J Biochem Technol (2026) 17(4): 116-124
https://doi.org/10.51847/uJ649BrdMA
ISSN: 0974-2328

Alternative Splicing and Proteoform Diversity in Cancer: Connecting Molecular Genetics, Proteomics, and Therapeutic Vulnerabilities

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Received: 20 September 2026 / Received in revised form: 24 October 2026, Accepted: 28 October 2026, Published online: 17 December 2026

Abstract

Cancer transcriptomes contain abundant alternative splice junctions, but the existence of an abnormal RNA does not establish an altered protein product, still less a therapeutically exploitable dependency. This integrative review follows evidence from splice-site choice and transcript fate through mass-spectrometric protein identification, isoform-specific function, and intervention. Genomic and RNA-based studies reveal recurrent splicing disruption, whereas proteogenomic analyses show that the transition from junction detection to uniquely assigned protein species is selective and method dependent. Mechanistic examples reveal distinct outcomes: degradation of aberrant transcripts with loss of normal protein, stable isoforms with altered signaling or cell states, and changes in antigen presentation. Perturbation studies can support vulnerability at the level of a splice-regulatory process, a downstream pathway, or a particular isoform; these are not interchangeable claims. This review therefore proposes an evidence-resolved interpretation that explicitly separates RNA association, protein identity, functional causation, and context-dependent therapeutic response. It identifies decisive experiments for resolving ambiguous peptide assignments, separating competing molecular mechanisms, and determining whether findings in model systems survive tumor heterogeneity and clinical translation. The practical conclusion is that therapeutic relevance depends on the experimentally demonstrated molecular entity and intervention context, not on the apparent abundance of alternative splicing alone.

Keywords: Splice-site selection, Protein isoforms, Proteoform identification, Top-down mass spectrometry, Isoform-specific rescue, Cancer dependency

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Introduction

The diversity of cancer-associated splice junctions presents a striking asymmetry between discovery and interpretation. Reanalysis of transcriptomic and genomic profiles across 8,705 tumors documented extensive altered junction use and detectable junction-derived peptides, but peptide detection represented only a restricted subset of the RNA landscape (Kahles *et al*., 2018). A complementary pan-cancer analysis linked mutations in splice-regulatory genes with alterations in RNA processing and downstream pathways (Seiler, Peng, *et al*., 2018). Neither result establishes that each altered transcript produces a stable, distinguishable protein, or that a tumor depends on that product.

This distinction matters because “isoform” describes more than one biological object. A transcript isoform is a specified RNA sequence; a protein isoform is a distinct translation product; a proteoform is a particular molecular form that may additionally differ by post-translational modification or processing. Detection of peptides shared among candidate proteins usually supports a protein group, not a unique intact proteoform. A single new exon junction can instead yield an unstable transcript, a shortened product, an altered domain, or an antigenic peptide without an accumulating full-length protein.

The central question is thus conditional: when is a cancer-associated splice alteration demonstrably linked to a protein-level mechanism, and what further evidence makes that mechanism an actionable vulnerability? The sections below distinguish descriptive prevalence from proteoform resolution and causal tests. A therapy directed against a globally disturbed spliceosome, for example, is conceptually different from a therapy that reverses the effect of one established protein isoform. Keeping these targets distinct prevents RNA signatures from being converted prematurely into clinical claims.

Review Approach and Proteogenomic Evidence Integration

This is a targeted critical, integrative review, not a systematic review or meta-analysis. Source selection was organized around five evidence questions: which cancer-associated splice changes occur; which generate identifiable protein products; which alter function; which survive perturbation and rescue; and which have credible intervention evidence. The source set contains 32 journal

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publications selected through topic-driven web and bibliographic discovery with publisher or PubMed metadata checks. Selection was purposive, prioritizing direct experimental and measurement studies alongside explanatory reviews. The 32-publication allocation must not be interpreted as the number of eligible studies in the field, and no exhaustive retrieval or formal study-quality score is claimed.

Evidence is considered across four linked but non-equivalent levels. RNA sequencing estimates splice-event usage and transcript consequences. Peptide-centric proteomics can identify sequence evidence while retaining uncertainty from shared peptides, database composition, and incomplete coverage. Intact-protein analysis potentially resolves coexisting sequence forms and modifications, subject to its own sensitivity and fragmentation limits. Finally, genetically or pharmacologically matched perturbation, rescue, and contextual comparison test functional necessity. Reviews describing clinical opportunities inform interpretation but are not counted as independent experimental replications (Anczukow *et al*., 2024).

