Alternative splicing in cancer drug resistance: Mechanisms and therapeutic prospects (Review)

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Abstract. Alternative splicing (AS) is one of the principal mechanisms of post-transcriptional regulation that confers transcriptomic plasticity and proteomic diversity in cancer, thereby enabling tumor adaptation to therapeutic pressure. However, two obstacles impede the translation of these findings into clinical benefit: The absence of systematic functional annotation of the numerous splice variants associated with drug resistance and the paucity of biomarkers capable of distinguishing *de novo* from acquired splice-mediated resistance. In the present review, the current mechanistic understanding of AS-driven drug resistance was briefly synthesized, and it was evaluated how existing strategies address these challenges. It was also described how knowledge of dysregulated splicing networks, due to mutations in cis-regulatory elements such as ESS, overexpression of trans-acting factors such as *SRSF1*, as well as mechanisms such as alternative trans-splicing, in which the spliceosome interacts with splice sites on two distinct RNA molecules and which can be driven by complementary sequences or other trans-acting factors, could be used to more accurately identify tumors dependent on aberrant splicing for survival. In addition, it was outlined how targeting aberrant splice variants to overcome therapeutic resistance can be achieved, such as through spliceosome inhibition (for example, H3B-8800) or antisense oligonucleotides directed to a specific exon or splice junction (for example, targeting exon 2 of *MET*, which is implicated in cis-regulated AS isoforms, or alternatively spliced isoforms of *BCL2L1*, *BRAF* and *CDYL*). However, therapeutic strategies to target adaptive resistance mechanisms such as AS remain limited, as intratumoral heterogeneity may facilitate the emergence of resistant subpopulations, and as most spliceosome inhibitors are not spliceosome-specific, they exhibit off-target effects. Importantly, it was also discussed how pan-cancer splicing databases and single-cell isoform expression profiling can be integrated with deep-learning models, thereby informing the design of therapeutic strategies to overcome splicing-mediated adaptive drug resistance. Notably, such integration will enable the rational design of isoform-specific combination regimens to dismantle drug-resistance circuits. It is anticipated that the present review will assist the scientific community, including both basic and translational researchers, in translating these findings into interventions that mitigate therapeutic failure in recalcitrant cancers.

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Abbreviations: ALL, acute lymphoblastic leukemia; AP, alternative promoter; APA, alternative polyadenylation; AR, androgen receptor; AS, alternative splicing; ASO, antisense oligonucleotide; ATS, alternative trans-splicing; CML, chronic myeloid leukemia; circRNA, circular RNA; DIs, detained introns; ecircRNA, exonic circRNA; EIciRNA, exon-intron circRNA; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; ES, exon skipping; HCC, hepatocellular carcinoma; HRR, homologous recombination repair; IR, intron retention; ISE, intronic splicing enhancer; ISS, intronic splicing silencer; LNP, lipid nanoparticle; MDS, myelodysplastic syndromes; MiS, minor spliceosome; MXE, mutually exclusive exons; NMD, nonsense-mediated decay; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; pre-mRNA, precursor mRNA; scRNA-seq, single-cell RNA sequencing; sQTL, splicing quantitative trait loci; SSM, splice-site mutation; TCGA, The Cancer Genome Atlas; TNBC, triple-negative breast cancer; UTR, untranslated region

Key words: AS, drug resistance, circRNA, spliceosome inhibitor, precision oncology

Contents

1. Introduction

Alternative splicing (AS) enables individual genes to produce multiple mRNA isoforms, substantially expanding eukaryotic

proteomic diversity and functional complexity (1,2). Occurring in ~95% of multi-exon genes, AS generates tissue- and context-specific variants that regulate key processes such as proliferation, apoptosis and differentiation (1,3,4). AS is a critical mechanism for regulating gene expression (5). Its biological importance is also highlighted by genomic studies and ~18% of cancer-associated single-nucleotide polymorphisms in the COSMIC database are located within splice-regulatory motifs (6). These mutations compromise exon-intron recognition, often causing the production of aberrant transcripts that support oncogenesis and resistance to therapy. In cancer, aberrant AS causes transcriptomic rewiring that supports tumor heterogeneity and adaptive evolution; for example, splice variants such as *BRCA1*-Δ11q (1) and *BCL2L12* (3) have been identified that interfere with apoptotic signalling, and variants of CD44v, which facilitate epithelial-mesenchymal transition (EMT) and metastasis. Pan-cancer analyses have allowed for the identification of more than 15,000 cancer-specific splice variants, numerous of which are associated with chemoresistance and stemness (7,8). Such alterations are often the result of mutations affecting spliceosomal components (for example, *SF3B1*) or dysregulated splicing factors (for example, *QKI* and *PTBP1*), which can rewire tumor phenotypes allowing them to be more resilient to cell stresses and to survive (8,9).

Despite significant progress in chemotherapy and targeted therapies, drug resistance continues to pose a major challenge in oncology and is responsible for more than 50% of cancer-related deaths, with relapse rates exceeding 70% in aggressive cancers such as pancreatic ductal adenocarcinoma (PDAC) and glioblastoma (3,8). Although numerous established resistance mechanisms involve secondary genetic mutations, including reversion mutations that restore homologous recombination in *BRCA1/2*-mutant cancers, increasing evidence underscores the clinical relevance of dysregulated RNA splicing. For instance, in *BRCA1*-mutant ovarian cancers, secondary splice-site mutations (SSMs) that cause exon skipping (AS) and produce resistant BRCA1 isoforms are detected in roughly 10% of patients following PARP inhibitor therapy, a considerable increase from the pretreatment frequency of ~1% (10). Pan-cancer analyses further reveal that AS produces diverse tumor-specific neoantigens and drug-resistant isoforms that promote immune evasion and treatment failure across cancer types. Although the exact proportion of splicing-mediated resistance has yet to be fully quantified and likely varies by cancer type and regimen, splicing dysregulation constitutes a major non-mutational pathway contributing significantly to the overall resistance burden, second only to genetic alterations (11,12).

AS-mediated mechanisms span diverse therapies: *BRCA1*-Δ11q confers resistance to PARP inhibitors and cisplatin in breast cancer (1), while c-Mpl-del splice variants promote chemoresistance in acute megakaryocytic leukemia (13). Under hypoxic conditions, LUCAT1 recruits *PTBP1* to alter *PARP3* splicing and foster oxaliplatin resistance in colorectal cancer (CRC) (9), paralleled by *FBXO7* stabilization of *RBFOX2* to drive pro-survival *FoxM1* and *POSTN* splicing in temozolomide-resistant glioblastoma (3).

Key splicing factors act as molecular hubs: *QKI* establishes basal-like splicing programs supporting PDAC chemoresistance (8), while SNHG family lncRNAs employ intron retention (IR) to suppress snoRNAs and enhance hepatoblastoma drug tolerance (14). Although hypoxia-induced LUCAT1 binds PTBP1 to modulate DNA damage response genes (9), the hierarchical organization of these regulatory networks remains undefined. Elucidating these mechanisms will require integrated approaches to identify master regulators and advance isoform-directed therapeutic strategies.

2. Molecular architecture of AS in cancer

Classification of AS events

Canonical AS classification and the splicing resistance map framework. Transcripts from nearly all human protein-coding genes undergo one or more modes of AS, such as inclusion or skipping of individual 'cassette' exons, selection between alternative 5' and 3' splice sites, differential retention of introns, mutually exclusive splicing of adjacent exons, and other, more complex patterns of splicing (15). These diverse outcomes are broadly classified into classical patterns of AS, such as ES, IR, alternative promoter (AP) usage, alternative polyadenylation (APA), alternative 3' splice site, alternative 5' splice site and mutually exclusive exons (MXE) (Fig. 1). These canonical splicing patterns constitute the foundational elements of the 'splicing resistance map', a unified landscape of AS events that collectively underlie therapeutic resistance across diverse cancers. In The Cancer Genome Atlas (TCGA) pan-cancer analysis, AP and ES were the most prevalent splicing patterns; importantly, these frequencies were derived from bulk tumor transcriptomes and therefore reflect composite signals from malignant and stromal/immune cells (16) (Fig. S1).

ES is reported to be one of the most common AS event, characterized by loss of functional domains/sites or a shift in the open reading frame, leading to a variety of human diseases (17), which involves the exclusion of specific exons during mRNA maturation, often modifying protein domains critical for tumor progression (18-20). ES events constitute a prominent component of the splicing resistance map, yielding isoforms that circumvent targeted therapies. For example, in triple-negative breast cancer (TNBC), *PRMT5* inhibition induces ES in AURKB transcripts, resulting in mitotic catastrophe and chemoresistance by disrupting chromatin stability (21). In the mesenchymal subtype of CRC (CMS4), retention of exon 17 in the *MYOF* gene and skipping of exon 11 in the *ENAH* gene underlie poor prognosis and enhanced chemoresistance by promoting EMT and augmenting tumor invasiveness (22). IR, once considered rare, is now recognized as a hallmark of aggressive malignancies (23-26). IR events represent a major axis of the splicing resistance map, particularly in advanced and therapy-refractory malignancies. In prostate cancer, IR induces the accumulation of aberrant transcripts (for example, SNHG19), which fosters castration resistance and stemness by impairing DNA repair pathways, enhancing chemoresistance, and activating oncogenic cascades, thereby nominating the spliceosome as a promising therapeutic target whose inhibitors reverse IR-mediated aggressive phenotypes (27,28).

APs, involve the use of different transcription start sites within a gene, thereby altering the 5'-untranslated regions (UTRs) sequences to generate distinct variants. Promoters not only determine when a gene is active and to what extent, but also regulate which gene isoforms are expressed (29).

