and improve the druggable targets [28]. Lastly, these technologies can be described as modular and programmable, which makes the process of personalized medicine faster, as treatments can be designed to match the individual's mutational profile [29].

4. CRISPR-Cas systems

4.1. Mechanism and variants

CRISPR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated proteins) has become the new genome engineering programmable site-specific DNA or RNA editing with an RNA-guided nuclease platform [37]. Cas9 is the most commonly used nuclease that creates double-stranded breaks (DSBs) at designated loci in the genome, which are then repaired via homology-directed repair (HDR) or non-homologous end joining (NHEJ), and thus allowing targeted gene knockout or repair [38]. Cas9 is the most widely used CRISPR-associated nuclease for genome editing, guided by a double-stranded RNA (sgRNA) to target specific DNA sequences adjacent to a PAM motif (5′-NGG-3′). It introduces double-stranded breaks (DBSs), which are repaired via non-homologous end joining (NHEJ) for gene knocked or homology-directed repair (HDR) for precise gene correction. Cas9 has been extensively applied in cancer research for disrupting

Table 1
Comparison of gene editing platforms in cancer.

Platforms	Mechanism	Applications in cancer	Advantages	Limitations	Ref.
CRISPR-Cas9	DNA DSB repaired by NHEJ or HDR	Knockout of oncogenes, repair of mutations	High efficiency, easy design	Off-target effects, delivery issues	[30]
Cas12	Single-stranded DNA cleavage	Diagnostic and therapeutic editing	High specificity	Less studied in vivo	[31]
Cas13	RNA cleavage	Silencing oncogenic fusion transcripts	Transcriptome editing, reversible	Delivery, transient effect	[32]
ZFNs	Protein-DNA binding with FokI nuclease	Oncogene disruption	Precise targeting	Complex design	[33]
TALENs	TALE protein-DNA recognition + FokI nuclease	Gene disruption in cancer models	Specific, flexible	Labor-intensive design	[34]
Base Editors	Direct nucleotide conversion (C → T, A → G)	Correction of point mutations	No DSBs	Limited to certain conversions	[35]
Prime Editors	Reverse transcriptase + guide RNA for insertions/deletions	Precise correction of mutations	Versatile editing	Delivery challenges	[36]

oncogenes, restoring tumor suppressor genes, and engineering CAR-T cells. However, concerns regarding off-target effects and genome instability due to DBSs remain significant challenges [39].

Outside of Cas9, Cas12 expands the zygote editing by cutting single-stranded DNA with specific Protospacer Adjacent Motifs (PAM) recognition patterns, and generates staggered double-stranded breaks with sticky ends, facilitating more precise DNA insertions. Unlike Cas9, it requires only a crRNA for targeting and exhibits collateral cleavage activity on single-stranded DNA, which has been exploited for diagnostic applications. Cas12 offers improved specificity in certain contexts, although its clinical applications are still less developed compared to Cas9 [40]. Cas13 is a unique RNA-targeting CRISPR system that enables sequence-specific cleavage of RNA without altering the genome. Guided by crRNA, Cas12 provides transient and reversible gene silencing, making it particularly suitable for applications where permanent DNA modification is undesirable. It has been explored for targeting oncogenic transcripts, fusion genes, and non-coding RNAs in cancer. However, its effects are temporary, and collateral RNA cleavage may lead to off-target activity [40,41]. Base editors (e.g., cytosine and adenine base editors), such that single-nucleotide replacements are made without DSB formation, are particularly useful to correct point mutations of oncogenes and tumor suppressors that can be repaired with base editors [42]. Prime editors with a catalytically defective Cas9 with a reverse transcriptase also increase this capacity by enabling a wider range of specific alterations of insertions, deletions, and replacements lacking a donor template [43]. The sum of all these versions of the CRISPR causes a uniquely powerful and versatile toolkit to repair the range of mutations that are detectable in cancers [44].

4.2. Applications in cancer gene knockout, repair, and functional genomics

CRISPR-Cas systems find wide use in the field of cancer biology and therapy. Tumor-promoting signaling has been inhibited with the help of the knockout of oncogenes such as KRAS, EGFR, and MYC [45]. On the other hand, tumor suppressors like TP53, BRCA1, or PTEN can have their position can be repaired using gene repair plans based on HDR or base editing, and could potentially revert malignant phenotypes [46]. However, translation of these findings in vivo remains challenging due to limitations in delivery efficiency, tumor heterogeneity, and off-target effects. Cas13-based RNA editing can be used at the transcript level to induce reversible silencing of transcripts that are linked to cancer and provide a non-permanent therapeutic option [47]. CRISPR has also found massive uses in functional genomics and target discovery, in addition to direct therapeutic intervention. High-throughput CRISPR knockout/activation screens allow the discovery of fundamental cancer genes, synthetic lethal partners, and weak points to drugs, systematically. CRISPR-based screens have been instrumental in identifying synthetic lethal interactions, such as vulnerabilities in BRCA1/2-deficient tumors, which have guided the development of targeted therapies like PARP inhibitors [48]. The findings can accelerate the drug discovery process and provide a reasonable means of combination therapy, e.g., by synergy with PROTAC-mediated protein degradation, checkpoint blockade, or delivery that is based on nanomedicine [49].

4.3. Delivery challenges and tumor-specific targeting

Even though it has the potential, there is a major challenge in the clinical translation of CRISPR-Cas systems in delivery. Cas proteins' large size, and requires delivery together with guide RNAs and, in some cases, donor DNA, which makes delivery efficiency difficult [50]. Adeno-associated virus (AAV) and lentivirus are the most frequently used viral vectors, which are associated with concerns about immunogenicity, genomic integration, and cargo effect. To overcome these barriers, non-viral systems (e.g., lipid nanoparticles, polymeric nanocarriers, and exosomes) are currently under investigation as they offer a higher level of biocompatibility and scalability [51]. In addition, mRNA-based delivery of CRISPR components has emerged as a promising strategy, offering transient expression, reduced risk of genomic integration, and lower immunogenicity compared to DNA-based systems [52].

The other key challenge is to target the tumor specifically. Genomic instability and unintended toxicity may occur when the genes of healthy tissues are off-target edited. More specificity is being achieved through the use of tumor-specific promoters, highly specific guide RNAs, and stimulus-responsive delivery vehicles (pH-, redox-, and enzyme-sensitive nanomaterials) [53]. Moreover, novel CRISPR switches (light- or small molecule-inducible systems) enable spatiotemporal control and are thus safer for therapeutic use [54].

5. PROTACs and targeted protein degradation

5.1. Mechanistic basis of PROTACs

PROTACs are a new type of therapeutic modality that seeks to take advantage of the cellular protein degradation machinery to destroy disease-relevant proteins [55]. Structurally, PROTACs are heterobifunctional small molecules having three parts (Fig. 1), (i) a ligand that binds to the protein of interest (POI), (ii) a ligand that interacts with an E3 ubiquitin ligase, and (iii) a linker to join the two [56]. Upon binding, PROTACs bring the POI into proximity with the E3 ligase, facilitating ubiquitination and subsequent degradation by the 26S proteasome [57]. Unlike classical inhibitors that require continuous occupancy of the target site, PROTACs function via an event-driven, catalytic mechanism where a single molecule can induce multiple rounds of protein degradation, resulting in high potency at sub-stoichiometric concentrations [58].

The next generation PROTACs with greater precision and control over protein degradation have been developed as a result of recent progress [59]. Different types of PROTACs (photocaged PROTACs (Fig. 2A), photoswitchable PROTACs (Fig. 2B), and CLIPTACs) are used in the treatment of cancers [60]. Photocaged PROTACs contain photolabile protecting groups that render them inactive until exposure to specific wavelengths of light, enabling controlled activation and targeted degradation of oncogenic proteins. This spatiotemporal regulation reduces off-target toxicity and enhances therapeutic selectivity in cancer models [61]. In contrast, photoswitchable PROTACs utilize a reversibly photosensitive linker (e.g., azobenzene), allowing dynamic switching between active and inactive conformations under different light conditions, thereby enabling fine-tuned modulation of protein degradation and associated signaling pathways [62].