The synthesis distinguishes reported observations from interpretive inferences. Claims made solely from abstracts or accessible summaries are restricted to those summaries; where necessary details of a rescue construct, confidence threshold, or patient-level validation are unavailable, that missing information is not inferred. This design deliberately prioritizes discriminating examples over estimated global effect sizes.

Splicing Alterations and Protein-Level Consequences

Altered splice-site use is not a uniform lesion. Mutations in core spliceosomal proteins, altered expression of RNA-binding regulators, and tumor-state transitions can each redistribute transcript abundance. Their biological consequences depend on reading frame, transcript surveillance, translation efficiency, protein stability, and the domain architecture of any surviving product. Comparing these routes before interpreting protein data is essential: an experiment may detect a strong loss of a canonical protein yet find no alternate protein at all.

Transcript Isoforms, Coding Consequences, and Regulation

Hotspot SF3B1 mutations favor cryptic 3′ splice sites through alternative branch-point use. Darman *et al*. (2015) reported that approximately half of their aberrantly spliced transcripts were subject to nonsense-mediated decay (NMD), resulting in reduced gene and protein expression. In this setting the biologically important consequence can be loss of the usual protein rather than gain of a novel stable species. SRSF2 mutations show a different recognition defect: altered sequence preferences change exon recognition, including EZH2 mis-splicing and NMD, with effects on hematopoietic differentiation (Kim *et al*., 2015).

Not all switches primarily act through transcript destruction. Comparative tumor analysis identified splicing changes that potentially alter interaction domains, but computational prediction of domain disruption is not direct protein-interaction measurement (Climente-González *et al*., 2017). An exon can remove a binding motif while leaving a translatable open reading frame; an alternative terminal exon can replace a regulatory tail; or intron retention can encode short peptides before transcript turnover. Each case calls for a different level of verification.

Intron-retention-derived peptides illustrate the distinction between translation and long-lived protein accumulation. Smart *et al*. (2018) used immunopeptidomics to show presentation of retained-intron-derived epitopes on major histocompatibility complex class I in cancer cell lines. This establishes processing and presentation of relevant peptides under the tested conditions; it does not establish clinical vaccine efficacy or the existence of an abundant intact protein carrying the entire predicted intron-derived sequence.

A further interpretation problem is cellular context. A splice change associated with tumor grade, cell lineage, or epithelial-to-mesenchymal transition (EMT) may be a consequence of changing cellular composition rather than a cell-autonomous initiating lesion. The proper unit of comparison includes cell state, regulatory background, normal-tissue usage, and experimental time point. **Table 1** therefore separates the molecular event from the protein species actually supported by the assay.

Table 1. Splice-event routes and the protein-level inference each permits

Event or route	Reported cancer-related example	Supported protein consequence	Unresolved inference
Cryptic 3′ splice-site use	Mutant SF3B1 redirects branch-point choice (Darman *et al*., 2015)	Reduced canonical protein through NMD for a subset	Stable alternate protein cannot be assumed
Altered enhancer recognition	Mutant SRSF2 changes EZH2 exon recognition (Kim *et al*., 2015)	Loss of normal expression linked to differentiation defects	A unique novel EZH2 proteoform is not established
Predicted domain-altering splice change	Tumor transcriptome domain analyses (Climente-González *et al*., 2017)	Domain consequence inferred from coding annotation	Direct expression and physical interactions require testing
Retained-intron translation	MHC-I peptides in cancer cell lines (Smart *et al*., 2018)	Presented peptide supported by immunopeptidomics	Intact stable source protein and patient efficacy unproven

Note. NMD = nonsense-mediated decay; MHC-I = major histocompatibility complex class I. Rows distinguish observed effects from inferred protein identity; they do not represent exhaustive event-class frequencies.

Protein Isoform Inference and Proteoform Identification

Proteomic inference is constrained by the information carried by the measured molecule. In bottom-up mass spectrometry, enzymatic digestion disconnects peptides that originated on the same intact protein. Identifying a unique junction-spanning peptide strengthens evidence for a particular translated sequence, but peptides shared across isoforms cannot determine which splice

product contributed the signal. Agosto *et al*. (2019) improved detection of splice-linked protein products using deep profiling and tailored sequence databases, although their activated T-cell system does not establish cancer-specific protein production.