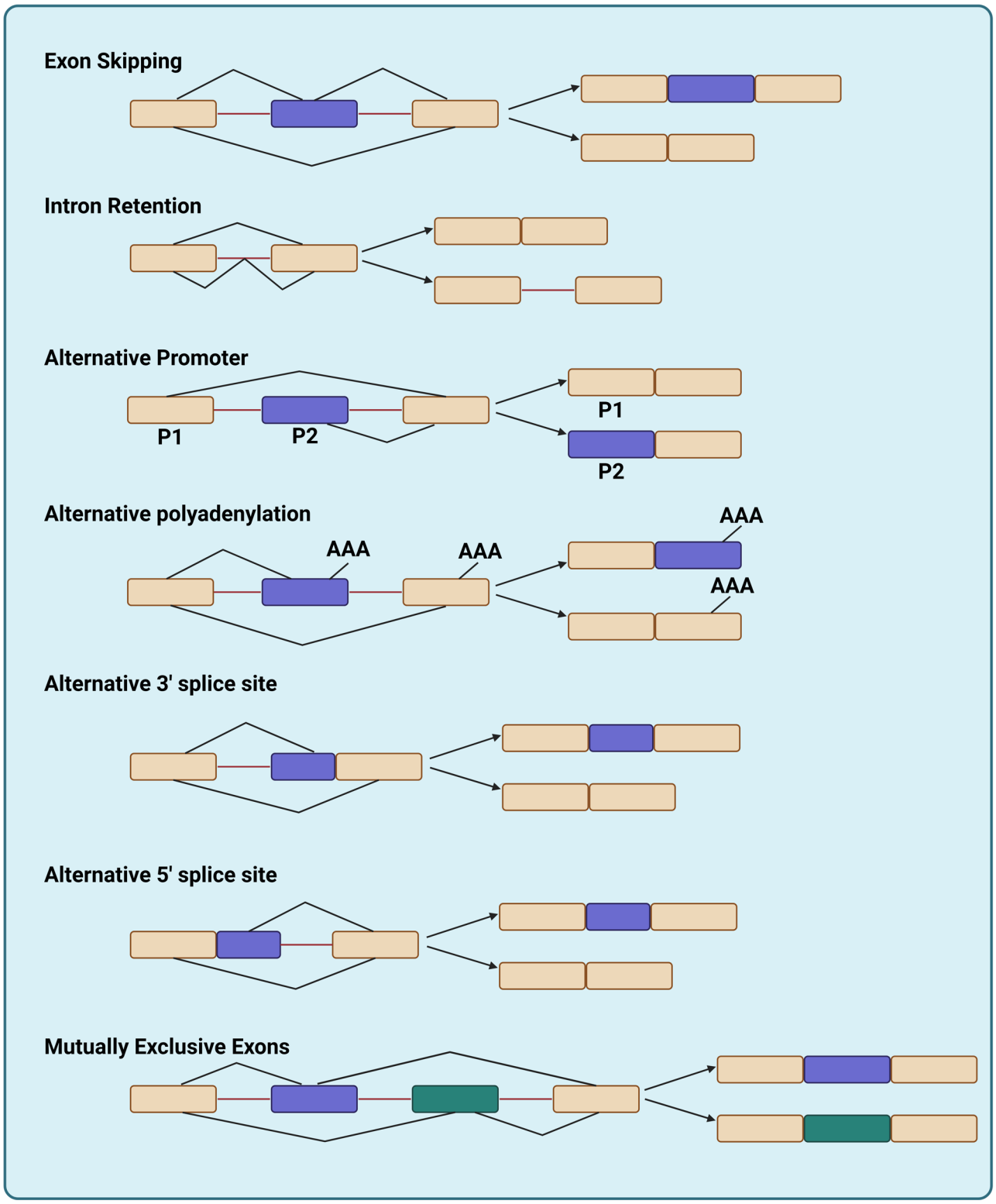

Figure 1. Splicing resistance map: Canonical classification of alternative splicing events. A schematic overview of the seven canonical types of AS events that constitute the foundational elements of the oncogenic 'splicing resistance map': Exon skipping also called cassette exon, intron retention, alternative promoter, alternative polyadenylation, alternative 3' splice site, alternative 5' splice site (5' SS) and mutually exclusive exons. This map depicts the repertoire of evolutionarily conserved AS mechanisms that are hijacked in cancer to enhance tumor survival, proliferation, and resistance to therapy. The pan-cancer prevalence of each event type is quantitatively summarized in Fig. S1. AS, alternative splicing.

AP-mediated isoform switching constitutes a central mechanism within the splicing resistance map, facilitating adaptive transcriptional reprogramming under therapeutic pressure. APs enable tissue-specific isoform expression, as observed in MET exon 14 skipping in non-small cell lung cancer (NSCLC), where splice site mutations produce oncogenic isoforms that evade kinase inhibitor targeting (30). In prostate cancer, distal APs (P2/P3) supersede the proximal promoter (P1) to drive

UGT2B17 transcription, generating n2/n3 isoforms, which contain additional 5' exons (for example, 1b/1c) through AS and encode full-length proteins, that dominate the androgen inactivation pathway in localized and metastatic tumors (31). In liver cancer, selective DNA methylation loss in CpG-poor regions increases chromatin accessibility, thereby enabling the binding of tissue-specific transcription factors and activation of APs, which drive tumor-specific transcription (32). These canonical AS patterns are systematically cataloged in large-scale projects such as TCGA, which demonstrated that >90% of cancer-related genes exhibit at least one AS event (33). Computational analysis of these splicing events employs specialized tools including the SpliceSeq package, which aligns RNA-Seq reads to a gene-specific exon junction graph and computes Percent Spliced In values to systematically quantify exon or splice junction inclusion across numerous tumor and normal samples (34). Long-read sequencing has recently revealed that 30% of isoforms in breast cancer contain unannotated ES or IR events, numerous of which are associated with HER^{2+} or TNBC subtypes (21,33). Other common splicing classes include MXE, exemplified by CD44 variants that promote ovarian cancer metastasis, and alternative 3'/5' splice site selection that alters EGFR kinase domains to confer tyrosine kinase inhibitor resistance (35).

Functionally classified isoforms in therapy resistance. New classification systems focus on AS events that directly influence therapeutic response, particularly those enabling drug resistance. Understanding these mechanisms requires precise definition of 'therapy-resistance-associated isoforms', alternatively spliced mRNAs experimentally demonstrated to confer resistance by modifying drug targets, activating compensatory pathways, or enhancing survival (10-12). To qualify, isoforms must satisfy three criteria: i) functional validation through drug sensitivity assays; ii) association with a specific treatment; and iii) identification in human tumors or clinically relevant models.

Numerous examples demonstrate this concept. In PARP inhibitor-resistant ovarian cancer, *BRCA1* exon 11 skipping generates Δ11q isoforms that restore homologous recombination repair (HRR) capacity, circumventing nonsense-mediated decay (10). Similarly, *NT5C2* exon 6a inclusion yields a hyperactive nucleotidase conferring 6-mercaptopurine resistance in leukemia (36). NSCLC with *KRAS* G12 mutations exhibits *MET* exon 14 skipping that activates RAS/ERK signaling to evade MET inhibitor effects (30). Splicing alterations also perturb epigenetic regulation: PRMT5 inhibition in CDK4/6 inhibitor-resistant breast cancer induces FUS-dependent IR, impairing DNA synthesis proteins such as PCNA and MCM2 (37). Given their clinical relevance, these mechanisms are emerging as therapeutic targets themselves, for instance, CRISPR/dCasRx-mediated exclusion of *TIMP1* exons counteracts SRSF1-driven splicing and attenuates metastasis in CRC (38). Such subclassifications are transforming precision oncology, with clinical trials currently evaluating splice-switching antisense oligonucleotides (ASOs) targeting *PTBP1* in glioblastoma (39). Additionally, APA events in *BCL2L1* produce anti-apoptotic isoforms in hepatocellular carcinoma (HCC), while poison exon inclusion in *MAP3K7* disrupts TGF-β signaling to foster chemoresistance (27,35). Isoforms are excluded from this category if evidence is purely computational or derived from correlative expression without direct functional proof, or if the resistance mechanism is indirect (for example, via general proliferation effects) (10-12).

Representative isoforms that satisfy these criteria, highlighting their drug contexts, functional validation methods, and clinical evidence levels are presented in Table I.

This systematic compilation underscores the diversity of splicing-mediated resistance mechanisms, which often coexist with genetic mutations and contribute substantially to treatment failure. Functional validation through targeted assays (for example, CRISPR and ASOs) is essential to establish causality, while clinical evidence ranges from preclinical models to direct patient data, highlighting the translational relevance of these isoforms (10,12,40).

Cis-regulatory elements and trans-acting factors

Cis-regulatory elements and trans-acting factors form the molecular scaffold of AS regulation in cancer. Cis-acting elements, including exonic/intronic splicing enhancers (ESE/ISE) and silencers (ESS/ISS), are nucleotide sequences within pre-mRNA that recruit trans-acting regulators (for example, SR proteins, hnRNPs) to govern splice site selection (41,42), Cell-type specific gene expression patterns are regulated by cis-regulatory elements such as promoters and enhancers (43) (Fig. 2). Moreover, the regulation of AS entails recognition of cis-acting RNA elements in the pre-mRNA by trans-acting protein splicing factors (44). These elements operate combinatorially to enhance or repress spliceosome assembly, with mutations or dysregulated expression commonly observed in cancer (12). For example, *BCL2L1* ESE mutations drive pro-survival *BCL2L1-L* isoform expression, conferring apoptosis resistance (40). Genome-wide analyses indicate that ~15% of cancer-associated mutations map to splicing regulatory elements, with ESE/ESS regions in oncogenes for example, *MET*) exhibiting recurrent alterations (12,45).

Integrated cis-trans-epigenetic mechanisms enable adaptive therapy resistance. Chronic myeloid leukemia (CML) cells exhibit ESS defects in SRSF1-targeted transcripts (for example, *PRKCH*), sustaining oncogenic signaling despite *BCR-ABL1* inhibition (46). Glioblastoma-associated *SF3B1* inhibition alters *BCL2L1* splicing, favoring anti-apoptotic isoform expression (47). High-throughput studies identify recurrent ESE/ESS mutations in drug resistance genes (for example, *ABCB1*), which disrupt repressive elements to circumvent therapy (44). For example, aberrant ESS motifs in TP53 exon 6 promote ES, generating dominant-negative isoforms that compromise chemotherapy-induced apoptosis (40). SR proteins (for example, SRSF1) and hnRNPs (for example, hnRNP A1) are frequently overexpressed in therapy-resistant cancers. In CML, SRSF1 upregulation enhances *BCL2L1* exon 2 inclusion, elevating pro-survival BCL2L1-S isoforms and undermining imatinib-induced apoptosis (46). SF3B1 dysfunction in glioblastoma drives BCL2L1-L overexpression via preferential 3' splice site selection, facilitating temozolomide evasion (47). Post-transcriptional modifications further modulate splicing factor activity: METTL3-mediated m6A methylation stabilizes SRSF1 mRNA in pancreatic cancer, potentiating cisplatin resistance (48). Epigenetic regulation, encompassing DNA methylation and RNA epitranscriptomics (for example, m6A), modulates splicing by altering

Table I. Exemplary therapy-resistance-associated isoforms.