The CLIPTACs (Click-formed PROTACs) (Fig. 3) are another variant in which two smaller cell-permeable precursors are then subjected to bioorthogonal click chemistry in the cellular environment to produce the active PROTAC molecule. This in situ assembly enhances

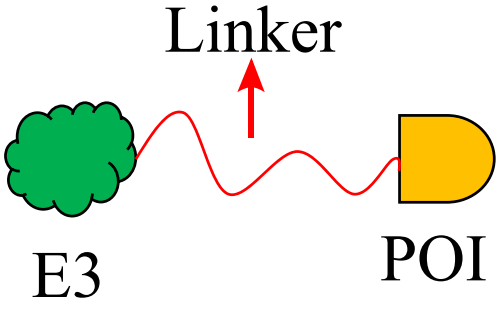

Fig. 1. The schematic figure depicts a PROTAC molecule, with the E3 ligase and protein of interest (POI) attached with the help of a linker. The connector connects the two parts, allowing ubiquitination and selective protein degradation.

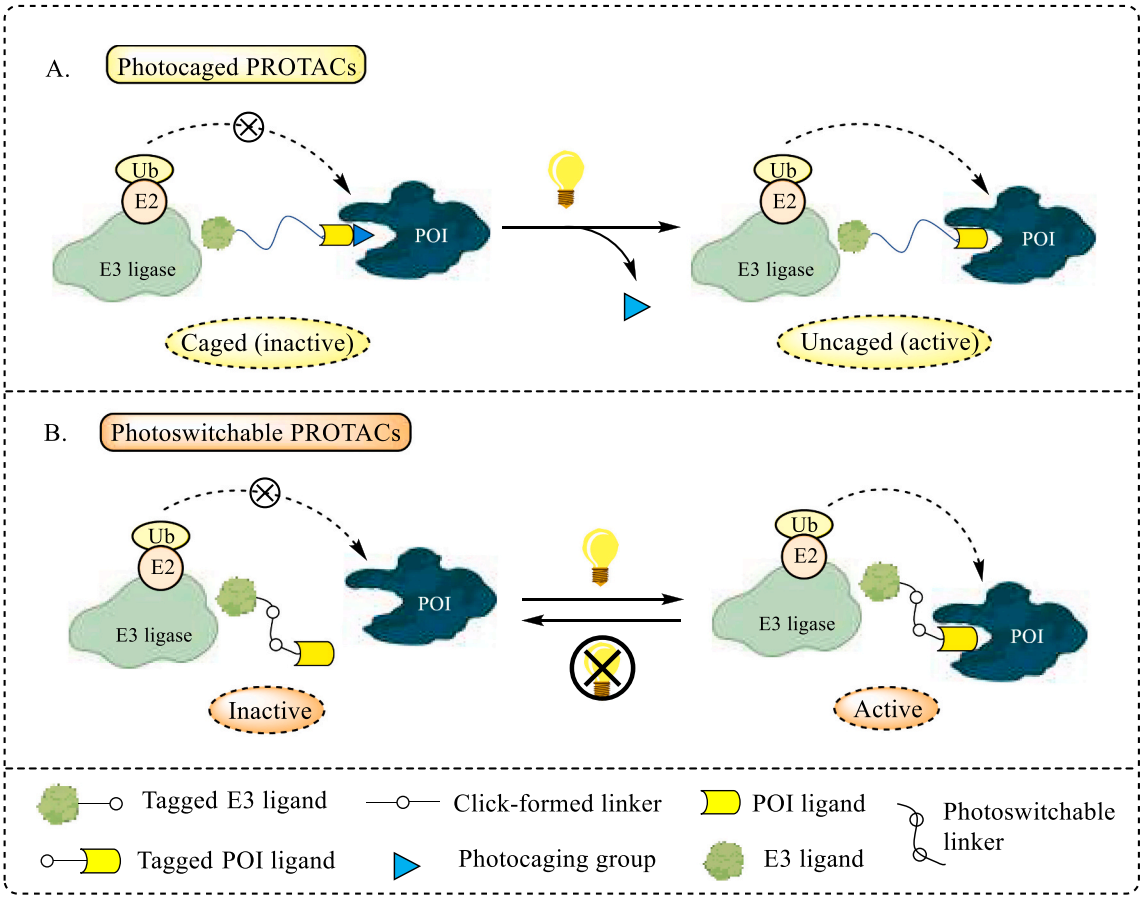

Fig. 2. Schematic illustration of next-generation PROTACs: (A) *Photocaged PROTACs* activated by light-induced cage removal, and (B) *Photoswitchable PROTACs* reversibly controlled by light [60].

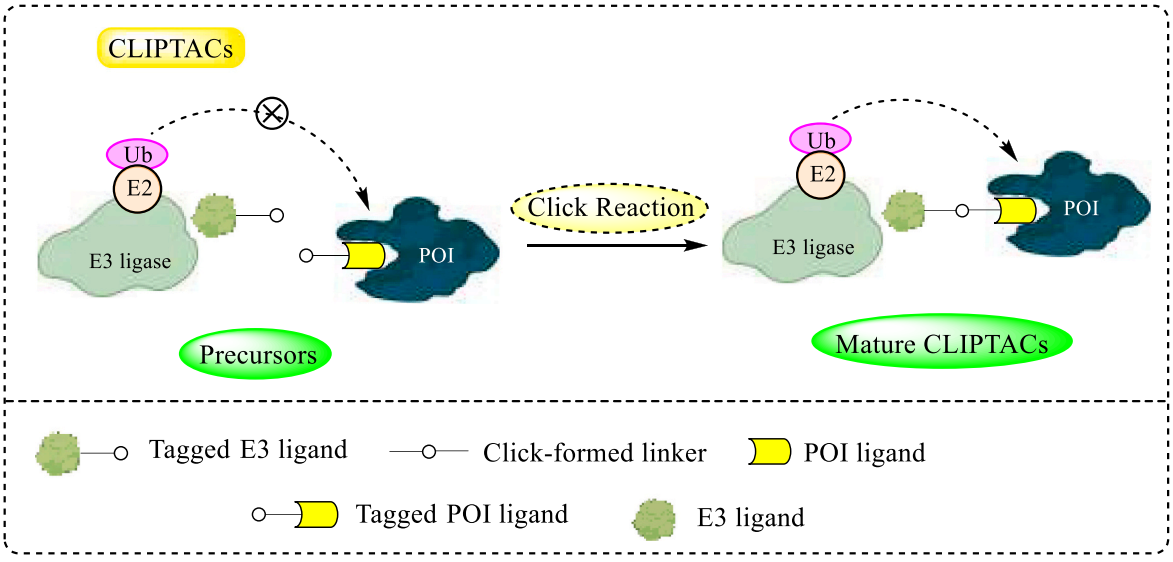

Fig. 3. Schematic illustration of next-generation PROTACs-*CLIPTACs* formed intracellularly via click chemistry for targeted protein degradation [60].

pharmacokinetic stability and cellular delivery, which partially overcomes the limitations of the delivery of entire PROTACs [2,63]. All of these creative versions increase the functional flexibility of the PROTAC technology, allowing more specific and controllable approaches to targeted cancer therapy in combination with more advanced gene-editing systems like CRISPR [2].

Despite these advances, a key limitation of PROTAC technology lies in its dependence on a relatively limited subset of E3 ubiquitin ligases. Although the human genome encodes over 600 E3 ligases, only a few (such as CRBN and VHL) are currently well-characterized and widely utilized in PROTAC design. This restricts the range of targetable proteins and may contribute to resistance mechanism or tissue-specific

variability, highlighting the need to expand the repertoire of exploitable E3 ligases for broader therapeutic applications [64].

