Fusion transcripts: Unexploited vulnerabilities in cancer?

Carla Neckles | Soumya Sundara Rajan | Natasha J. Caplen

Abstract
Gene fusions are an important class of mutations in several cancer types and include genomic rearrangements that fuse regulatory or coding elements from two different genes. Analysis of the genetics of cancers harboring fusion oncogenes and the proteins they encode have enhanced cancer diagnosis and in some cases patient treatment. However, the effect of the complex structure of fusion genes on the biogenesis of the resulting chimeric transcripts they express is not well studied. There are two potential RNA-related vulnerabilities inherent to fusion-driven cancers: (a) the processing of the fusion precursor messenger RNA (pre-mRNA) to the mature mRNA and (b) the mature mRNA. In this study, we discuss the effects that the genetic organization of fusion oncogenes has on the generation of translatable mature RNAs and the diversity of fusion transcripts expressed in different cancer subtypes, which can fundamentally influence both tumorigenesis and treatment. We also discuss functional genomic approaches that can be utilized to identify proteins that mediate the processing of fusion pre-mRNAs. Furthermore, we assert that an enhanced understanding of fusion transcript biogenesis and the diversity of the chimeric RNAs present in fusion-driven cancers will increase the likelihood of successful application of RNA-based therapies in this class of tumors.

1 | INTRODUCTION

Gene fusion events that deregulate protein expression or generate a chimeric protein are associated with the pathology of several cancer types, accounting for approximately 20% of tumors overall (Mitelman, Johansson, & Mertens, 2007). Most tumorigenic gene fusions comprise of regulatory and protein-encoding sequences from two different genes and, depending on the fusion gene, one or both the partner genes can contribute to oncogenesis (Mertens, Johansson, Fioretos, & Mitelman, 2015). Examples of pathologically relevant gene fusion events include the juxtaposition of promoter and enhancer sequences close to a proto-oncogene, the disruption of a tumor suppressor gene, or the creation of a fusion protein with aberrant functionality.
careful assessment of RNA-related vulnerabilities inherent to fusion-driven cancers, it should be possible to develop RNA-focused strategies to treat genetic diseases (Crooke, Witztum, Bennett, & Baker, 2018; Setten, Rossi, & Han, 2017). This recent success leads us to ask the question—Is this the time to reassess the feasibility of similar therapeutic strategies to directly target fusion transcripts in cancers that depend on their translated products?

Here, using case studies, we highlight how the genetic organization of fusion oncogenes impacts the generation of translatable mature mRNAs. We discuss how the complexity of fusion transcripts expressed in specified tumor types may influence disease pathology and treatment, and how this may complicate the application of RNA-based strategies to target fusion-driven cancers. We also emphasize how little we know about the proteins required for processing transcripts that contain regulatory sequences derived from two different genes. Furthermore, using our recent studies of the EWS-FLI1 fusion oncogene, we demonstrate how functional genetic approaches could be used to address this lack of knowledge. Finally, we aim to show that with careful assessment of RNA-related vulnerabilities inherent to fusion-driven cancers, it should be possible to develop RNA-based therapies against fusion transcripts for some tumors that are dependent on the activity of their translated proteins.

2 | ONCOGENIC FUSION TRANSCRIPTS—THE JOINING OF PARTS

Despite a significant increase in the ability to characterize the genomic structure of fusion genes and detect the transcripts they express, the complex molecular processes required for the generation of a translatable product from these atypical genes remains relatively unexplored. Most factors that modulate splicing of the wild-type counterpart transcripts will affect the
processing of the corresponding fusion transcripts. However, it is currently ambiguous how exon/intron definition around fusion transcript junctions influence the RNA splicing outcomes required for the expression of an in-frame fusion product. Furthermore, many fusion genes express transcript variants that potentially impact disease pathology or the response to treatment. But, the proteins responsible for regulating the alternative splicing events that give rise to these transcript variants remain poorly characterized. Bridging this gap could have profound implications, as it would allow for the development of new therapies that block the biogenesis of the fusion transcript and deplete expression of the oncoprotein it encodes. Before discussing the layer of complexity observed when a cancer cell expresses a fusion precursor messenger RNA (pre-mRNA), we will briefly describe the processes utilized to generate the diversity of mRNAs expressed in all human cells.

Precursor messenger RNA splicing is a vital process that intricately regulates gene expression and the protein diversity observed in complex organisms (Braunschweig, Gueroussov, Plocik, Graveley, & Blencowe, 2013; Irimia & Blencowe, 2012; Nilsen & Graveley, 2010). Splicing of pre-mRNAs involves the recognition and removal of noncoding regions (intron excision) and joining of coding regions (exon ligation) to generate a mature mRNA. The spliceosome, a dynamic ribonucleoprotein machine that incorporates the stepwise assembly and disassembly of several hundred proteins and five small nuclear RNAs, catalyzes this process. For further details, please see the following reviews: Jurica and Moore (2003), Shi (2017), and Sperling (2017). The architecture of pre-mRNAs determines whether the spliceosome components recognize splice sites across introns or exons and modifies spliceosome assembly (Berget, 1995; Zhu et al., 2009). If exons are long and separated by short introns (<250 bp), then the splicing machinery is more likely to form across introns—the intron definition model. In contrast, if exons are short and separated by long introns (>250 bp), then the splicing machinery can form across exons—the exon definition model. The efficiency of juxtaposing smaller units of either exons or introns compared to larger units is considered a critical determinant of these splice-site pairing mechanisms. In addition to the importance of splice-site recognition and pre-mRNA architecture in spliceosome assembly, short degenerate sequences found within introns and exons can recruit trans-splicing auxiliary proteins, such as members of the serine/arginine-rich (SR) or heterogeneous nuclear ribonucleoproteins (hnRNPs) protein families to regulate alternative splicing. These sites are exonic/intronic splicing enhancers and exonic/intronic splicing silencers, which can synergistically or antagonistically change the efficiency of spliceosome formation. For more information on elements that influence spliceosome assembly and pre-mRNA splicing, we refer the reader to the following advanced reviews by De Conti, Baralle, & Buratti, 2013 and Keren, Lev-Maor, & Ast, 2010.