A database that contains only canonical proteins can miss genuine altered sequence products; conversely, adding many predicted isoforms can enlarge the search space and create ambiguous assignments. Deep multi-protease sequencing expanded accessible human sequence coverage, while still showing why even broad peptide coverage is not equivalent to direct reconstruction of every intact molecular form (Sinitcyn *et al*., 2023). The inferential claim must include uniqueness of supporting spectra, candidate database provenance, sequence coverage, and false-discovery controls.

Top-down mass spectrometry examines intact protein ions and fragments, bringing sequence variants and modifications into a shared molecular context. Chen and Liu (2021) coupled RNA-based sequence information with top-down identification in colorectal cancer cells. Distinct cancer-cell proteoform patterns were subsequently observed using deep top-down analyses, but differences between metastatic and nonmetastatic cell lines alone do not demonstrate a splice-derived causal driver (McCool *et al*., 2022). Mammary carcinoma studies extend intact-protein profiling toward tumors, while noting practical limits for large proteins and complex samples (Knott *et al*., 2025).

One caution is that shorter proteins need not be splicing products. McWhite *et al*. (2024) identified short proteoforms whose origins could involve transcript processing or proteolytic cleavage. Post-translational modification further multiplies molecular forms independently of splice choice. Native approaches can preserve information about complexes and proteoforms: their use in endocrine-resistant breast cancer offers a route toward assembly-aware interpretation, not automatic proof of isoform-dependent resistance (Gomes *et al*., 2025). **Table 2** links each measurement class to the highest defensible identity claim.

Table 2. Proteogenomic measurement strategies and defensible identity boundaries

Measurement	Directly observed	Strongest defensible inference	Critical ambiguity
RNA-seq / junction analysis	Splice junctions and transcript abundance	Candidate coding isoform (Kahles *et al*., 2018)	Translation, turnover, protein presence
Bottom-up MS, shared peptides	Fragment sequences matching a protein group	Expression of a set of compatible proteins (Agosto *et al*., 2019)	Assigning one specific isoform
Bottom-up MS, unique junction peptide	Peptide crossing splice-derived sequence	Local translation support (Sinitcyn *et al*., 2023)	Intact chain, co-occurring modifications
RNA-guided top-down MS	Intact mass and informative fragments	More specific candidate proteoform (Chen & Liu, 2021)	Incomplete fragmentation, co-isolation
Native / complex-aware MS	Protein or complex-level features	Proteoform or assembly context (Gomes *et al*., 2025)	Stoichiometry, source isoform, functional causality

Note. MS = mass spectrometry. "Strongest" indicates a conditional inference given adequate spectra and quality control, not a universal guarantee. Assignments remain database and sample dependent.

Functional Mechanisms and Therapeutic Vulnerabilities

For a splice-dependent therapeutic mechanism, the causal question is not merely whether changing splicing alters growth. One must locate the intervention's effective molecular level. Restoring one protein may explain a tumor phenotype; inhibiting a shared spliceosomal component may affect hundreds of transcripts and expose broad regulatory stress. The latter can be therapeutically meaningful without establishing the former.

Localization, Interactions, Signaling, and Cell-State Effects

CD44 offers a discriminating isoform-switch example. During EMT, epithelial cells shifted from CD44 variant isoforms toward CD44s, with ESRP1 regulating the switch. In functional experiments, CD44s rather than an alternative variant restored EMT-associated behavior after CD44 depletion (Brown *et al*., 2011). The result supports isoform-dependent cell-state control in tested systems, while expression correlations in human tumors are weaker evidence for individual-patient causation. By contrast, a CD44v-positive stem-like subpopulation exhibited enhanced lung metastasis in another breast-cancer system (Hu *et al*., 2017). The divergent contexts caution against a universal ranking of CD44 isoforms.

MKNK2 alternative last exons yield Mnk2a and Mnk2b with different signaling functions. Mnk2a contains a MAPK-interacting region and promoted p38α-MAPK signaling and tumor-suppressive effects in the tested transformation models; Mnk2b did not share that response (Maimon *et al*., 2014). Here, alternative coding sequences are tied to distinct protein properties and a relevant phenotype. Even so, a molecule that reverses an isoform ratio does not necessarily engage only one downstream signaling node.