First author/s, year	Isoform	Gene	Drug context	Functional assay	Clinical evidence level	(Refs.)
Nesic *et al*, 2024	Δ11q	BRCA1	PARP inhibitors (olaparib, rucaparib)	RNA-seq, minigene assays, CRISPR/Cas9	Patient biopsies, PDX models	(10)
Bradley and Anczuków, 2023	BQ323636.1	NCOR2	Tamoxifen, aromatase inhibitors (anastrozole)	RNA-seq, immunohistochemistry, siRNA knockdown	Patient tissue microarrays, cell lines	(11)
Sciarrillo *et al*, 2020	AR-V7	AR	Androgen receptor inhibitors (enzalutamide, abiraterone)	RT-qPCR, RNA-sequencing, circulating tumor cell analysis	Patient circulating tumor cells	(40)
Kahles *et al*, 2018	PKM2	PKM	Metabolic inhibitors (for example, glycolysis inhibitors)	siRNA knockdown, CRISPR screening	Pan-cancer TCGA data, cell lines	(12)
Sciarrillo *et al*, 2020	HER2Δ16	ERBB2	Trastuzumab	Immunoblotting, colony formation assays	Breast cancer patient biopsies	(40)
	BRAF p61	BRAF	Vemurafenib	RNA-seq, protein immunoprecipitation	Melanoma biopsies	
	CD44v6	CD44	5-FU, radiation therapy	Antibody blockade, RNA interference	Patient tumor samples, cell lines	
	MENAINV	ENAH	Paclitaxel	Invasion assays, isoform-specific antibodies	Breast cancer metastases	
	BARD1β	BARD1	PARP inhibitors	Immunoblotting, homologous recombination assays	Colon cancer cell lines	
	KLF6-SV1	KLF6	Sorafenib, cisplatin	Apoptosis assays, xenograft models	Hepatocellular carcinoma samples	
	CHK1-S	CHEK1	DNA-damaging agents (for example, topotecan)	Kinase activity assays, survival analysis	Patient tumor tissues	
	RonΔ165	MST1R	MET inhibitors (crizotinib)	Migration assays, immunoblotting	PDX models, NSCLC cell lines	
	FGFR1β	FGFR1	FGFR inhibitors (erdafitinib)	Proliferation assays, reverse transcription PCR	Bladder cancer models	
	IL-6R soluble	IL6R	Tocilizumab	ELISA, signaling pathway analysis	Patient serum samples	
	CD19e2Δ	CD19	CAR-T therapy	Flow cytometry, sequencing	B-ALL patient samples at relapse	
	CD22e2Δ	CD22	CAR-T therapy	Isoform-specific PCR, cytotoxicity assays	B-cell malignancy models	
	USP5s	USP5	Gemcitabine	Splicing modulation, drug sensitivity tests	Glioma cell lines	
	MDM4-S	MDM4	MDM2 inhibitors (nutlin-3)	Apoptosis assays, xenograft studies	Patient tumor tissues	
	SRSF3-S	SRSF3	Paclitaxel	Colony formation, ASO treatment	Oral squamous cell carcinoma	
	circCDYL2	CDYL2	Cisplatin, radiation	RNAi, miRNA sponging assays	Patient plasma, xenograft models	
	VEGFxxxb	VEGFA	Anti-angiogenic therapies (bevacizumab)	Splicing reporter assays, tube formation assays	Patient serum and tumor tissues	

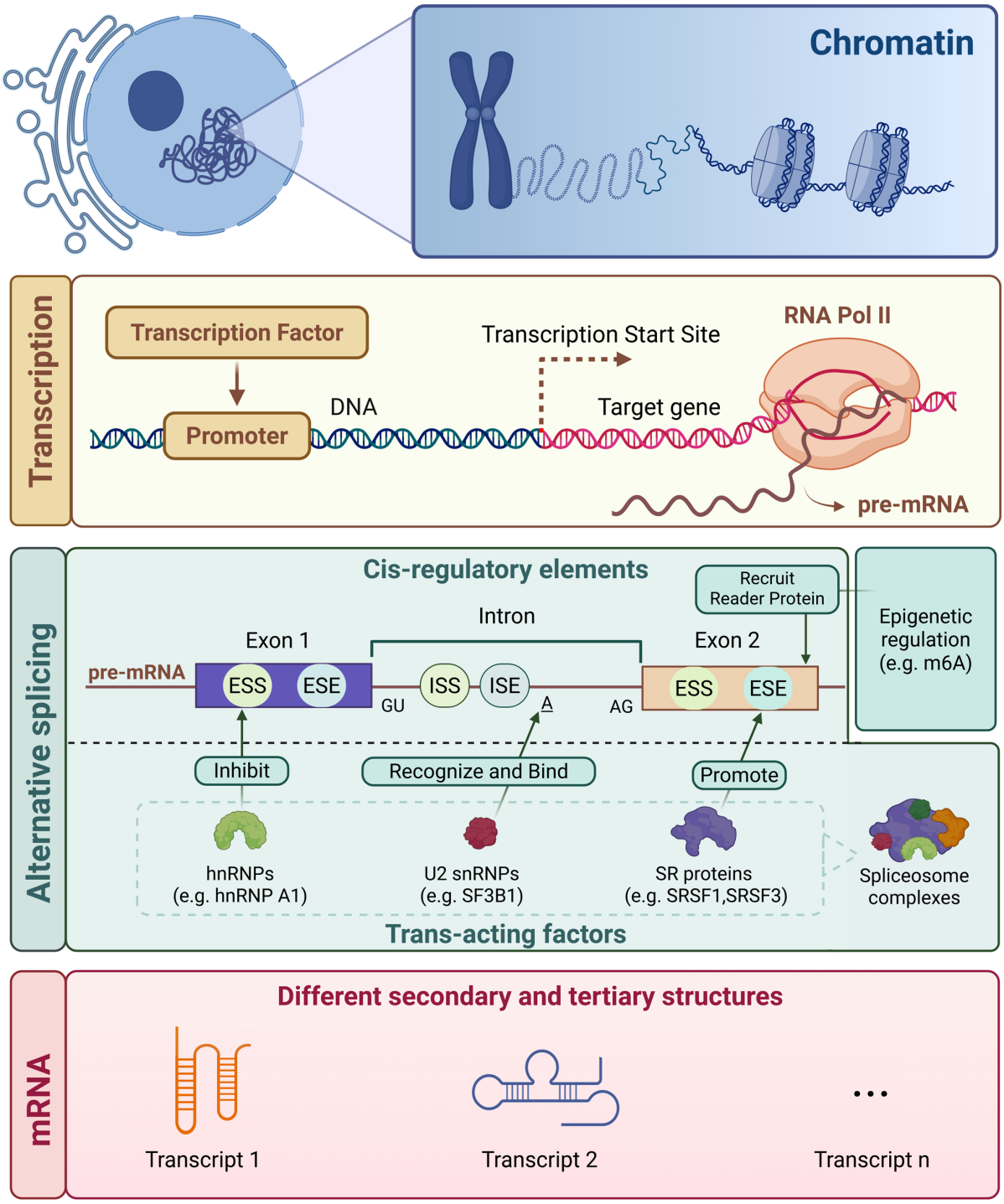

Figure 2. Alternative splicing mechanism and spliceosome complex proteins with their functions. Pre-mRNA splicing is the process of removal of intronic regions and joining the exons to form mature mRNA. Trans-acting factors consist of splicing factors which recognize conserved premRNA sequences (cis-regulatory elements) and recruit the core spliceosome machinery which coordinates and executes the excision of introns. U2 snRNP (for example, SF3B1) recognizes and binds to branch point A, initiating spliceosome assembly and splicing reactions; SR proteins (for example, SRSF1) primarily bind to ESE elements (with partial assistance from ISE elements) to promote splicing; hnRNPs (for example, hnRNP A1) inhibit splicing by binding to ESS/ISS elements; epigenetic regulation (for example, m6A modification) acts on exons through recruiting reader proteins (for example, YTHDC1). These factors collectively act on pre-mRNA, regulating alternative splicing and ultimately generating distinct mRNA transcripts. ESE, exonic splicing enhancer; ISE, intronic splicing enhancer; ESS, exonic splicing silencer; ISS, intronic splicing silencer.

RNA-protein interactions (49-51). In gastric cancer, *SNORA37* promotes *CMTR1-ELAVL1* interaction to drive CD44 AS and tumor progression (52). Dysregulation of the epigenome drives aberrant transcriptional programs that promote cancer onset and progression (53,54). *METTL3* deposits m6A on *QSOX1* exon 14, recruiting YTHDC1 to enhance exon inclusion and β-catenin activation, thereby conferring gefitinib resistance in NSCLC (55). Conversely, ALKBH5 erases m6A from *FOXO1* mRNA, enabling SRSF3 binding and anti-apoptotic isoform generation (55). Chromatin remodelers (for example,

SWI/SNF) also influence splicing: *ARID1A* loss induces *BRD9* ES, activating Wnt signaling in ovarian cancer (44). These findings collectively define a dynamic 'splicing code' that integrates cis-regulatory mutations, trans-factor dysregulation and epigenetic modifications, a multilayered system that enables tumor cells to rapidly adapt and develop resistance to targeted therapies, chemotherapy, and epigenetic drugs (Fig. 2).

Splicing dysregulation: Protein alterations and cis-element mutations. Altered spliceosome proteins due to somatic mutations, overexpression or post-translational modifications are key drivers of aberrant splicing that promotes tumour survival and therapy resistance. Mutations in *SF3B1* in glioblastoma lead to ES such as *BRCA1-Δ11q* that results in a hypomorphic isoform that provides resistance to PARP inhibition (12). In CML, SRSF1 is overexpressed in leukemic blasts and IL-3 maintains SRSF1 expression following *BCR-ABL1* inhibition, leading to imatinib resistance via PKC- and PLC-dependent mechanisms (46). These events promote proliferation and inhibit apoptosis providing a selective advantage in cancer.