5.2. Examples of oncogenic protein degradation

PROTAC technology has been extensively investigated for its ability to degrade oncogenic drivers that were once considered "undruggable". One key target is BRD4 (Bromodomain-containing protein 4), a transcriptional regulator of oncogenes such as MYC. In contrast, BET-targeting PROTACs (Fig. 4), including ARV-771 and dBET1, effectively degrade BRD4 and have demonstrated potent antitumor activity in leukemia, prostate, and breast cancers [65]. Another notable application involves BCL-XL, an anti-apoptotic member of the BCL-2 family that is frequently overexpressed in tumors and contributes to chemotherapy resistance. The PROTAC molecule DT2216 selectively degrades BCL-XL while sparing platelets, thereby reducing the toxicity associated with conventional BCL-XL inhibitors [66].

Mutant KRAS, particularly $KRAS^{G12C}$ and $KRAS^{G12D}$ variants, has long been regarded as an undruggable oncogene. Recent PROTAC strategies have targeted KRAS indirectly by degrading its upstream effectors or binding partners. These emerging PROTACs and molecular glue degraders hold promise in overcoming resistance to existing KRAS inhibitors such as sotorasib [67]. Additionally, androgen receptor (AR) and estrogen receptor (ER) degradation via PROTACs represents a major advance in the management of hormone-driven cancers. Compounds such as ARV-110 (targeting AR) and ARV-471 (targeting ER) have shown strong degradation efficacy and therapeutic potential, offering alternatives for prostate and breast cancers resistant to standard endocrine therapies [68].

5.3. PROTACs in clinical and preclinical cancer pipelines

The rapid translation of PROTAC technology into clinical evaluation underscores its significant therapeutic potential in oncology. Several PROTAC candidates have progressed into human clinical trials for cancer treatment (Table 2). Among them, ARV-110 (bavdegalutamide), a PROTAC targeting the androgen receptor, is being evaluated for metastatic castration-resistant prostate cancer. Recent phase I/II clinical data have demonstrated promising antitumor activity along with manageable safety profiles [69]. Similarly, ARV-471 (vepdegestrant), designed to degrade the estrogen receptor, is under investigation for ER-positive breast cancer. Updated Phase II clinical findings have shown improved progression-free survival and sustained estrogen receptor degradation compared to fulvestrant, with a favourable tolerability profile, including predominantly grade 1–2 advers events such as fatigue and nausea, and a low incidence of severe toxicities [70].

Another noteworthy candidate, DT2216, functions as a BCL-XL degrader and is in preclinical development. It selectively targets tumor cells while sparing normal platelets, thereby overcoming a major limitation associated with traditional BCL-XL inhibitors [71]. In addition, various preclinical candidates, including BET degraders (such as dBET6 and MZ1) and kinase-targeting PROTACs aimed at EGFR, BTK, and CDK9, are being evaluated across multiple cancer models [72]. Despite these encouraging advances, challenges remain in optimizing pharmacokinetic properties, enhancing oral bioavailability and tissue penetration, and minimizing off-target effects and drug resistance. Nonetheless, the successful transition of PROTACs from bench to bedside emphasizes their impactful potential as a new paradigm in cancer therapy by enabling the selective degradation of oncogenic proteins [73].

6. Other gene editing and regulatory tools

6.1. TALENs and ZFNs: early genome editing applications in cancer

Before the emergence of CRISPR-Cas systems, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)

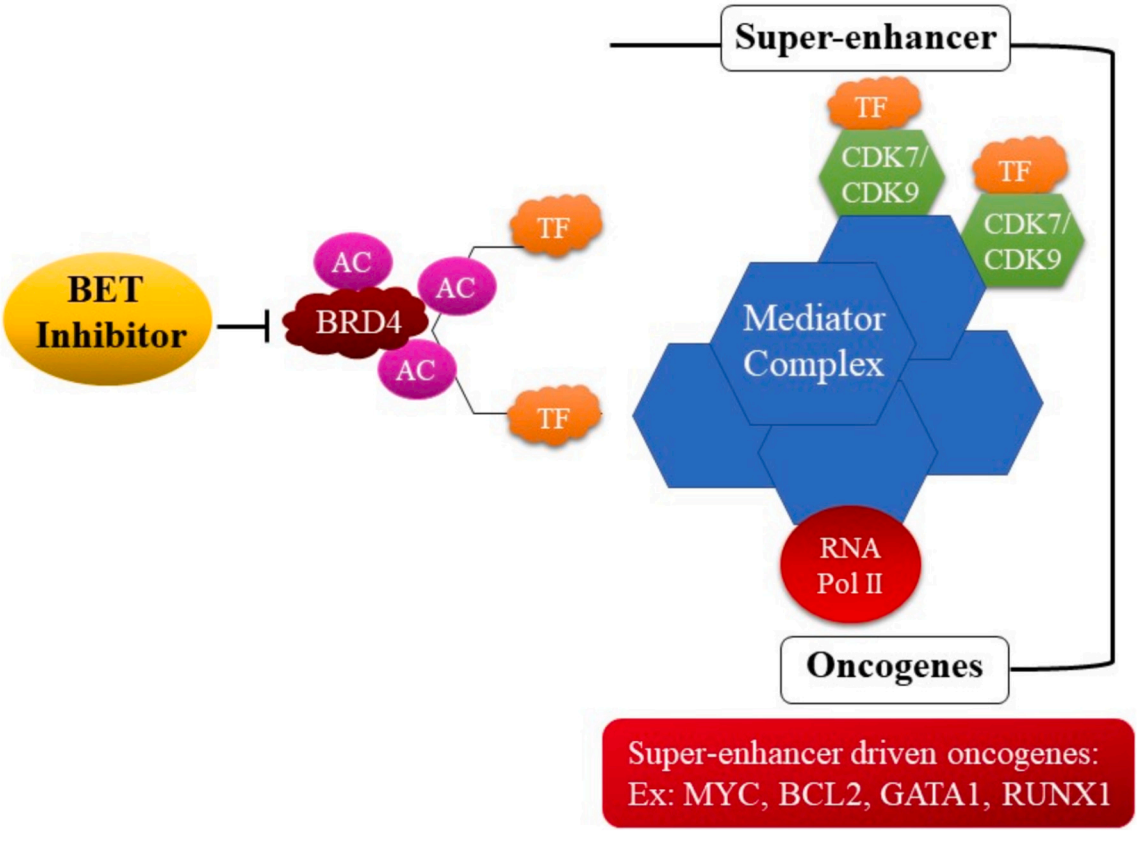

Fig. 4. Mechanism of BET inhibition and conceptual transition toward PROTAC-mediated degradation of BRD4.

Table 2
Representative PROTAC candidates in cancer.

Target protein	PROTAC candidate	Cancer type	Trial stage	Key outcomes	Ref.
BRD4	ARV-771	Leukemia, solid tumors	Preclinical	Effective degradation, tumor regression	[100]
AR (Androgen Receptor)	ARV-110	Prostate cancer	Phase I/II	Reduced PSA levels	[101]
ER (Estrogen Receptor)	ARV-471	Breast cancer	Phase II	Well tolerated, promising efficacy	[102]
BCL-XL	DT2216	Hematological cancers	Preclinical	Selective degradation, reduced toxicity	[71]

represented the first generation of programmable genome-editing technologies applied in cancer research [74,75]. Both systems utilize engineered DNA-binding domains fused to the bacterial type IIS restriction endonuclease (*Fok*I), which induces double-strand breaks (DSBs) at specific genomic loci. The resulting breaks can be repaired through either non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling targeted gene modification [76,77]. ZFNs have been utilized to inactivate oncogenes such as VEGF-A, thereby inhibiting angiogenesis and reducing tumor-mediated immune suppression. However, their complex architecture, restricted targeting range, and potential off-target effects have limited their widespread application [78,79]. In contrast, TALENs offer improved specificity and modularity compared to ZFNs [79]. In oncology, TALENs have been employed to disrupt PD-1 in T cells, enhancing antitumor immune responses, and to engineer chimeric antigen receptor-T (CAR-T) cells with reduced susceptibility to graft-versus-host disease [80,81]. Although CRISPR technology has largely supplanted TALENs and ZFNs due to its simplicity and efficiency, these earlier platforms continue to play valuable roles in clinical-grade cell engineering, given their high precision and relatively low immunogenicity [82].