The lung cancer regulator RBM10 provides another mechanistic axis. Loss of RBM10 shifted EIF4H exon usage, and experiments distinguished the contribution of EIF4H-L from EIF4H-S to lung adenocarcinoma phenotypes (Zhang *et al*., 2020). Such findings come closer to assigning phenotype to a specific coding isoform, but changes in parallel RBM10 targets and model-specific expression remain plausible contributors unless matched rescue and off-target controls constrain them.

Perturbation, Rescue, and Targetability

Molecular specificity becomes consequential when a perturbation is used as a therapeutic proxy. Spliceosome-modulator sensitivity in SF3B1-mutant cells, including a knock-in mouse model,

demonstrates that a regulatory genotype can alter response to broad perturbation (Obeng *et al*., 2016). Lee *et al.* (2018) similarly described convergent vulnerabilities associated with cancer-linked spliceosomal mutations. These experiments establish context-dependent splicing-system dependence; they cannot be reclassified as selective inhibition of one splice-derived protein species.

Downstream causal chains offer different targets. Inoue *et al*. (2019) found that mutant SF3B1 induced a BRD9 poison exon, NMD, and reduced full-length BRD9, disrupting the noncanonical BAF complex. Importantly, endogenous tagging did not support production of a predicted truncated BRD9 protein. The relevant protein consequence was a deficit, not a novel stable isoform. In another setting, SF3B1-mutant cells displayed altered serine-pathway metabolism and sensitivity to serine deprivation linked to reduced PHGDH, with evidence from cultured systems and xenografts (Dalton *et al*., 2019). This is a downstream metabolic vulnerability, not proof of a uniquely targetable mutant proteoform.

Isoform-directed intervention is more tightly specified. Splice-switching oligonucleotides shifted MKNK2 toward Mnk2a in glioblastoma models and inhibited tumor-associated phenotypes; pharmacological interference with p38 signaling partially rescued the effects, strengthening the proposed pathway link (Mogilevsky *et al*., 2018). Conversely, a TRA2β poison-exon-targeting oligonucleotide produced antitumor phenotypes that were not fully mimicked by ordinary TRA2β knockdown. The induced poison-exon RNA acted in part as a long noncoding RNA, demonstrating that a splice intervention may act through an RNA product rather than a newly synthesized proteoform (Leclair *et al*., 2025).

These examples imply a practical hierarchy: verify splice switching, identify the changed RNA or protein entity, measure a coherent pathway effect, and test a matched perturbation with rescue. For broad drugs, comparator genotypes and normal cells also matter; for splice-switching oligonucleotides, sequence-specific binding and independent oligonucleotides help distinguish intended events from off-target actions. **Table 3** compares what the selected experiments actually support rather than assigning artificial quality scores.

Table 3. Mechanistic interventions and the level at which vulnerability is established

Experimental system	Perturbation and relevant observation	Functional support	Boundary of target claim
SF3B1-mutant hematopoietic models	Spliceosome modulator selectively affected mutant cells (Obeng *et al*., 2016)	Genotype-linked drug sensitivity	Broad spliceosome effect, not one proteoform
SF3B1-mutant cancer cells and xenografts	Serine limitation impaired growth with PHGDH-associated metabolic defect (Dalton *et al*., 2019)	Downstream metabolic dependence	Diet/model context; no isoform-selective drug shown
SF3B1-mutant cancer models	BRD9 poison exon caused depletion; no truncated BRD9 detected (Inoue *et al*., 2019)	Protein-loss and complex-disruption mechanism	Novel BRD9 protein species unsupported
Glioblastoma models	MKNK2 splice-switching oligonucleotide altered isoform ratio; p38 inhibition partly reversed phenotype (Mogilevsky *et al*., 2018)	Isoform-linked pathway intervention	Incomplete rescue; preclinical only
Preclinical tumor models	TRA2β poison-exon targeting; knockdown did not phenocopy all effects (Leclair *et al*., 2025)	Functional RNA-product component	Not exclusively protein depletion or new proteoform

Note. Entries summarize distinct experimental contexts, not directly comparable treatment effect estimates. “Partly” and “not fully” preserve reported mechanistic limitations.