DNA cis-element mutations (for example, in exonic splicing silencers) disrupt trans-factor binding, leading to aberrant splicing and drug resistance. In acute lymphoblastic leukemia (ALL), *NT5C2* exon 6a inclusion creates a gain-of-function isoform with elevated nucleotidase activity, driving resistance to 6-mercaptopurine (36). These mutations are enriched in relapse samples and represent promising targets for ASOs due to their sequence-specific nature; ASOs can be designed to bind mutant cis-elements or aberrant splice sites, blocking incorrect splicing and restoring normal isoform expression. This re-sensitizes cells to therapy, as demonstrated in leukemia models where ASOs reversed chemoresistance by correcting *NT5C2* splicing (Figs. 3B and 4) (36).

3. AS-driven drug resistance mechanisms

Cis splicing in drug resistance

Overview of cis splicing. In cancer, dysregulated cis splicing promotes tumor progression and therapy resistance by altering protein function, stability, or subcellular localization (56). For instance, aberrant splicing of apoptosis regulators, oncogenic kinases and drug transporters directly affects treatment efficacy and disease relapse (10). The clinical relevance of cis-splicing is underscored by its association with poor prognosis and resistance to targeted therapies, immunotherapies and chemotherapy (57).

ES. AS-mediated exclusion of *ABL1* exon 2 results in an in-frame deletion of SH3 domain residues. This 31-amino-acid deletion in BCR-ABL1 weakens asciminib binding by 18-fold and impairs the autoinhibitory conformation required for drug efficacy, conferring resistance in CML (58).

In *BRCA1*, secondary SSMs induce exon 11 skipping, producing truncated Δ11 and Δ11q isoforms that restore HRR capacity. These isoforms allow tumor cells to bypass PARPi-induced synthetic lethality (10).

In relapsed ALL, the inclusion of exon 6a introduces a novel phosphorylation site that promotes *NT5C2* dimerization, leading to a 15-fold increase in enzymatic activity of the protein compared with the wild-type protein (36).

IR. IR and the more recent concept of 'detained introns' (DIs), a type of IR, are critical splicing events that drive resistance to cancer therapies (59,60). DIs are introns that are retained in polyadenylated transcripts owing to a stalled co-transcriptional splicing process. These splicing events act as rapid and reversible regulators of gene expression, whereas classical IR, is defined by persistent IR in mature mRNAs.

In glioblastoma, multiple of splicing mechanisms cooperate to mediate both acute and chronic therapy resistance. Chemoradiation with agents such as temozolomide stalls RNA polymerase II elongation and induces DIs in *MLH1* through H3K9 lactylation-dependent upregulation of *LUC7L2* (62), which introduces a frameshift mutation truncating the protein and leading to mismatch repair (MMR) dysfunction. The functional loss of MMR leads to acute chemoresistance. These DIs are capable of acting as rapid stress sensors, such that inhibition of lactylation rescues *MLH1* splicing and re-sensitizes the tumors to treatment (63). In addition, NONO-induced IR in *GPX1* results in the loss of antioxidant activity and an increase in reactive oxygen species which leads to apoptosis (63). Radiation further stalls splicing progression, converting the acute DI response into chronic IR in an epigenetically dependent manner, highlighting how stress duration mediates resistance plasticity (61).

In CRC, SRSF5-induced IR in *PKM* intron 9 leads to the upregulation of PKM2, which increases glycolysis and enables survival under 5-fluorouracil-induced oxidative stress (56).

In AML, loss of *RBM39* leads to widespread IR promoting the nonsense-mediated decay (NMD) of pro-leukemic transcripts including *HOXA9* targets (64). IR in spliceosome mutant cells mediates their acute vulnerability to this mechanism, providing a measurable biomarker of therapeutic response. Chronic *RBM39* inhibition leads to the consolidation of the IR state promoting continual NMD.

In myelodysplastic syndromes (MDS), loss of *ZRSR2* causes IR in a subset of minor introns in *LZTR1* inducing NMD and subsequent RAS pathway activation, which drives leukemogenesis in these patients (65). Taken together, chemoradiation stalls co-transcriptional splicing, inducing DIs as an acute, reversible response to therapy (61). Chronic stress promotes the conversion of DIs into stable IR states, allowing chronic resistance to be established. This dichotomy explains how tumors adapt rapidly (via DIs) and persist (via IR) under therapeutic pressure (Fig. 3A and B).

Back-splicing and circRNA-driven drug resistance.

Back-splicing: Mechanisms, classification and functional roles in drug resistance. Back-splicing is a non-canonical RNA processing mechanism distinct from linear splicing, wherein a downstream splice donor site covalently joins an upstream splice acceptor site, forming a closed-loop RNA structure called circular RNA (circRNA) (66). Numerous protein-coding genes in higher eukaryotes are capable of producing circRNAs through back-splicing of exons (67-69). This process competes with canonical splicing for splice sites (70-72), resulting in exonuclease-resistant circRNAs that lack 5' caps and 3' poly(A) tails, thereby enhancing their stability and allowing secretion via extracellular vesicles (73,74). Experimental evidence underscores the remarkable stability of circRNAs; for instance, Enuka *et al* (75) demonstrated, using metabolic labeling that the