7. RNA-based tools: siRNA, shRNA, and RNA editing

RNA-targeting strategies are used in conjunction with DNA editing technology because they offer momentary and reversible regulation of oncogenic transcripts with no modifications in the genome. Small interfering RNA (siRNA) and short hairpin RNA (shRNA) work on a mechanism of RNA interference (RNAi), mediating post-transcriptional gene silencing by promoting degradation of target mRNA molecules. siRNA-based therapy has been investigated in cancer therapy to silence cancer oncogenes, including BCL2, KRAS, and VEGF, but the problem of delivering this drug efficiently, stability, and off-target effects remains an impediment to clinical implementation [83,84]. Notably, the clinical success of siRNA therapeutics, such as the FDA-approved Patisiran, has validated the feasibility of RNAi-based strategies in humans, particularly when delivered via LNP systems. [85]

Simultaneously, technologies based on synthetic RNA editing with engineered adenosine deaminase that acts on RNA (ADAR) enzymes have also been developed as more specific methods of nucleotide site-modification, e.g., adenosine-to-inosine (A-to-I) or cytidine-to-uridine (C-to-U) conversions. In this method, oncogenic point mutations of RNA level can be fixed without permanently altering the DNA, eliminating the possibility of the occurrence of off-target genomic instability [86]. Equally, there is evidence of ADAR-mediated RNA editing being researched to repair TP53 mutations in preclinical cancer models, and this is likely to be a more versatile and safer alternative to DNA-based genome editing [87].

8. Epigenome editing: dCas9-fused modifiers for silencing/activation

Epigenome editing utilizes catalytically inactive Cas9 (dCas9), fused with epigenetic effector domains, to regulate gene expression without altering the underlying DNA sequence. This system enables reversible and tunable control, making it suitable for cancer therapy [88]. For repression, dCas9-KRAB recruits regulators such as KRAP1/TRIM28 and SETDB1, inducing chromatin compaction and long-term gene silencing. Oncogenes like MYC have been effectively suppressed, and repression can be enhanced using dCas9-DNMT3A [89]. For activation (CRISPRa), dCas9 is combined with activators such as VP64, p300, or multi-component systems like SAM and VPR. These approaches enable strong and tunable activation of tumor suppressor genes such as CDKN1A/p21 [90].

In the case of therapeutic translation, it is necessary to have temporal and controlled delivery to reduce long-term exposure and off-target effects [91]. More recent reports have demonstrated that transient delivery of epigenome editors on an mRNA basis, ribonucleoprotein complexes (RNPs), non-integrating viral vectors, or lipid nanoparticles can induce long-term transcriptional effects without exposing the nuclease [92]. It is interesting to note that, in preclinical models, transient exposure to epigenetic effectors has provided permanent silencing of genes in vivo by setting up a self-sustaining chromatin state, an approach called the hit-and-run paradigm of delivery [93]. Chem-CRISPR and other chemical- or small-molecule-inducible systems can also be used to obtain additional temporal precision [93].

8.1. Base and prime editing: precision modification for oncogenic mutations

Base editing and prime editing are state-of-the-art CRISPR-based genome engineering methods that are more precise and safer than the traditional CRISPR-Cas9 systems. Base editors are catalytically inert Cas9 (nick) complexed with a deaminase to produce extremely brief substitutions of single nucleotides in the absence of the creation of double-strand breaks (DSBs). Cytosine base editors (CBEs) mediate CG-TA conversions, and adenine base editors (ABEs) mediate AT-GC conversions, the most effective way of repairing point mutations. They have specific potential in treating mutations that are recurrent and are linked to cancer, including TP53 R273H and KRAS G12D [94,95]. Prime editing has been applied to repair oncogenic EGFR mutations and to restore loss-of-function BRCA1 alleles successfully in preclinical cancer models, indicating its therapeutic relevance [96]. Relative to conventional CRISPR systems, base and prime editing reduce by far the incidence of chromosomal rearrangements and insertion-deletion mutations (indels), and thus provide a less dangerous and more controlled method of genome editing in the future [97]. Prime editing typically shows lower efficiency than base editing due to its complex, multi-step mechanism involving reverse transcription and DNA integration. In contrast, base editing enables direct single-base conversion, making it generally faster and more efficient, especially in in vivo settings [98]. Compared with conventional CRISPR, these approaches reduce the risk of chromosomal rearrangements and indels, making them safer for therapeutic development [99].

9. Expanding the toolkit: emerging platforms

Besides the traditional genome and transcriptome editing technologies, there are some new directions to enhance therapeutic possibilities in the field of oncology [103]. The goals of these strategies are to address the shortcomings of off-target toxicity, limited target space, and difficulty in delivering drugs. A structured distinction between different toolkits (molecular glues, LYTACs, PROTACs, and AUTACs is given in Table 3.

Table 3
Targeted protein degradation modalities.

Feature	Molecular glues	PROTACs	LYTACs	AUTACs
Full Form	–	Proteolysis Targeting Chimeras	Lysosome Targeting Chimeras	Autophagy Targeting Chimeras
Molecular Type	Small monovalent molecules	Heterobifunctional molecules	Bifunctional ligands (protein + receptor binder)	Small molecules with degradation tags
Mechanism	Stabilize interaction between E3 ligase and target protein	Recruit E3 ligase to ubiquitinate target protein	Bind extracellular/membrane proteins and direct them to lysosomes via receptors	Tag intracellular proteins/organelles for selective autophagy
Degradation Pathway	UPS (proteasomal degradation)	Ubiquitin-proteasome system (UPS)	Lysosomal degradation (endocytosis pathway)	Autophagy–lysosome pathway
Target Scope	Intracellular proteins (limited to neosubstrate compatibility)	Intracellular proteins (cytosolic/nuclear)	Extracellular and membrane proteins	Intracellular proteins, aggregates, organelles
Catalytic Activity	Yes (event-driven)	Yes (event-driven, recyclable)	Not strictly catalytic (receptor-mediated trafficking)	Semi-catalytic (depends on autophagic flux)
E3 Ligase Requirement	Yes (e.g., CRBN)	Yes (e.g., CRBN, VHL)	No (uses lysosome-targeting receptors like CI-M6PR)	No direct E3 requirement
Typical Targets in Cancer	IKZF1/3 (thalidomide analogs). GSPT1	BRD4, AR, ER, BCL-XL	EGFR, PD-L1, HER2 (membrane proteins)	Misfolded proteins, mitochondrial proteins
Clinical Status	Clinically validated (thalidomide class)	Advanced (e.g., ARV-110, ARV-471)	Early preclinical	Preclinical/early research
Key Advantage	Simple structure, good bioavailability	Expands “undruggable” intracellular targets	Targets extracellular/membrane proteins	Removes aggregates and organelles
Key Limitation	Limited target scope	Large size, PK issues	Delivery and receptor specificity	Less precise targeting

10. Molecular glues

Molecular glues are small molecules that favor or stabilize protein-protein interactions and, in many cases, result in a selective degradation or functional modulation of the disease-relevant proteins [104]. Unlike traditional inhibitors that block enzymatic activity, molecular glues redirect the cellular degradation machinery, particularly the ubiquitin-proteasome systems, to eliminate oncogenic proteins. In contrast to PROTACs, which are heterobifunctional molecules requiring two distinct ligands connected by a linker, molecular glues are monovalent compounds that induce or stabilize interactions between a target protein and an E3 ubiquitin ligase without the need for a linker, resulting in simpler structures and often improved pharmacokinetic properties [105,106]. As an example, thalidomide derivatives are molecular glues and recruit cereblon, an E3 ubiquitin ligase, to degrade neosubstrates that play a role in multiple myeloma [107]. Oral bioavailability is also attainable because of their versatility and low molecular weight, which makes them attractive anticancer agents [108,109].