The branching paths summarized in **Figure 1** make an additional point: evidence can end legitimately at transcript decay, peptide presentation, or an unresolved protein assignment. A solid relationship in the figure represents experimentally supported association or intervention within the cited model, not universal causality. A dashed link identifies an additional test or proposed translational inference. By allowing these dead ends, the map prevents apparent transcript novelty from being mistaken for an actionable molecular species.

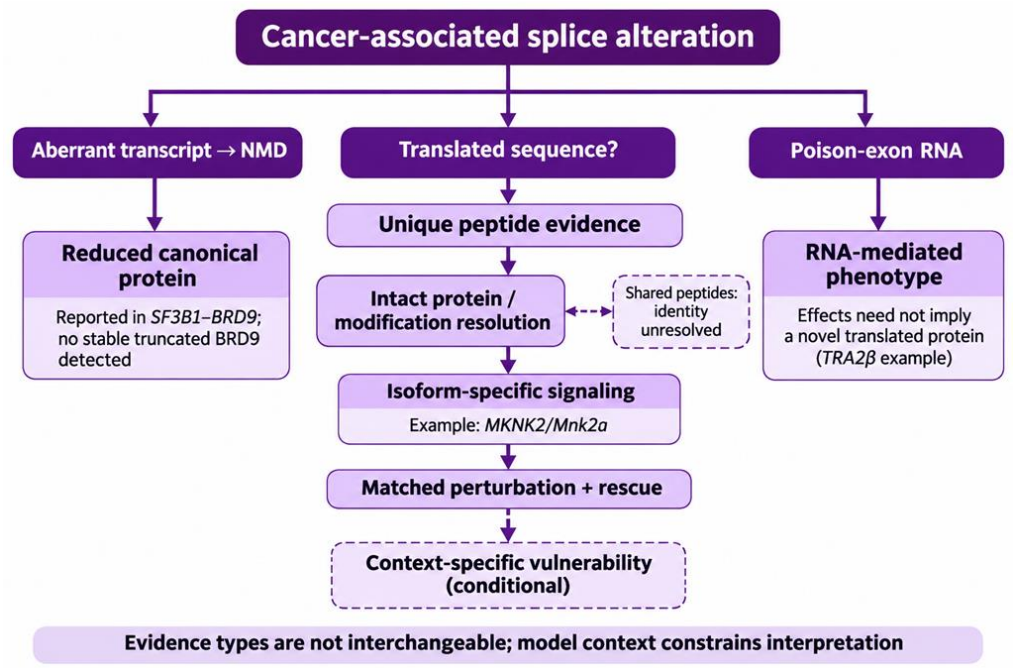


Figure 1. Distinct evidence paths from splice alteration to protein identity and conditional vulnerability

Note. Branched interpretation of alternative splicing in cancer. Reported paths include NMD-mediated protein loss (Darman *et al*., 2015; Inoue *et al*., 2019), localized splice-peptide detection (Agosto *et al*., 2019), isoform-linked signaling (Maimon *et al*., 2014), and RNA-product-mediated drug effects (Leclair *et al*., 2025). Solid connectors indicate experimentally observed relationships in their stated contexts; dashed connectors denote additional validation required before asserting species-specific therapeutic dependency. Neither peptide detection nor a transcript switch alone establishes a uniquely resolved intact proteoform. This is an integrative interpretation, not a quantitative pathway model.

An Evidence-Resolved Translational Synthesis

A clinically useful splice-associated marker should specify both what is measured and which decision it changes. In matched cancer RNA–proteomics datasets, Karakulak *et al*. (2026) reported mass-spectrometric support for 12.1% of identified cancer-associated dominant transcript switches across melanoma, ovarian cancer, and acute myeloid leukemia. Their predicted losses of protein or drug interactions are hypotheses generated from isoform annotations and network models, not measured patient drug resistance. The gap between RNA switching and unique-peptide detection thus remains a substantial translational bottleneck even in an integrated dataset.

Clinical association, molecular identity, and actionable dependence may also diverge. In metastatic castration-resistant prostate cancer, circulating-tumor-cell AR-V7 messenger RNA was associated with poor response to enzalutamide or abiraterone (Antonarakis *et al*., 2014). The splice variant lacks the conventional ligand-binding domain, making the association biologically plausible. Nevertheless, an RNA-based resistance marker cannot alone distinguish every coexisting androgen-receptor protein form or prove that selectively removing AR-V7 would reverse resistance. Those would require additional protein-specific and intervention studies.