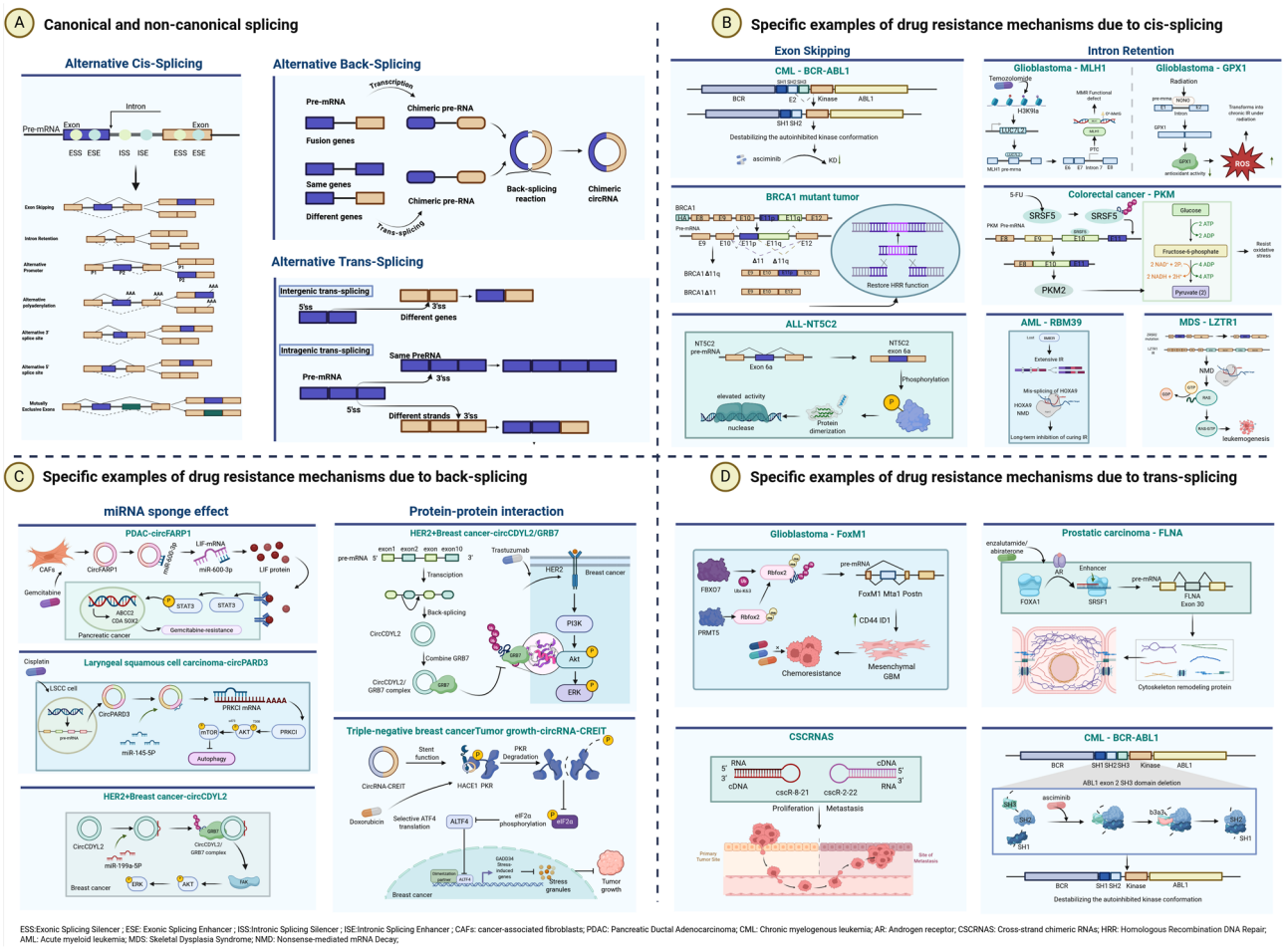

Figure 3. Canonical vs. non-canonical splicing events and their associated resistance mechanisms. (A) The AS can be classified into two categories, canonical splicing events: ACS and non-canonical splicing events: ATS and ABS. There are seven subtypes of ACS, consistent with the content in Fig. 1. ABS is a non-canonical RNA processing mechanism distinct from linear splicing, wherein a downstream splice donor site covalently joins an upstream splice acceptor site, forming a closed-loop RNA structure called circular RNA (circRNA). ATS connects fragments from two distinct primary RNA transcripts generating chimeric RNAs. ATS can be classified into two types based on the origin of the primary RNA transcripts, involving intragenic and intergenic trans-splicing. (B) Specific examples of drug resistance mechanisms due to cis-splicing. Exon skipping (left panel) confers resistance through alterations in protein structure and function. Examples include: deletion of the SH3 domain in BCR-ABL1, which reduces asciminib binding in chronic myeloid leukemia (CML); skipping of exon 11 in BRCA1, which restores homologous recombination repair (HRR) and leads to PARP inhibitor (PARPi) resistance; and inclusion of exon 6a in NT5C2, which promotes dimerization and enhances enzymatic activity in acute lymphoblastic leukemia (ALL). Intron retention (right panel) facilitates resistance via rapid stress response and regulation of gene expression. Key instances are: temozolomide-induced detained introns in MLH1, resulting in mismatch repair (MMR) deficiency in glioblastoma; radiation-induced intron retention in GPX1, mediated by NONO, that impairs its antioxidant function, elevates reactive oxygen species (ROS), and promotes radiation resistance in glioblastoma; SRSF5-mediated retention of intron 9 in PKM, upregulating PKM2 to enhance glycolysis and confer oxidative stress resistance in colorectal cancer; widespread intron retention following RBM39 loss, which triggers nonsense-mediated decay (NMD) of oncogenic transcripts in acute myeloid leukemia (AML); and finally, intron retention in LZTR1 in myelodysplastic syndromes (MDS) that evades NMD, thereby activating the RAS pathway and driving leukemogenesis. (C) Specific examples of drug resistance mechanisms due to back-splicing. The miRNA sponge mechanism (left panel) is illustrated by the following: exosomal circFARP1 derived from cancer-associated fibroblasts sequesters miR-660-3p, leading to upregulation of LIF and activation of STAT3 signaling, thereby promoting stemness and gemcitabine resistance in pancreatic ductal adenocarcinoma; circPARD3 acts as a sponge for miR-145-5p, activating the PRKCI/AKT/mTOR pathway to suppress autophagy and induce cisplatin resistance in laryngeal squamous cell carcinoma; and circCDYL2 sequesters miR-199a-5p, resulting in elevated GRB7 expression and subsequent FAK signaling activation, which contributes to trastuzumab resistance in HER2-positive breast cancer. Protein-protein interactions (right panels) are demonstrated through two examples: circCDYL2 directly binds to and stabilizes GRB7, sustaining HER2/AKT/ERK signaling; and circRNA-CREIT scaffolds the HACE1-mediated degradation of PKR, which disrupts stress granule formation and promotes doxorubicin resistance in triple-negative breast cancer. (D) Specific examples of drug resistance mechanisms due to trans-splicing. In glioblastoma, Rbfox2 stabilized by FBXO7 promotes trans-splicing, generating FoxM1 variants that upregulate stemness markers (for example, CD44, ID1) and confer chemoresistance. In prostate cancer, FOXA1 facilitates the inclusion of FLNA exon 30 via trans-splicing, resulting in cytoskeletal remodeling and resistance to androgen receptor inhibitors. Trans-splicing between sense and antisense transcripts also produces cross-strand chimeric RNAs (such as cscR-8-21 and cscR-2-22), which contribute to cancer cell survival and have been implicated in therapy resistance. Furthermore, in chronic myeloid leukemia, trans-splicing gives rise to truncated BCR-ABL1 isoforms (for example, b3a3) that lack the SH3 domain, leading to constitutive kinase activity and resistance to allosteric inhibitors such as asciminib. ACS, alternative cis-Splicing; ATS, alternative trans-splicing; ABS, alternative back-splicing

median half-life of circRNAs in mammary cells ranges from 18.8-23.7 h, significantly longer than the 4.0-7.4 h observed for linear RNAs. Their exceptional longevity makes circRNAs well-suited as biomarkers, a feature demonstrated across multiple studies to stem from their covalently closed circular architecture which confers intrinsic resistance to exonuclease degradation (76-78). CircRNAs are broadly classified into three subtypes: Exonic circRNAs (ecircRNAs), intronic

circRNAs and exon-intron circRNAs (EIciRNAs), with ecircRNAs being the most prevalent and functionally characterized in cancer (79) (Fig. 3A). Their aberrant expression in cancers provides insights into roles in tumorigenesis (80), and they can act as miRNA sponges, protein scaffolds or peptide templates (74,81). For example, circCDYL2 in *HER*$^{2+}$ breast cancer stabilizes GRB7 to sustain HER2/AKT/ERK signaling and confers trastuzumab resistance (73), while circPARD3 in laryngeal squamous cell carcinoma sponges miR-145-5p to activate PRKCI/AKT/mTOR signaling and suppress autophagy, promoting chemoresistance (79). The conservation of back-splicing and its dysregulation in tumors highlight its role in therapy evasion, with circRNAs like circRNA-CREIT in TNBC serving as biomarkers for poor prognosis under doxorubicin treatment (74).

Role of the circFARP1 miRNA sponge effect in gemcitabine resistance through the miR-660-3p/ leukemia inhibitory factor (LIF) axis. CircRNAs may act as competing endogenous RNAs to sponge miRNAs and relieve repression of oncogenic targets. In PDAC, cancer-associated fibroblasts secrete exosomal circFARP1, which sponges miR-660-3p to upregulate LIF, activating STAT3 signaling to induce stemness and gemcitabine resistance (81). In this way, circFARP1 may bind miR-660-3p to derepress the cytokine LIF, which in turn promotes IL-6/STAT3 survival signaling pathways (81). Likewise, circPARD3 acts as a sponge for miR-145-5p, leading to upregulation of PRKCI to activate AKT/mTOR signaling and inhibit autophagy to enhance cisplatin resistance in laryngeal squamous cell carcinoma (79). Such circRNA/miRNA/protein signaling axes are often amplified in tumor cells that are resistant to therapy, for example, circCDYL2 sponges miR-199a-5p to upregulate growth factor adaptor protein GRB7 and focal adhesion kinase signaling in *HER*$^{2+}$ breast cancer (73). CircRNA sponging can also be blocked in tumor cells to sensitize them to chemotherapy such as with anti-circFARP1 small interfering RNA (siRNA) to to restore miRNA activity (Fig. 3C) (81).

CircRNA-protein interactions in therapy resistance: CircCDYL2/growth factor receptor-bound protein 7 (GRB7) complex formation and circRNA-CREIT-mediated protein kinase R (PKR) degradation. CircRNAs also participate in direct protein interactions that modulate cellular function. In *HER*$^{2+}$ breast cancer, circCDYL2 binds GRB7, protecting it from ubiquitination, and thereby maintaining HER2/AKT/ERK signaling in the presence of trastuzumab (73). This leads to *HER*$^{2+}$ tumors exhibiting resistance to *HER*$^{2-}$ targeted therapies (73). In TNBC, circRNA-CREIT acts as a molecular scaffold to bring HACE1 (HACE1 ubiquitin protein ligase) and PKR together, leading to degradation of PKR and disruption of stress granules, and thereby allowing the tumor to exhibit resistance to doxorubicin (74). These protein-interaction mechanisms establish circRNAs as versatile regulators of therapeutic resistance, suggesting that targeting such interfaces, for instance through GRB7 inhibition, may represent a promising approach to reestablish treatment sensitivity (Fig. 3C).

Trans-splicing. Definition, mechanisms and classification of alternative trans-splicing (ATS). ATS generates chimeric mRNAs by joining exons from two distinct pre-mRNA transcripts through spliceosome-mediated ligation (82,83). Unlike conventional cis-splicing, ATS connects splice sites located on different RNA molecules, often mediated by complementary base pairing or trans-acting factors (4,84). The process involves coordinated recruitment of spliceosomal components, including U1 and U2 snRNPs, to donor and acceptor sites on distinct pre-mRNAs, enabling exon joining via transesterification reactions (11). ATS events are classified as intergenic when combining exons from different chromosomal loci or intragenic when linking exons from alternative transcripts of the same gene (4,12) (Fig. 3A). This mechanism is thus expected to substantially increase transcript diversity in cancers and likely underlies oncogenic adaptation in treatment-resistant tumors (84). Although fusion genes such as *ABL/BCR* are widely considered to arise from translocations such as the Philadelphia chromosome, ATS alone can generate similar chimeric transcripts at the RNA level.

In CML, ATS gives rise to fusion transcripts such as CLEC12A-MIR223HG, which has been found in both malignant and normal cells, demonstrating its capacity to diversify the transcriptome in the absence of DNA rearrangement (85). ATS variants of the *BCR-ABL* oncogene may escape detection or cause resistance to imatinib due to an altered configuration of the kinase domain (58). By augmenting the functional transcriptome beyond what the genome can encode, ATS can increase the adaptability of tumor cells for oncogenic adaptation under therapeutic pressure (86,87).

Mechanisms of ATS in drug resistance. ATS confers drug resistance through the production of oncogenic chimeric transcripts or via the disruption of drug-target interactions. In glioblastoma, *FBXO7* stabilizes *RBFOX2* to induce trans-splicing events that produce FOXM1 variants, which further promote the expression of stemness-related markers such as CD44 and ID1, thereby conferring chemotherapy resistance (3) (Fig. 3D). Apart from resistance to chemotherapy, ATS may also play a role in immune escape mechanisms. Pan-cancer analyses have identified tumor-specific splicing junctions, or 'neojunctions', that produce peptides not found in normal tissues, and computational predictions suggest these peptides may serve as ligands for MHC-I proteins and thereby influence the response to immune checkpoint inhibitors (12). Trans-splicing between *BCL2L1* and adjacent genes generates truncated BCL2 variants that impair chemotherapy-induced apoptosis (11). These mechanisms highlight how ATS diversifies the transcriptome to promote survival under therapeutic pressure.

Case studies of ATS in drug-resistant cancers. Specific ATS events are linked to therapy resistance. In prostate cancer, FOXA1 orchestrates ATS by binding enhancer regions of splicing factors such as SRSF1, leading to exon 30 inclusion in *FLNA*, which stabilizes isoforms promoting cytoskeletal remodeling and resistance to androgen receptor (AR) inhibitors (88). Additionally, FBXO7-driven trans-splicing in glioblastoma stabilizes RBFOX2, which coordinates splicing of mesenchymal genes such as *POSTN* to augment temozolomide resistance through integrin-mediated survival signaling (3). Furthermore, cross-strand chimeric RNAs (cscRNAs) produced by ATS between sense and antisense transcripts have been identified in multiple cancer cells. For instance, in prostate cancer PC3 cells and HCC Huh7 cells,