11. LYTACs (LYsosome-TArgeting Chimeras) and AUTACs (AUtophagy-TArgeting Chimeras)

LYTACs make use of bifunctional molecules, which bind extracellular or membrane proteins and target them to lysosomal degradation through lysosome-shuttling receptors (e.g., CI-M6PR). This strategy enables the selective removal of cell-surface and secreted oncogenic proteins that are not accessible to proteasome-dependent technologies like PROTACs [110,111]. Recent preclinical studies have demonstrated the ability of LYTACs to degrade membrane receptors such as EGFR and PD-L1, highlighting their potential in targeting tumor-related signaling pathways.

Similarly, AUTACs exploit the autophagy pathway of the cell by labeling undesired proteins with degradation signals that are sensed by the autophagic receptors. The route is especially helpful when removing aggregated, misfolded, or membrane-bound proteins associated with tumor development [112]. AUTAC-based strategies have shown promise in degrading aggregated, misfolded, and membrane-associated proteins, as well as in the selective clearance of damaged mitochondria (mitophagy), which is increasingly linked to cancer progression [113]. However, both LYTACs and AUTACs remain emerging and largely preclinical technologies, with challenges related to delivery, stability, and in vivo efficacy still under investigation. Despite these limitations, they represent promising complementary approaches to expand the scope of targeted protein degradation beyond intracellular proteins.

12. RNA-targeted CRISPR (Cas13 systems)

In contrast to the non-editing CRISPR systems (Cas12), the Cas13 family affects the relationship with RNA, allowing the operation of gene expression with temporary and reversible efficiency without definitive genome editing. This renders Cas13 specially adapted to therapeutic use, in which safety and reversibility are of great importance [47]. Cas13 has been investigated in cancer in silencing oncogenic fusion transcripts (e.g., BCR-ABL in leukemia), degradation of viral oncogenes (e.g., transcripts of HPV in cervical cancer), and controlling noncoding RNAs that control tumor growth or immune evasion [114]. Cas13 is an exciting technology to enable next-generation RNA therapies in oncology because of its transcriptome-wide activity, programmable specificity, and the potential to combine it with delivery systems (e.g., LNPs). However, efficient in vivo delivery of Cas13 components remains a significant challenge, as the system requires co-delivery of Cas13 protein or mRNA along with guide RNAs. Similar to other CRISPR platforms, delivery strategies such as LNPs, viral vectors, and exosome-based systems are being explored, but issues including limited tissue-specific targeting, rapid clearance, and potential immune responses must be addressed to achieve clinical translation [115].

13. Synthetic biology circuits

Synthetic biology uses engineering ideologies to construct gene regulatory systems that are capable of detecting and acting on tumor microenvironment (TME) signals, including hypoxia, acidic pH, or cytokines. These circuits function as regulatory control systems that ensure therapeutic genes or editing devices are activated only in cancer cells, thus causing minimal harm to normal cells [116]. Examples include logic-gated CAR-T cells that require the presence of multiple tumor antigens for activation, as well as advanced SynNotch receptor systems, which enable programmable antigen recognition and conditional activation of CAR expression, thereby enhancing tumor specificity and reducing off-target toxicity. In addition, CRISPR-based editing systems controlled by tumor-specific promoters or microRNA signatures, along with self-destructive “kill-switches” incorporated into engineered cells to avoid unregulated activity once the therapeutic goal is achieved [117].

14. Synergistic approaches and combination therapies

14.1. CRISPR + PROTAC (dual gene/protein regulation)

The integration of CRISPR gene editing with PROTAC-mediated protein degradation has been validated in recent proof-of-concept studies, providing concrete evidence of dual genomic-proteomic control in cancer (Table 4) [118]. For instance, a 2024 CRISPR screen paired with the BET PROTAC ARV-825 identified mTOR pathway ablation as synergistic in cholangiocarcinoma, yielding >70% tumor growth inhibition in xenografts by blocking metabolic rewiring [119]. For instance, CRISPR could disrupt *KRAS* mutations, while PROTACs degrade compensatory proteins such as *BRD4* or *BCL-XL*. Such an approach addresses both genetic and proteomic contributors to malignancy, thereby reducing the likelihood of resistance [120]. Recent proof-of-concept studies have also demonstrated the use of PROTACs to control the stability of Cas9 proteins, providing a "switchable" CRISPR system with improved safety profiles [121]. A 2024 CRISPR screen paired with the BET PROTAC ARV-825 identified mTOR pathway ablation as synergistic in cholangiocarcinoma, yielding >70% tumor growth inhibition in xenografts by blocking metabolic rewiring [122]. Similarly, CRISPR activation uncovered FBXO22 for novel PROTACs degrading BRD4 (>90% reduction), enhancing selectivity in leukemia models [123]. PROTACs stabilizing Cas9 via inducible degradation further enabled safe, temporal CRISPR-KRAS editing in prostate xenografts, achieving durable regression without prolonged nuclease exposure [124]. These examples underscore reduced resistance and superior efficacy over monotherapy.

Permanent edits of CRISPR (72 h kinetics) can be paired with the rapid degradation of PROTACs (4-24 h DC50) to allow temporal control: e.g., CASPROTAC-6 can be used to clear SpCas9 after editing, slice OTEs without affecting 70% of on-target [125]. POM-Cas9-degron (PMC 2025) reversibly reduces Cas9 3–5× in 4 h, optimal in cancer models such as KRAS/BRD4 dual hits. This off-switch reduces OTEs noted in the manuscript, increasing safety [126].

14.2. CRISPR-based screens for PROTAC target discovery

High-throughput CRISPR knockout and activation screens have become indispensable for identifying essential cancer genes and uncovering synthetic lethal interactions. These screening approaches are increasingly applied to expand the utility of PROTACs by revealing novel druggable vulnerabilities and new E3 ligases suitable for targeted protein degradation [127]. Although the human genome encodes more than ~600 E3 ubiquitin ligases, current PROTAC design largely relies on a limited subset, primarily CRBN and VHL, thereby restricting the diversity of targeted proteins [123]. Recent CRISPR activation studies have identified FBXO22 as a promising E3 ligase, which has been successfully utilized for PROTAC-mediated degradation of oncogenic proteins. Additionally, other ligases such as DCAF15, RNF114, and KEAP1 are being explored, highlighting the potential of CRISPR-based functional genomics to broaden the E3 ligase repertoire and enhance targeted degradation strategies [128].

14.3. Integration with immunotherapy (CAR-T, checkpoint inhibitors)

The intersection of gene editing, targeted protein degradation, and immunotherapy offers significant therapeutic synergy. CRISPR engineering of T cells enables knockout of immune checkpoint regulators such as *PD-1* or *LAG-3*, improving T-cell persistence and cytotoxicity against tumors. Simultaneously, PROTACs can degrade checkpoint proteins (e.g., PD-L1) or immunosuppressive transcription factors within the tumor microenvironment, thereby augmenting antitumor immune responses [129]. In addition, CRISPR-edited CAR-T [130] cells can be combined with PROTACs that degrade tumor resistance proteins, enabling a two-pronged strategy where engineered immune cells directly attack tumors while PROTACs dismantle mechanisms of immune evasion [131,132]. Such combinational designs may be particularly valuable in solid tumors, where immunotherapy efficacy is often limited by hostile microenvironments [133].