The validation experiment depends on the proposed therapeutic object. For a candidate coding isoform, the strongest sequence assignment available should be paired with a depletion or splice-switch perturbation, a resistant-construct rescue, and normal-cell controls. For an RNA-mediated mechanism, transcript localization and separation of RNA effects from translated products become central. For drugs that alter spliceosome function, sensitivity must be tested across matched mutant and wild-type contexts, rather than attributed to a single proteoform. The priorities in **Table 4** are this review's proposed discriminating tests, not claims that all source studies performed them.

Table 4. Translational claims and experiments that would distinguish competing explanations

Proposed use	Existing evidence anchor	Crucial unresolved alternative	Discriminating next test
Tumor-associated isoform biomarker	Matched RNA and peptide detection for a subset of switches (Karakulak *et al*., 2026)	Shared peptides or reference-database bias	Validate unique sequences in independent matched tumors with orthogonal protein assays

Treatment-resistance stratification	Circulating AR-V7 RNA associated with antiandrogen resistance (Antonarakis *et al*., 2014)	Coexisting resistance drivers and protein-form ambiguity	Prospective marker-defined therapy comparison plus protein-specific testing
Splice-switch therapeutic candidate	REST-directed antisense design in neuroendocrine cancer models (Mishima *et al.*, 2024)	Off-target or context-dependent effects	Independent oligonucleotide, rescue, delivery, and normal-tissue comparison
Personalized antisense selection	Pancreatic-cancer organoid splice-switch screens (Wan *et al*., 2024)	Organoid-specific exposure and clone selection	Patient-matched organoid replication and in vivo distribution testing
Broad spliceosome inhibition	Preclinical H3B-8800 sensitivity versus limited clinical response (Seiler, Yoshimi, et al., 2018; Steensma *et al*., 2021)	Model-to-patient pharmacodynamic and tolerance differences	Exposure-linked splice biomarkers and clinically meaningful responder-enrichment studies

Note. This is a proposed validation matrix anchored to published observations; its tests are recommendations, not reported completed experiments. No row asserts proven patient benefit.

The resulting relationships are conditional rather than linear. **Figure 2** places molecular identity inside cell-state, tumor-composition, delivery, and clinical-evidence boundaries: a credible peptide assignment may remain clinically irrelevant when the tumor does not depend on that species, whereas a reproducible downstream vulnerability may be actionable without an individually resolved proteoform. This distinction should shape trial biomarkers and preclinical endpoints.