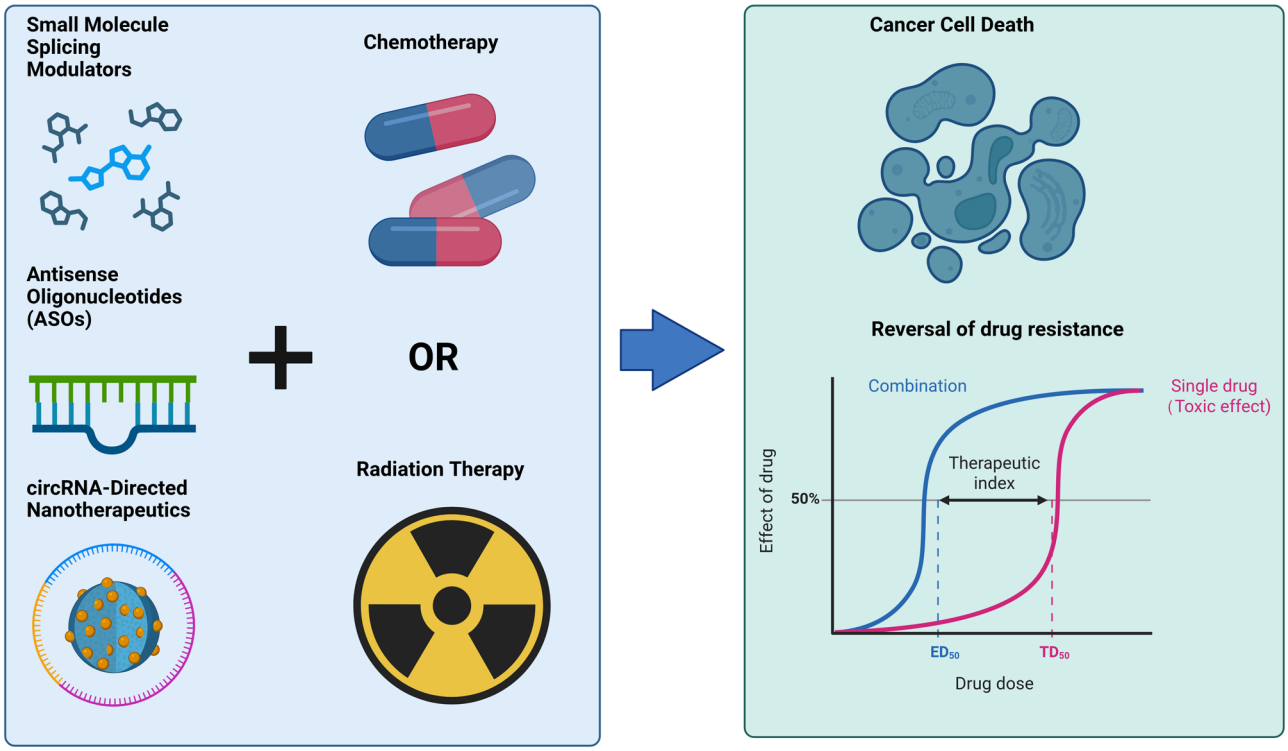

Figure 4. Multimodal strategies to overcome cancer drug resistance. When combined with standard chemotherapy or radiotherapy, appropriately dosed splicing modulators, antisense oligonucleotides, and circRNA-directed nanotherapeutics produce synergistic antitumor responses. This approach counteracts drug resistance by correcting aberrant splicing and concurrently reduces off-target toxicity in healthy tissues, leading to a significantly widened therapeutic index.

specific cscRNAs such as cscR-8-21 and cscR-2-22 are generated through the fusion of transcripts from opposite DNA strands. Knockdown of these cscRNAs using siRNA significantly diminishes cell proliferation, colony formation and migration, as validated by reverse transcription PCR, Sanger sequencing, RNA fluorescence *in situ* hybridization, and functional assays, supporting their role in promoting cancer cell survival and potentially contributing to therapy resistance (89). In CML, canonical splicing of *BCR-ABL* produces p210 or p190 transcripts, but ATS can generate truncated isoforms for example, *BCR-ABL1/b3a3*) that lack *ABL1* exon 2-encoded SH3 domain residues, leading to constitutive kinase activity and resistance to allosteric inhibitors such as asciminib by destabilizing the autoinhibited kinase conformation (58). Moreover, EZH2-mediated repression of splicing factors such as CELF2 in CML results in aberrant splicing patterns that promote stem cell-like properties and diminish sensitivity to tyrosine kinase inhibitors (Fig. 3D) (87). These ATS-derived variants activate alternative signaling pathways, such as dynamic phosphorylation events, thereby contributing to treatment failure and relapse (86). These findings demonstrate that ATS actively contributes to the development of aggressive, therapy-resistant cancers.

ATS and its prominence compared with chromosomal abnormalities. AS events including trans-splicing, are prevalent somatic perturbations in cancer that promote tumor heterogeneity and therapy resistance (90). By contrast, hereditary cancer syndromes are frequently driven by germline chromosomal abnormalities, including *RB1* deletions in retinoblastoma, *TP53* mutations in Li-Fraumeni syndrome, *NF1* deletions in neurofibromatosis type 1, *APC* mutations in familial adenomatous polyposis, and *BRCA1/BRCA2* germline mutations in hereditary breast cancer (91-93). AS predominantly occurs as a somatic event that broadly reprograms gene networks, while chromosomal abnormalities generally underlie inherited cancer syndromes with high penetrance. In neurofibromatosis type 1, mis-splicing of *NF1* exon 23a disrupts neurofibromin function in high-grade gliomas, thereby activating the Ras/MAPK pathway to drive tumor formation (94).

Similarly, retinoblastoma exhibits upregulated spliceosome activity regulated by pRB/E2F3a under oncogenic stress (90). Genomic studies establish AS dysregulation as a nearly universal feature of tumorigenesis, whereas chromosomal abnormalities primarily drive ~10-20% of hereditary cancer syndromes (91-93). This predominance establishes AS as a central mechanism in sporadic cancers and a promising therapeutic target, as exemplified by metabolic vulnerabilities conferred by *TP53* splicing variants in Li-Fraumeni syndrome (95).

4. Splicing regulation in cancer immunity, computational discovery, and systems crosstalk

Splicing-derived neoantigens and resistance to immunotherapy. Somatic mutations in core spliceosomal components including *SRSF2* and *SF3B1* produce recurrent mis-splicing events that create novel immunogenic peptides, these splicing-derived neoantigens are presented by MHC class I molecules and trigger $CD8^+$ T-cell responses in myeloid malignancies (96,97). While these neoantigens provide effective targets for immunotherapy, tumors can develop resistance through selection of low-immunogenicity splice variants or

impaired antigen presentation through mechanisms such as aberrant HLA-I splicing (98). Pharmacological spliceosome modulation with agents such as indisulam or MS-023 counteracts this resistance by restoring neoantigen presentation, and exhibits synergistic effects with anti-PD-1 therapy to enhance T-cell-mediated tumor clearance in preclinical models (97).

Mis-splicing of microexons, short exons typically regulated in tissue-specific patterns, represents an important disease mechanism that disrupts essential protein interaction networks, in autism spectrum disorder, CPEB4 microexon mis-splicing drives protein aggregation and functional impairment (99). While this mechanism has been primarily characterized in neurological disorders, similar processes likely operate in cancer biology, where microexon alterations may influence oncogenic signaling pathways and immune evasion capabilities, thereby creating opportunities for novel biomarkers and therapeutic strategies (98,99). These observations underscore splicing-derived neoantigens as promising biomarkers of response to immune checkpoint blockade response and as a basis for emerging approaches such as personalized cancer vaccines and combination therapies that target spliceosome activity is targeted (97,98).

Computational deconvolution and single-cell dissection of splicing networks. Computational methods have been developed to enable systematic study of splicing heterogeneity with both bulk and single-cell RNA sequencing data (scRNA-seq). Deep learning models such as APARENT-Perturb can predict splicing from sequence and uncover key regulatory mechanisms including the CFIm complex, as well as clinically relevant splicing signatures associated with drug resistance (100). Integrating these models with large-scale genomic repositories such as TCGA enables systematic pan-cancer studies that associate specific splicing patterns with clinical outcomes.

Single-cell technologies provide an unprecedented resolution of splicing dynamics among cell populations in the tumor microenvironment (TME). Reference atlases such as Tabula Sapiens show cell-type-specific splicing of *MYL6* and other genes, and highlight the required resolution to resolve complex cellular heterogeneity (101). In aplastic anemia, scRNA-seq enables detection of aberrant splicing in the rare population of hematopoietic stem cells, and can provide mechanistic hypotheses for clonal evolution and treatment resistance (102).

Recent work in computational biology has led to the development of advanced frameworks for integration of multi-omics data and the mapping of splicing regulatory networks. The model APARENT-Perturb relies on deep neural networks to predict the effect of genetic perturbations on splicing outcomes, and has uncovered functional interactions between regulatory elements, as well as sequence features influencing polyadenylation site selection (100). Integration of splicing data with other molecular measurements using tools such as CellPhoneDB enables systematic investigation of networks of cell-cell communication that underlie splicing heterogeneity in tissue microenvironments (101).

Systemic crosstalk of pre-mRNA splicing with metabolism, cellular stress and epitranscriptome. Splicing regulation can dynamically respond to metabolic and stress signals through adaptive systems such as the minor spliceosome (MiS), which is activated during cancer progression. In prostate cancer, a progressively dysregulated MiS, as evidenced by elevated U6atac snRNA levels, has been associated with disease progression and splicing reprogramming under metabolic stress (103). Cellular stressors, including androgen deprivation therapy and AR pathway inhibition, have been shown to further enhance the efficiency of minor intron splicing, a clear indication of how treatment-related stress can remodel the spliceosome to facilitate the development of therapeutic resistance (103). This dynamic regulation of splicing in turn selectively reprograms the splicing network to express additional antiapoptotic isoforms, firmly positioning MiS at the center of the adaptive response in cancer.

The U12-type spliceosome functions as a specialized regulatory system for processing stress and oncogenic signals via minor intron splicing. The structural analyses of the MiS point to MiS-specific components, such as SCNM110 and PPIL2, as key factors that ensure a stable catalytic core required for the accurate processing of genes involved in cell-cycle checkpoint and DNA repair pathways (104,105). The evolutionary conservation of this system and the mutational vulnerability of its key components underscore the importance of MiS in preserving cellular homeostasis under metabolic and therapeutic stress (103,104).

In conclusion, splicing serves as an integrator of metabolic and stress signals, and the U12-type spliceosome offers a compelling model of how an ancient system of gene regulation can influence the tumor adaptive response. Further work is needed to elucidate the direct links between metabolism and splicing as well as the epitranscriptomic layer of regulation, both of which still remain to be fully characterized in the current literature.

5. Therapeutic targeting of aberrant splicing

Perturbations in splicing patterns are well documented in cancer, and mutations in spliceosomal proteins and cancer driver genes are enriched across malignancies (106). Aberrant AS contributes to tumor progression and therapeutic resistance by generating tumor-specific splice variants that evade conventional therapies. New therapeutic modalities are under development to directly target dysregulated splicing machinery or its downstream effects. These include small molecules that inhibit the activity of specific splicing factors or the assembly of the spliceosome, ASOs that block the selection of pathogenic splice sites, and nanoparticle-delivered circRNAs that leverage the endogenous splicing machinery.