14.4. Combination with nanocarriers and smart drug delivery

Efficient and selective delivery remains a central challenge for both CRISPR and PROTAC-based therapeutics [124]. Nanocarrier systems, including lipid nanoparticles, polymeric micelles, dendrimers, and exosome-mimetic vesicles, offer versatile platforms for co-delivery of nucleic acids (Cas9 mRNA, gRNA) and small molecules (PROTACs) [134,135]. Smart nanocarriers responsive to tumor-related stimuli (pH, enzymes, redox gradients) further enhance spatiotemporal control, ensuring therapeutic activity primarily within the tumor microenvironment. Notably, the clinical success of LNP-based mRNA delivery systems, as demonstrated by the Pfizer-BioNTech COVID-19 vaccine and Moderna COVID-19 vaccine, has validated their scalability, safety, and translational potential, supporting their application in CRISPR-based

Table 4
Emerging integration of CRISPR and PROTAC technologies for precision cancer therapy.

Strategy	CRISPR target/ function	PROTAC target	Mechanistic synergy	Cancer model/ application	Translational potential	Current limitations/ barriers	Ref.
CRISPR knockout + PROTAC degradation	Disrupts oncogene transcription (e.g., MYC, KRAS, BCL2)	Degrades the corresponding overexpressed proteins	Dual suppression at the genomic and proteomic levels reduces compensatory resistance mechanisms	Breast, prostate, and colorectal cancers	High – enables durable, multi-layered inhibition of oncogenic signaling	Co-delivery challenges (Cas9 + PROTAC), off-target editing, E3 ligase dependency.	[143]
Epigenomic editing (dCas9-KRAS) + PROTACs	Silences promoter/ enhancer regions of driver genes	Removes residual protein pools post-silencing	Combines reversible gene repression with targeted protein degradation	Leukemia and glioblastoma	Moderate – reversible control with minimal genomic damage	Transient epigenetic effects, delivery efficiency, incomplete silencing.	[144]
Base editing + PROTAC targeting mutant proteins	Corrects oncogenic point mutations (e.g., TP53, KRAS G12D)	Eliminates mutated protein remnants	Prevents relapse through correction–degradation dual action	Pancreatic and lung cancer models	High – mutation-specific precision therapy	Editing efficiency variability, risk of bystander edits, complex formulation.	[145]
CRISPRa (dCas9–VP64) + PROTACs	Activates tumor-suppressor genes (e.g., p53, PTEN)	Degrades inhibitors of those suppressors	Restores tumor suppressor pathways via dual modulation	Melanoma and hepatocellular carcinoma	Promising – enhances endogenous repair pathways	Risk of overactivation, off-target transcriptional effects, delivery complexity.	[13]
CRISPR screening + PROTAC library screening	Identifies essential gene–protein pairs for degradation	Validates druggable protein degradation targets	Accelerates discovery of synthetic lethal interactions	High-throughput cancer cell panels	High – drives rational design of next-gen degraders	Data interpretation complexity, scalability, limited E3 ligase diversity.	[146]

cancer therapies [136,137]. However, challenges such as preferential liver accumulation, limited tissue-specific targeting, transient expression, and potential immune activation remain significant barriers that must be addressed to fully translate LNP-based systems for CRISPR and PROTAC delivery in cancer therapy [138]. For example, lipid nanoparticles could deliver CRISPR components to knock out oncogenic mutations, while the same carrier could be co-loaded with PROTACs for the degradation of resistance-mediating proteins [139]. The convergence of nanomedicine, CRISPR editing, and targeted protein degradation thus holds potential to overcome delivery barriers and maximize therapeutic efficacy in cancer [140].

15. Delivery platforms for gene editing tools in cancer

The clinical translation of gene editing technologies such as CRISPR-Cas systems, base/prime editors, and RNA-targeting tools is heavily dependent on the development of safe and efficient delivery platforms (Table 5) [141]. Effective delivery must overcome barriers such as nucleic acid instability, large cargo size, cellular uptake, immune clearance, and tumor-specific targeting. Current approaches can be broadly divided into viral vectors, non-viral systems, and tumor-targeted or stimuli-responsive carriers (Fig. 5) [142].

15.1. Viral vectors (AAV, lentivirus)

Adeno-associated virus (AAV) and lentivirus are widely used delivery systems for gene editing in cancer research due to their high transduction efficiency and ability to deliver genetic material directly into target cells [51]. AAVs are non-pathogenic and have relatively low immunogenicity. They are suitable for in vivo delivery of Cas9 variants and guide RNAs, due to their favourable safety profile, low immunogenicity, and ability to mediate long-term gene expression. However, their limited packaging capacity (~4.7 kb) poses challenges for large editors like SpCas9, often requiring smaller Cas variants (e.g., SaCas9) [82,147]. Capsid engineering strategies primarily include directed evolution, rational design, and peptide insertion. Directed evolution involves generating large libraries of AAV variants followed by in vivo selection to identify capsids with enhanced tropism for specific tissues such as tumors, liver, or central nervous system. For example, engineered variants like AAV-PHP.B and AAV-PHP.eB have demonstrated improved transduction efficiency in neuronal tissues [148].

Lentiviruses integrate into the host genome, providing stable and long-term expression of editing components, which is useful for ex vivo engineering of immune cells, such as CRISPR-edited CAR-T cells. However, risks of insertional mutagenesis and strong immune responses limit their use in systemic delivery for solid tumors [149,150].

Table 5
Comparison of in vivo vs. ex vivo gene editing and CAR-T strategies.

Parameter	Ex vivo engineering	In vivo delivery
Approach	T cells isolated, edited outside the body, then reinfused	Direct delivery of gene-editing tools into patients
Editing Control	High (pre-validation possible)	Limited (occurs inside the body)
Targeting Specificity	High (engineered immune cells)	Variable; depends on delivery system
Delivery Systems	Viral vectors (lentivirus), electroporation, RNPs	LNPs, AAVs, polymeric NPs
Clinical Status	Clinically established (CAR-T therapies)	Early-stage/preclinical to early clinical
Advantages	High precision, safety screening, durable response	Scalable, less complex, no cell handling
Key Challenges	Cost, manufacturing complexity, and time-intensive	Liver accumulation, off-target effects, lower efficiency
Recent Advances	Base-edited allogeneic CAR-T, multiplex CRISPR editing	Targeted LNPs, in vivo CAT-T programming, SORT NPs

15.2. Non-viral systems (lipid nanoparticles, polymeric carriers, hydrogels)

In order to overcome the shortcomings of viral vectors, a wide set of non-viral delivery platforms have been established, which are safer, scalable, and flexible [151]. The lipid nanoparticles (LNPs) are the most clinically mature systems as demonstrated by their effective use in mRNA-based vaccines and new gene-editing therapeutics [152]. The LNPs consist of lipids that are ionizable, helper lipids, cholesterol, and polyethylene glycol (PEG)-lipids, which combine to promote effective encapsulation, resistance to nuclease degradation, as well as endosomal escape of nucleic acid cargo [153]. Another important factor in CRISPR delivery mediated by LNPs is the type of cargo format, specifically, whether Cas9 is presented as messenger RNA (mRNA) or ribonucleoprotein (RNP) complex, as this directly affects the editing kinetics, nuclease activity duration, and off-target risk [154].

The Cas9 mRNA delivery together with guide RNA (gRNA) mediated by LNPs facilitates the transient expression of the Cas9 protein into the cell after cytoplasmic translation. It is a method to efficiently edit the genome without introducing genomic integration, but the timeframe of Cas9 activity is rather long (usually ranging between 24 and 72 h), which can enhance the likelihood of off-target cleavage events, particularly in highly proliferative tumor cells [92]. However, mRNA-based delivery has advantages of increased encapsulation efficiency, better stability and simplicity in mass production. More recent preclinical efforts have been able to adopt LNPs to deliver Cas9 mRNA targeting of oncogenic mutations including KRAS in pancreatic cancer models, with a high degree of tumor growth inhibition and gene-editing success [155].