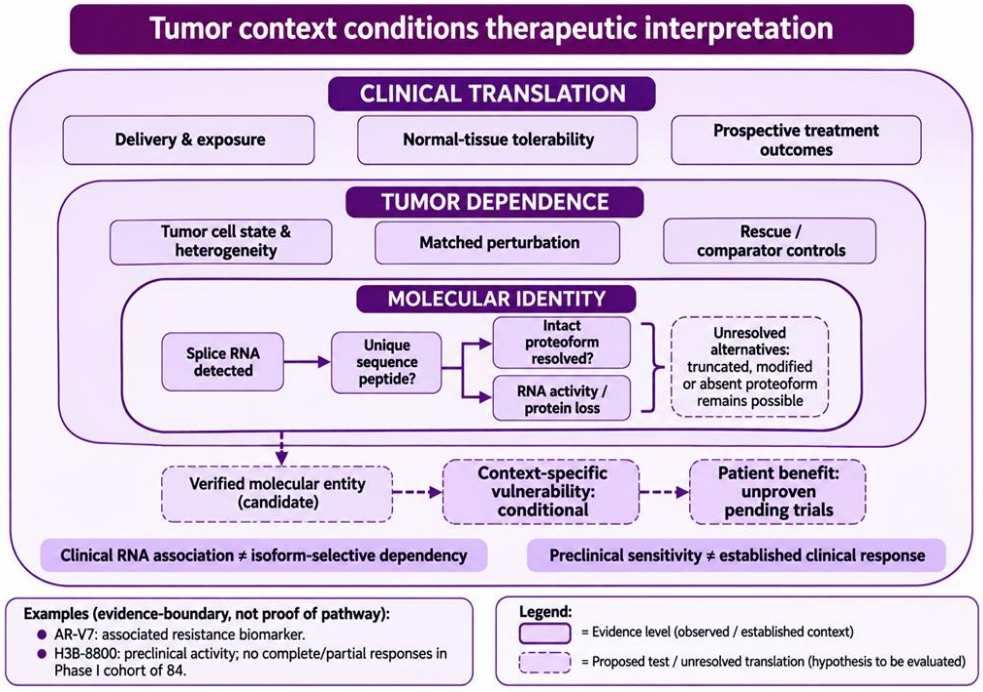


Figure 2. Tumor context determines when protein evidence becomes a therapeutic claim

Note. Nested conceptual map of three separate questions: whether the altered molecular species is resolved, whether a matched tumor system depends on the affected mechanism, and whether an intervention reaches relevant tissue with clinically meaningful consequences. Reported examples span molecular/functional proof and conditional resistance association (Antonarakis *et al*., 2014; Karakulak *et al*., 2026). Dashed links are proposed validation steps, not established effects. Arrows among molecular questions represent investigative alternatives, not biological prerequisites: RNA activity or protein loss can occur without a newly detected peptide. The figure is an original evidence-integration synthesis without numerical efficacy claims.

Results and Discussion

The strongest splicing-to-vulnerability examples do not all lead to an isoform-selective drug. SF3B1-linked splice defects can deplete BRD9 or shift serine metabolism; MKNK2 switching can redirect signaling; and a poison-exon transcript can exert RNA-mediated effects. These mechanisms invite different therapeutic classes and cannot be pooled as one "proteoform-targeting" strategy. In pancreatic cancer models, elevated or dysregulated SF3B1 was associated with sensitivity of tumor and stem-like cells to a splice-targeting agent (Alors-Perez *et al*., 2021). Such activity supports exploration of splicing-system vulnerability, but not selective engagement of a particular protein isoform.

Preclinical efficacy and clinical efficacy must be reported separately. H3B-8800 produced activity in spliceosome-mutant preclinical models (Seiler, Yoshimi, *et al*., 2018). In the subsequent Phase I study of 84 patients with myeloid neoplasms, no complete or partial responses by the stated criteria were observed, although some transfusion-dependent patients experienced transfusion-free intervals (Steensma *et al*., 2021). This is not evidence that spliceosome modulation has no potential; it is evidence that model-linked sensitivity cannot be substituted for demonstrated clinical response. The observed patient outcomes, dose schedules, and tolerability constrain any translational interpretation.

Isoform-specific manipulation is likewise not one established treatment class. Mishima *et al*. (2024) studied REST splice-switching in neuroendocrine cancer models, whereas Wan *et al*. (2024) developed organoid-based antisense screening in pancreatic cancer. These approaches can prioritize candidates and reveal delivery or cellular-state dependencies, but model selection and oligonucleotide uptake can favor effects not reproduced in whole tumors. An informative development pathway couples orthogonal isoform measurement to rescue, multiple models, and pharmacodynamic evidence at the intended site.

Limitations

This targeted review does not estimate the field-wide prevalence of translated aberrant isoforms or the proportion of therapeutic hypotheses that succeed. The curated reference allocation is not a systematic sampling frame, and some source claims were checked at abstract rather than complete experimental-figure level. Across primary studies, discordant genome annotations, sequence databases, proteomic coverage, sample purity, and changes in tumor cellular composition complicate comparison. Spatially restricted clones or time-dependent treatment adaptations can be lost in bulk RNA and proteomic averages. Particularly for short peptides, cleavage products, antigen presentation, and intact protein accumulation cannot be treated as interchangeable observations. Animal models, organoids, and cell lines also differ from patient tumors in immune environment, dosing, and tolerability; neither positive nor negative findings transfer automatically.

Conclusion

Alternative splicing creates plausible therapeutic opportunities only when the relevant molecular consequence and biological dependence have been established at the level required by the claim. Transcript discovery nominates candidates; selective peptide or intact-protein measurement resolves some of them; perturbation and rescue establish mechanism; and clinical testing determines benefit in context. Some credible vulnerabilities arise from protein loss or RNA activity rather than a novel stable proteoform. Preserving these distinctions offers a more reliable route from cancer splice variation to actionable evidence.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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