Together these modalities aim to reestablish physiologic splice patterns and counteract tumor-promoting isoforms to overcome therapeutic resistance (Fig. 4). The development of these agents reflects an increasing focus on splicing-targeted therapeutics that integrate mechanistic insights into splicing dysregulation with recent advances in drug design and delivery (Table II).

Small molecule splicing modulators. Small molecule splicing modulators are an emerging class of therapeutics designed to correct or exploit dysregulated pre-mRNA splicing in cancer. These molecules bind to core components of the spliceosome, the macromolecular machinery that mediates intron removal

Table II. Clinical trials of splicing-targeted therapies in cancer.

First author/s, year	Therapeutic agent	Target	Cancer type	Clinical trial identifier and phase	Key Findings / Status	(Refs.)
Steensma *et al*, 2021; Seiler *et al*, 2018	H3B-8800	SF3B1 (Spliceosome)	Myelodysplastic syndromes, leukemia	NCT02841540 (Phase I)	Established maximum tolerated dose (MTD); reversible QTcF prolongation and gastrointestinal (GI) toxicity as common adverse events. Showed antitumor activity in SF3B1-mutant patients.	(107,108)
Chi *et al*, 2017	Custirsen	Clusterin (via ASO)	Metastatic castration-resistant prostate cancer	SYNERGY (Phase III)	Failed to improve overall survival when combined with docetaxel, highlighting delivery challenges for antisense oligonucleotides (ASOs) in solid tumors.	(109)

and exon ligation, thereby either directly altering splice site selection or modulating the kinetics of spliceosome assembly. One prominent target is the SF3b complex, a subcomplex of the U2 small nuclear ribonucleoprotein (U2snRNP) that is responsible for recognizing the branchpoint sequences during the early stages of spliceosome assembly. Inhibitors of SF3b, such as pladienolide derivatives including H3B-8800 and E7107, stabilize a conformation that prevents its interaction with pre-mRNA, leading to widespread IR or ES (108,110). The effect of these inhibitors is amplified in cancer cells harboring mutations in spliceosomal genes (for example, *SF3B1, U2AF1, or SRSF2*) since these cells exhibit heightened dependence on already compromised spliceosome function. Recent studies also highlight the role of nuclear condensates, which are phase-separated compartment enriched in splicing factors, in potentially concentrating splicing modulators selectively in cancer cells (111). In addition to SF3b-targeting molecules, several small molecules have been identified that modulate other components of the splicing machinery, such as SR proteins, or even target upstream RNA modification pathways, such as *METTL3*, which mediates N6-methyladenosine (m6A) methylation, a post-transcriptional modification known to regulate splicing efficiency and pre-mRNA transcript stability (112,113). Consequently, the diversity of mechanisms underlying the action of splicing modulators suggests their potential efficacy across a wide range of splicing-driven malignancies.

The therapeutic efficacy of SF3b inhibitors is exemplified in MDS and chronic lymphocytic leukemia (CLL), where recurrent *SF3B1* mutations drive aberrant splicing and oncogenesis. H3B-8800, an orally bioavailable pladienolide derivative, selectively eliminates spliceosome-mutant cells by causing retention of short GC-rich introns in genes that encode spliceosome proteins and increase splicing stress (108). Preclinical studies in *SF3B1*-mutated pancreatic cancer and leukemia models have shown that H3B-8800 reduced viability by greater than 50% at nanomolar concentrations, with only mild effects in wild-type cells. Resistance to H3B-8800 was caused by mutations in other components of SF3b, such as PHF5A-Y36C, indicating on-target activity (108). In MDS, the *SF3B1* mutations lead to a hypersensitivity to splicing perturbation, as these cells depend on residual activity of the spliceosome to produce transcripts critical for the survival of mutant cells (110). In early clinical trials in MDS, H3B-8800 treatment led to a reduction in blasts and an improvement in other hematologic parameters in patients with *SF3B1*-mutated MDS (108).

However, the Phase I first-in-human dose-escalation study of H3B-8800 (NCT02841540) completed dose escalation with defined dose-limiting toxicities, including ocular toxicity observed with prior pladienolide derivatives (for example, E7107). The trial identified reversible QTcF prolongation and gastrointestinal disturbances (diarrhea, nausea and vomiting) as common treatment-emergent adverse events, with the maximum tolerated dose established at 30 mg for a 5-days-on/9-days-off schedule and 14 mg for a 21-days-on/7-days-off schedule (107). Despite safety considerations, these findings validate SF3b as a therapeutically tractable node in spliceosome-mutant cancers and provide a roadmap for targeting splicing vulnerabilities in other malignancies.

Aberrant splicing also underlies resistance to targeted therapies, as observed in *BRAF*-mutant melanomas treated with vemurafenib. ~30% of resistant tumors express *BRAF* splice variants (for example, *BRAF3-9*), which lack the RAS-binding domain and dimerize independently of upstream signals (113). A C-to-G mutation in intron 8 of *BRAF* creates a cryptic branchpoint, promoting *BRAF3-9* expression through enhanced recognition by mutant SF3B1 (113). The splicing modulators, including spliceostatin A (SSA), reverse the shift in isoform expression by inhibiting SF3b and thus re-establishing sensitivity to BRAF inhibitors. In vemurafenib-resistant melanoma xenografts, SSA treatment decreased BRAF3-9 levels by 60% and slowed tumor growth by 40%, showing that splicing modulation can overcome drug resistance (113). The splicing modulators and BRAF inhibitors are enriched in nuclear condensates containing MED1 and BRD4, which enhance the efficacy of the combination therapy (111). Parallel studies implicate RNA m6A methylation in regulating *BRAF* splicing, as *METTL3* knockdown reduces *BRAF3-9* expression and synergizes with vemurafenib (112). These insights highlight the dual utility of splicing modulators: As monotherapies in spliceosome-mutant cancers and as adjuvants to reverse adaptive splicing in drug-resistant tumors.

ASOs. ASOs are short synthetic nucleic acids. Specifically, ASOs are single-stranded, chemically modifiable short nucleic acid sequences usually in the range of 18-25 nucleotides in length (114). They are designed to bind pre-mRNA and modulate splicing events by blocking splice-site recognition or altering exon inclusion. These molecules are chemically modified (for example, phosphorothioate backbones, 2'-MOE/cEt modifications) to enhance stability and binding affinity. For example, in HCC, ASO1-cEt/DNA targeting *PKM* pre-mRNA induced a splice switch from the oncogenic PKM2 isoform to tumor-suppressive PKM1, suppressing glycolysis and tumor growth in xenografts (115). Similarly, in *KRAS* Q61K-mutant cancers, ASOs disrupting exonic splicing enhancer motifs near cryptic splice sites induced ES, generating non-functional *KRAS* transcripts and sensitizing tumors to osimertinib (116). These studies underscore ASOs' ability to target splicing-dependent oncogenic drivers with high specificity.

A key design strategy is to target variants that mediate resistance to therapy. In gallbladder cancer, PTBP3 mediates the skipping of *IL-18* exon 3, leading to the expression of a tumor-specific *ΔIL-18* isoform that in turn is responsible for the suppression of the tumour-fighting activity of $CD8^+$ T-cells. ASO4, designed to block the splice site of the *ΔIL-18* isoform, was shown to restore expression of full-length *IL-18* and also to induce improved tumor clearance by T-cells *in vivo* (117). Likewise, in B-cell tumors, two CD20 mRNA isoforms with different 5'-UTRs (V1/V3) have been shown to control protein translation. In these isoforms, splice-switching morpholinos selecting for the translation-competent V3 isoform raised CD20 levels, the increased CD20 expression resensitized rituximab-resistant cell lines (118). These examples demonstrate how ASOs can reverse resistance by reprogramming splicing toward nonpathogenic isoforms.

Clinical progress in solid tumors mirrors advances observed with nusinersen (a spinal muscular atrophy therapy). In ovarian cancer, BUD31 sustains antiapoptotic *BCL2L12* expression by promoting exon 3 inclusion. BUD31-targeting ASOs induced ES, triggering nonsense-mediated decay of *BCL2L12* mRNA and apoptosis in xenograft models (119). Meanwhile, in HCC, systemic delivery of a mouse-specific ASO (mASO3-cEt/DNA) redirected *PKM* splicing, inhibiting tumorigenesis without toxicity (115). Similarly, in lung cancer models, the addition of the *MERTK* ASO to XRT^+anti-PD1 and XRT^+ anti-CTLA4 profoundly slowed the growth of both primary and secondary tumors and significantly extended survival (120). Challenges remain in tumor-specific delivery, but chemical conjugates (for example, GalNAc for hepatic targeting) and lipid nanoparticles (LNPs) show promise.

Previous advances in delivery platforms have significantly enhanced the *in vivo* efficacy of ASOs by improving targeted delivery and splice-switching efficiency. GalNAc conjugates, which facilitate hepatocyte-specific uptake through asialoglycoprotein receptor-mediated endocytosis, have demonstrated a 10-fold improvement in gene silencing potency in murine liver models, highlighting their specificity for hepatic applications (121). LNPs, engineered to encapsulate splice-switching oligonucleotides, have achieved over 60% splice correction in tumor xenografts, as measured by functional recovery of target genes, underscoring their utility in oncology settings (122). Exosomes allow delivery of nucleic acids to neurons by exosomes, which can be functionalized with RVG peptides to target delivery to the brain. It has led to ~50% reduction in models of neurodegenerative disorders (123). These approaches allow for delivery to be more precise, and for the systemic levels of agents to be lower, reducing off-target effects and improving therapeutic indices.