Conversely, pre-formed Cas9gRNA ribonucleoprotein (RNP) complexes delivered using LNPs presents a more regulated and transient editing profile [156]. Genome editing can take place rapidly upon cytoplasmic release of the protein since the Cas9 protein has already been synthesized, which can happen in a matter of hours; the protein will then be rapidly degraded. This low intracellular half-life not only increases the number of genetic editing specificity but also decreases the amount of time off-target activity is possible [157]. Notably, the mRNA/RNP decision may be personalized depending on the therapeutic objective: mRNA-based systems prove to be beneficial in cases where increased editing activity is needed, but RNP-based delivery is needed in the case when off-target effects and transient exposure are crucial, including precision oncology applications [158].

In addition to LNPs, polymeric carriers such as biodegradable polymers like polyethyleneimine (PEI), poly(lactic-co-glycolic acid) (PLGA), and dendrimers have also been studied extensively to deliver CRISPR. These systems can be tuned to provide physicochemical properties, and functionalized with targeting ligands (e.g., folate, peptides, antibodies) in order to promote tumor-targeting uptake. Nevertheless, issues of reduced cytotoxicity (especially with PEI) and reduced transfection efficiency in comparison to LNPs are still challenges [159].

Another approach that could be used to deliver CRISPR components locally and in a sustained fashion is injectable hydrogel-based delivery systems. On these platforms, direct intratumoral delivery of Cas9-GRNA complexes or nucleic acids can be achieved, allowing increased retention at the tumor location and reducing the off-target effects and systemic exposure [160]. Recent publications have shown that hydrogel-mediated delivery increases the local editing efficiency and therapeutic outcome in solid tumor models, and are especially appealing to accessible malignancies.

15.3. Tumor-targeted and stimuli-responsive systems

To enhance the accuracy and safety of CRISPR-based cancer therapy, scholars are building tumor-targeted and stimuli-response delivery vehicles [161]. Nanocarriers in ligand-mediated targeting the tumor-specific ligand folate, transferrin, antibodies, or peptides are

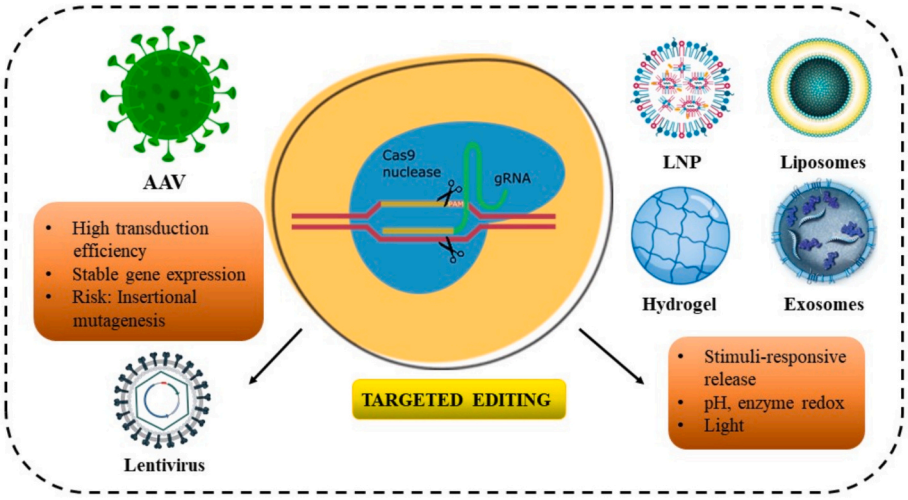

Fig. 5. Viral (AAV, lentivirus) and non-viral (lipid nanoparticles (LNP), liposomes, hydrogels, exosomes) delivery systems for CRISPR and PROTAC-based cancer therapy. Non-viral carriers enable safer, tumor-targeted, and stimuli-responsive delivery for precise gene and protein modulation.

functionalized on nanocarriers to allow their selective uptake by cancer cells and not by normal tissues [162]. The receptor-targeted approach has proven effective in targeting tumors with HER2- or EGFR-expressing receptors with minimal adverse off-target effects of Cas9 mRNA or RNPs. The control of stimuli-responsive systems has been further increased by activation of gene-editing cargo based on tumor microenvironmental signals, such as acidic pH, high glutathione, or enzyme activity (e.g., matrix metalloproteinases), in a manner that ensures local activation of CRISPR machinery [163].

Besides this, exosome-based delivery provides a natural and biocompatible surface, whereby engineered exosomes are able to be loaded with aspects of Cas9 and customized to home directly to tumor sites. Exosome-based delivery systems offer a complementary strategy, providing a natural, biocompatible platform for transporting CRISPR components. Engineered exosomes can be loaded with Cas9 mRNA or RNPs and modified to enhance tumor-specific homing. A recent in vivo study demonstrated the biodistribution profile of engineered exosome-based CRISPR/Cas9 delivery systems using fluorescent (DiR)-labeled exosomes in mouse models [164]. Following intravenous administration, exosomes showed rapid accumulation in the liver within 6 h, with additional distribution observed in the spleen and lungs, consistent with uptake by the mononuclear phagocyte system. Importantly, when exosomes were engineered with tissue-specific targeting ligands (e.g., integrin α6), enhanced accumulation in target tissues such as the lungs was observed, accompanied by efficient Cas9-mediated gene editing and minimal systemic toxicity. Collectively, these new approaches add more specificity to tumors and minimize systemic toxicity along with being major steps toward realizing safe and effective clinical delivery of CRISPR therapeutics [165,166].

16. Challenges and limitations

Despite their influential potential, gene editing and targeted protein degradation tools face several critical barriers that must be addressed before they can be widely implemented in cancer therapy.

16.1. Off-target mutations and genomic instability

One of the primary concerns with CRISPR-Cas systems and other nucleases (TALENs, ZFNs) is the risk of off-target cleavage. Even small mismatches between the guide RNA and unintended genomic loci can result in insertions, deletions, or chromosomal rearrangements, potentially leading to oncogenic transformation or disruption of essential genes [167]. While advances such as high-fidelity Cas9 variants, base/prime editing, and improved gRNA design have reduced off-target activity, absolute precision in complex cancer genomes remains challenging. To address this, several high-throughput detection methods, including GUIDE-seq (Genome-wide, Unbiased Identification of DSBs Enabled by Sequencing), Digenome-seq, and SITE-seq, have been developed to map off-target cleavage sites with high sensitivity and genome-wide resolution, thereby improving the safety assessment of gene editing systems. Additionally, unintended genomic instability from double-strand breaks or integration events can complicate clinical translation [168,169].

16.2. Drug resistance and tumor heterogeneity

Inherent genetic and phenotypic heterogeneity of cancer contributes to therapy resistance. Editing a single oncogene may not be sufficient, as tumors can activate compensatory signaling pathways [170]. Similarly, while PROTACs efficiently degrade target proteins, resistant mutations in the protein of interest or in E3 ligases can limit efficacy [171]. Subclonal variations within tumors further complicate editing outcomes, as some cells escape modification and drive relapse [172]. Advanced approaches, such as single-cell RNA sequencing (scRNA-seq), are increasingly being utilized to characterize intratumoral heterogeneity at high resolution, enabling the identification of resistant subpopulations and guiding the design of more effective, personalized therapeutic strategies. Addressing heterogeneity may require combinational approaches (e.g., CRISPR + PROTAC + immunotherapy) or multiplexed editing strategies targeting multiple cancer drivers simultaneously [128,173].