However, the translation of ASO medicines into the clinic for the treatment of solid tumors is significantly hampered by challenges that arise during systemic delivery. This includes poor tissue penetration, limited uptake and poor transport of ASOs into and within cells. A key clinical failure for systemic delivery of ASOs was the SYNERGY Phase 3 trial of custirsen, a clusterin-targeting ASO, in combination with docetaxel and prednisone in patients with metastatic castration-resistant prostate cancer. It was observed that custirsen had no impact on overall survival in these patients, a finding that underscores the need to overcome delivery barriers through means such as ligand conjugates and nanocarriers (109).

circRNA-directed nanotherapeutics. Circular RNAs (circRNAs) are covalently closed RNA molecules formed by back-splicing of precursor mRNAs. CircRNAs are highly stable molecules that are resistant to exonucleases. CircRNAs are involved in the development of cancers by regulating the expression of target genes through microRNA sponging, protein interactions and transcriptional regulation (124,125). CircRNA-directed nanotherapeutics is a preclinical proof-of-concept approach that employs RNA interference technology in combination with a delivery system that integrates nanoparticles to target oncogenic circRNAs. CircRNA-directed nanotherapeutics utilizes siRNAs or ASOs encapsulated in biocompatible nanoparticles to enhance stability, targeted delivery and mitigate off-target effects relative to free RNA agents. This strategy holds promise to overcome issues such as rapid renal clearance and enzymatic

degradation of free RNA, and has been shown to enable specific targeting of dysregulated circRNAs in tumors (126,127).

Recent studies have provided proof-of-concept evidence that circRNA-targeted nanomedicines can be effective in different cancer types in mouse models. In gastric cancer, lipid-polymer hybrid nanoparticles carrying siRNAs to knockdown circ_0008315 resensitized tumors to cisplatin by disrupting *miR-3666/CPEB4* signaling. This nano-formulation exhibited 2.3-fold higher tumoral accumulation than free siRNA and significantly decreased tumor volumes in a patient-derived xenograft model (65.8 mm^3 vs. 132.4 mm^3 in free siRNA-treated mice; with no effects on organ function) (124). In HCC, poly(β-amino ester)-based nanoparticles carrying circMDK siRNA achieved (78% target gene knockdown) inhibited tumor growth by upregulating *ATG16L1* via an m6A-associated mechanism. The nanoparticles were designed with pH-sensitive components, so that 92% of the siRNA payload could be released specifically in the TME, suggesting potential superiority over conventional chemotherapy treatment in an orthotopic tumor model (126). These examples underscore the potential, at a proof-of-concept stage, for nanocarriers to improve the pharmacokinetic properties of circRNA-targeting agents in a preclinical model.

There are other examples of preclinical validation of circRNA nanotherapeutics, whereby multifunctional nanoparticle designs provide supporting evidence (Table III). For instance, delivery of *circRHBDD1* siRNA in PLGA-PEG nanoparticles effectively blocked the the *IGF2BP2/PD-L1* axis and increased infiltration of $CD8^+$ T cells by 4.1-fold in murine gastric cancer, achieving complete tumor regression in 40% of treated animals when combined with anti-PD-1 antibody (125). PLGA nanoparticles were also used to deliver si-*cSERPINE2* to breast cancer cells in orthotopic mouse models. This treatment attenuated *IL-6*-mediated crosstalk between breast cancer cells and tumor-associated macrophages, and inhibited lung metastasis by 72% (127). Current early-phase clinical trials are beginning to validate such alternative strategies. For instance, a Phase I clinical trial of LNP-formulated circRNA inhibitors was conducted in solid tumors, and indicated a favorable safety profile, but was undertaken as an exploratory study and was not further validated in large animal studies.

6. Conclusions and future perspectives

The detailed association between dysregulation of AS and cancer drug resistance is increasingly appreciated, wherein numerous splicing events, including ES, IR, and selection of alternative splice sites, can directly influence therapeutic response. Recent advances in long-read sequencing methods, particularly Oxford Nanopore Technologies and PacBio sequencing, have enabled the resolution of full-length transcript isoforms, allowing cancer-specific AS events to be precisely identified. For instance, in spatial isoform transcriptomics of esophageal squamous cell carcinoma (ESCC), the isoform switching of CD74 was found to contribute to chemoresistance by promoting the interaction of $C1QC^+$ tumor associated macrophages and exhausted $CD8^+$ T-cells, which in turn fosters an immunosuppressive TME (129). In PDAC, the circRNA hsa_circ_0007919 was found to be involved in gemcitabine resistance by recruiting the transcription factors *FOXA1* and *TET1* to demethylate the *LIG1* promoter, thereby boosting DNA repair by regulating AS (128). These observations align with splicing quantitative trait loci (sQTL) studies in CRC, in which a genetic variant was found to impact the splicing of *PRMT7*, which in turn was implicated in the MAPK signalling pathway and chemotherapy resistance (27). Importantly, recent structural analyses of the minor spliceosome have identified several components specific to U11 or U12 snRNPs (for example, 35K and 48K) and lactylated nucleolin (NCL) that are required for stabilizing the recognition of U12-type introns, which in turn drive the oncogenic splicing programs (104,130). Together, these findings highlight the dual role of AS in both driving molecular diversity and representing a potential vulnerability for therapeutic intervention in cancer.

In the future, it will be essential to optimise long-read sequencing protocols to improve their accuracy and scalability in studying spatially resolved isoform diversity in the metastatic niche. A key priority would be to study how the minor spliceosome, responsible for excising a minority of spliceosomal introns (U12, accounting for ~0.5% of all human genes) that often include oncogenes and tumor suppressors, is regulated. Cryo-electron microscopy studies have elucidated the structure of the spliceosome responsible for processing U12-dependent introns, which revealed that some of the components appear to be functionally orthologous to the SF3a complex that stabilizes branchpoint recognition in the major spliceosome, notably *SCNM1*, and demonstrated that a U-box protein PPIL2 is essential for the assembly of the catalytic core (104). On a more clinical note, it might also be valuable to examine spatially resolved splicing that is sex or tissue specific, as such splicing patterns could provide useful biomarkers for precision medicine. Multi-ancestry sQTL studies have shown that the spliceosome component *PRPF8* is regulated in an ancestry-specific manner in TNBC where *DHX9* suppresses circRNA-CREIT and induces chemoresistance (27,74). Furthermore, combining single-cell isoform sequencing with spatial transcriptomics in tumor microenvironments could be explored to identify how subclonal splicing variants drive adaptive resistance. Yang *et al* (130) showed that in cholangiocarcinoma, lactate-driven NCL lactylation reprograms the RNA splicing machinery via *MADD* isoform switching, which could be a potential therapeutic target. Such studies will require collaboration between basic scientists and clinicians to standardize isoform annotation pipelines and cross-validate key splicing nodes in multiple models.

Therapeutic strategies specifically targeting AS-mediated drug resistance are rapidly evolving with promising approaches being investigated in preclinical models that target the cis-regulatory elements, trans-acting factors and various spliceosome inhibitors. Small molecules such as the *SRPK1* kinase inhibitor NVP2 act by normalizing *VEGF* splicing in PDAC to resensitize tumour cells to cisplatin (128). ASOs targeting the pathogenic isoform in ESCC, such as CD74v6, which is known to promote immune evasion via macrophage apoptosis, demonstrated efficacy in suppressing metastasis in preclinical trials (129). Inhibition of lactylation-dependent activation of NCL by SGC3027 also abrogated oncogenic *MADD* splicing in cholangiocarcinoma and synergized with

Table III. Summary of therapeutic strategies targeting alternative splicing in cancer.

First author/s, year	Therapeutic modality	Specific agent/ approach	Molecular target/action	Resistance mechanism addressed	Proof-of-concept/model	Clinical status	(Refs.)
Steensma *et al*, 2021; Seiler *et al*, 2018	Small molecule inhibitors	H3B-8800	SF3b complex modulator	Induces lethal splicing errors in spliceosome-mutant cells	SF3B1-mutant leukemia and pancreatic cancer models	Clinical Phase I	(107,108)
Salton *et al*, 2015		Spliceostatin A	SF3b complex inhibitor	Reverses BRAF3-9 isoform expression	Vemurafenib-resistant melanoma xenografts	Preclinical	(113)
Xu *et al*, 2023		NVP2 (SRPK1 inhibitor)	Inhibits SR protein kinase	Normalizes VEGF splicing	Pancreatic ductal adenocarcinoma models	Preclinical	(128)
Ma *et al*, 2022	ASOs	PKM-targeting ASO	Switches PKM2 to PKM1 isoform	Suppresses oncogenic glycolysis	HCC xenografts	Preclinical	(115)
Zhao *et al*, 2024		IL-18-targeting ASO	Corrects pathogenic IL-18 splicing	Restores $CD8^+$ T-cell function	Gallbladder cancer models	Preclinical	(117)
Chi *et al*, 2017		Custirsen	Clusterin mRNA (ASO)	Aimed to inhibit anti-apoptotic protein	Patients with metastatic castration-resistant prostate cancer	Clinical Phase III (Failed)	(109)
Sun *et al*, 2023	CRISPR/Splice-Switching	CRISPR/dCasRx	Targets TIMP1 pre-mRNA	Reverses SRSF1-driven pro-metastatic splicing	Colorectal cancer models	Preclinical	(38)
Fei *et al*, 2024; Li e*t al*, 2024; Du *et al*, 2022	Nanotherapeutics	siRNA-loaded nanoparticles	Targets oncogenic circRNAs (for example, circ_0008315, circRHBDD1)	Reverses chemoresistance or immune evasion	Gastric cancer, HCC xenograft models	Preclinical	(124-126)

ASOs, antisense oligonucleotides; HCC, hepatocellular carcinoma.

chemotherapy to reduce tumor burden (130). Combinatorial approaches are also gaining traction; for instance, pairing spliceosome modulators for example, pladienolide B) with immune checkpoint blockers enhances T-cell infiltration in tumors with high *SF3B1* mutation burden (27). Structural information about the minor spliceosome will also provide a framework for rational drug design as has been recently demonstrated for the interaction of PPIL2-U5 snRNA to inhibit tri-snRNP assembly (104). However, the main concern regarding these drugs is mitigating off-target effects due to the ubiquitous nature of the splicing machinery in normal cells. Clinical trials should aim to develop isoform-specific therapies, and leveraging CRISPR-based screens that identify synthetic lethal interactions between splicing factors and chemotherapeutic drugs could be employed. As the field progresses, integrating splicing-centric therapies with conventional regimens will be pivotal for overcoming the adaptive plasticity of cancer cells and improving patient outcomes.

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Authors' contributions

WZhu, ZW and CL conceptualized the study. WZhu wrote the original draft. BB, WZha and GC wrote, reviewed and edited the manuscript. HY and HA supervised the research work, and contributed to the study methodology, investigation and data curation. FL and ZL solicited for funding, contributed to project administration, validation and provision of resources. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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