16.3. Immunogenicity and safety concerns

The delivery of gene editing tools raises significant safety issues. Viral vectors (AAV, lentivirus) can trigger immune responses, and pre-existing immunity to viral capsids may reduce efficacy [174]. Cas proteins themselves are derived from bacteria (e.g., *Streptococcus pyogenes*, *Staphylococcus aureus*), and immune recognition could result in clearance of edited cells or systemic inflammation [175]. For PROTACs, off-target protein degradation may disrupt normal physiological functions, leading to toxicity. Moreover, long-term safety data are limited, and the potential for unanticipated adverse events remains a major barrier to clinical adoption [176].

16.4. Manufacturing, scalability, and regulatory hurdles

Producing gene editing and targeted degradation tools at clinical

grade and scale is complex and resource-intensive [177]. Manufacturing challenges include ensuring stability of CRISPR components (Cas mRNA, gRNA, or RNPs), maintaining batch-to-batch consistency in nanoparticle formulations, and scaling up production under Good Manufacturing Practices (GMP) [178]. PROTACs, although chemically synthesizable, often exhibit suboptimal pharmacokinetic properties due to their relatively large molecular weight and polarity, which can complicate formulation and dosing [179].

On the regulatory side, gene editing therapies face rigorous evaluation for safety, ethical acceptability, and long-term monitoring. Recent regulatory guidance from agencies such as the U.S. Food and Drug Administration emphasizes a comprehensive assessment of off-target effects, durability of gene edits, immunogenicity, and long-term follow-up in patients receiving CRISPR-based therapies. In addition, regulatory frameworks continue to evolve with respect to distinctions between somatic and germline editing, with current consensus strongly restricting clinical applications to somatic cells. Key considerations also include informed consent, risk-benefit assessment for irreversible interventions, and post-treatment surveillance to monitor delayed adverse effects [180].

17. Disease-specific considerations in cancer therapeutics

The use of advanced genetic and molecular therapeutic approaches in cancer biology is context-specific and varies greatly among cancer types with regard to biological feasibility, delivery limits, and therapeutic responses. Thus, referring to cancer as a uniform entity can potentially blur important translational issues and opportunities [181]. The hematologic malignancies, e.g. the leukemias and lymphomas, are especially susceptible to ex vivo genetic engineering efforts, due to the availability of malignant cells, and their compatibility with reinfusion-based treatments [182]. In contrast, solid tumors pose considerable obstacles, such as ineffective delivery in vivo, stromal heterogeneity, hypoxia, and immunosuppressive tumor microenvironments, all of which hinder the success of analogous strategies [183]. Furthermore, the different treatment approaches address radically different molecular mechanisms. As an example, editing oncogenic fusion transcripts (e.g. BCR-ABL in chronic myeloid leukemia) is a direct approach to eradicate driver mutations, but overcoming gene regulatory and functional integration challenges are needed to restore tumor suppressor activity (e.g. TP53 reactivation through gene editing or mRNA delivery) [184]. Similarly, specific protein degradation technology, e.g., PROTACs, has demonstrated potential in degrading estrogen and androgen receptors in breast and prostate cancers, respectively [185].

Simultaneously, immune checkpoint editing (e.g., PD-1/PD-L1 editing or regulation) is another type of therapy that utilizes host immunity, but not tumor cells themselves. New CRISPR-based approaches seek to boost T-cell persistence and overcome exhaustion, but their effectiveness in hematologic and solid tumors differs widely [81]. More recent developments also highlight the role of tumor-specific delivery systems, such as lipid nanoparticles, viral vectors, and tumor-targeted nanocarriers, now being streamlined to overcome anatomical and microenvironmental barriers of solid tumors. Although there is encouraging preclinical evidence, off-target effects, inefficiency in delivery, and heterogeneity of tumors are still a limiting factor in clinical translation [163].

18. Future perspectives

The future of gene editing and targeted protein degradation in cancer therapy is expected to be shaped by advances in artificial intelligence (AI), synthetic biology, personalized medicine, and clinical translation. AI and machine learning are being increasingly used to optimize guide RNA design, predict off-target effects, and identify novel targets within the cancer genome, while also accelerating the discovery of PROTAC molecules through the virtual screening of linker chemistries and E3 ligase binding domains. Synthetic biology further enhances this field by enabling programmable circuits that respond to tumor-specific signals, allowing CRISPR-based editing or PROTAC-mediated degradation to be activated only in malignant cells, thereby improving precision and safety. In parallel, personalized medicine approaches are being advanced through next-generation sequencing and single-cell genomics, which enable the design of individualized therapies that correct patient-specific mutations or degrade proteins associated with resistant tumor subclones. Already, several CRISPR-based therapies, such as edited CAR-T cells and early PROTAC candidates like ARV-110 [186] and ARV-471 [187], are in clinical trials, demonstrating the feasibility of these approaches in oncology. In the near term (approximately 3–5 years), further Phase II/III clinical data are expected to clarify safety and efficacy profiles, while broader clinical adoption and regulatory approvals may be anticipated within the next decade, depending on continued technological and translational advancements. Looking forward, the convergence of durable genome editing with dynamic protein degradation, supported by innovative nanocarriers and immune cell engineering, is likely to redefine cancer therapy. However, achieving this vision will require overcoming regulatory, safety, and scalability challenges. With sustained progress, the integration of CRISPR, PROTACs, and other gene editing platforms holds the potential to establish a new paradigm in precision oncology.

19. Conclusion

Gene editing and targeted protein degradation technologies have emerged as transformative platforms in cancer therapy, offering the ability to intervene at the genetic, transcriptomic, and proteomic levels with unprecedented precision. Tools such as CRISPR-Cas systems, base and prime editors, TALENs, RNA editing strategies, and PROTACs collectively provide complementary modes of action, ranging from permanent correction of oncogenic mutations to reversible elimination of pathogenic proteins. Their synergistic use holds particular promise in overcoming tumor heterogeneity, drug resistance, and the limitations of conventional therapies.

However, despite these advances, several challenges remain. Issues related to delivery efficiency, off-target effects, long-term safety, immunogenicity, and tumor-specific targeting continue to limit widespread clinical translation. In addition, the complexity of tumor biology, interpatient variability, and potential resistance mechanisms necessitate further validation in large-scale clinical studies.

Looking ahead, the successful integration of these tools into clinical oncology will depend on the development of safe and efficient delivery platforms, reduction of off-target effects, and careful navigation of immunogenicity and regulatory challenges. Advances in artificial intelligence, synthetic biology, and nanomedicine are expected to accelerate this transition, enabling the design of highly personalized and programmable therapeutic systems. Ultimately, the roadmap for future cancer therapy points toward a paradigm in which genome editing and protein degradation strategies converge to deliver durable, precise, and patient-specific treatments, while continued efforts to address existing limitations will be critical for realizing their full clinical potential.

Abbreviations

AAV	Adeno-Associated Virus
ABEs	Adenine Base Editors
AI	Artificial Intelligence
AUTACs	AUtophagy-TArgeting Chimeras
CAR-T	Chimeric Antigen Receptor-T
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DSB	Double-Stranded Breaks
GMP	Good Manufacturing Practices
HDR	Homology-Directed Repair
LNPs	Lipid Nanoparticles

LYTACs LYsosome-TArgeting Chimeras
NHEJ Non-Homologous End Joining
PAM Protospacer Adjacent Motifs
PROTACs PROteolysis-TArgeting Chimeras
RNPs Ribonucleoproteins
TALENs Transcription Activator-Like Effector Nucleases
TME Tumor Microenvironment
ZFNs Zinc Finger Nucleases

CRediT authorship contribution statement

Akash Vikal: Writing – original draft, Data curation. **Rashmi Maurya:** Writing – review & editing, Formal analysis. **Pradeep Kumar:** Visualization, Conceptualization. **Navneet Verma:** Supervision. **Arun Kumar Mishra:** Visualization.

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