RNA processing of transcripts that arise from gene fusions adds another layer of complexity to the splicing process. The intronic and exonic regulatory sequences derived from two different genes need to be compatible with the efficient formation of the spliceosome to initiate canonical and alternative splicing. This compatibility is essential at regulatory sites nearby and surrounding the fusion junction sites. Figure 1 depicts plausible splicing events that may lead to the generation of the predominant and/or variant fusion transcripts.

**FIGURE 1** Canonical and alternative splicing events for a representative fusion transcript. Boxes and solid lines indicate exons and introns, respectively. The dashed lines indicate possible splicing events in the context of exons adjacent to a fusion breakpoint.
Analysis of the mRNAs expressed from many fusion oncogenes has shown the expression of variant transcripts resulting from alternative splicing, including those expressed by the **BCR-ABL1**, **RUNX1-RUNX1T1**, and **TMPRSS2-ERG** fusion genes. These findings are not surprising since ~95% of multiexon genes are alternatively spliced (Pan, Shai, Lee, Frey, & Blencowe, 2008). In fact, to date, there are over 11,000 chimeric transcripts associated with cancer in the ChiTaRS database (Gorohovski et al., 2017). While many represent minor transcript species, in some cases, the dominant oncogenic fusion transcript that encodes the protein required for the initiation and maintenance of tumorigenesis depends on a specific RNA processing event (e.g., exclusion of one or more exons). Thus, dissecting the RNA and protein factors that antagonistically and synergistically decide the fate of exon/introns in these fusion transcripts could lead to potential targetable vulnerabilities to treat various cancer types. Overall, these factors are potentially driven by (a) distinctive RNA sequence and/or structural features within fusion transcripts and (b) protein factors that participate in a discrete splice-site pairing when processing the fusion transcript. The following sections discuss some of these features in the context of representative fusion genes associated with different cancers and their potential relevance to disease development and current or future treatment.

### 3 | ONCOGENIC FUSION TRANSCRIPTS—PARTNERS IN CANCER

#### 3.1 | **BCR-ABL1** in leukemia

The discovery of fusion oncogenes began with the study of hematological malignancies that harbor recurrent gross chromosomal rearrangements (Rowley, 1984), the first being **BCR-ABL1** (Ben-Neriah, Daley, Mes-Masson, Witte, & Baltimore, 1986). **BCR-ABL1** is generated from a reciprocal translocation, t(9;22)(q34;q11), and is a hallmark of chronic myeloid leukemia (CML) (Figure 2a). Soon after the identification of the **BCR-ABL1** fusion oncogene, reports of variant transcripts derived from the use of alternative **ABL** 5' exons emerged (Shtivelman, Lifshitz, Gale, Roe, & Canaani, 1986). However, further studies demonstrated that while translocation of the 5' end of the **ABL** gene can result in the inclusion of different first exons at a genomic level, upon RNA processing, the majority of transcripts include one 5' **ABL1** exon, which is typically referred to as **ABL1** exon a2 (reviewed in Barnes & Melo, 2002; Kurzrock, Gutterman, & Talpaz, 1988; Melo, 1996). In contrast, the three regions in the **BCR** locus that are the target of rearrangements can lead to the expression of different transcripts encoding the principal **BCR-ABL1** isoforms defined by their approximate molecular weights, p210, p190, and p230. Each **BCR-ABL1** isoform persistently enhances tyrosine kinase activity resulting in altered downstream signaling pathways and transformation (Branford, Rudzki, & Hughes, 2000; Chan et al., 1987; Demehri et al., 2005; Hochhaus et al., 1996; LaFiura et al., 2008; S. Li, Ilaria, Million, Daley, & Van Etten, 1999; Saglio et al., 1996).

The variant **BCR-ABL1** transcripts predominantly observed in CML involve exons 13 or 14 of **BCR** (13a2 or 14a2) that encode the p210 isoforms. Some tumors can express both the 13a2 and 14a2 transcripts as a result of alternative splicing. The e1a2 **BCR-ABL1** fusion is observed in CML but is found more frequently in acute lymphoblastic leukemia. The p230-coding transcripts that result from breakpoints in intron 19 of **BCR** are relatively rare, as are other transcripts that account for less than 2% of **BCR-ABL1** positive tumors (Weerkamp et al., 2009). A recent retrospective analysis of the **BCR-ABL1** fusion transcripts expressed in over 45,000 newly diagnosed CML cases showed that 37.9% of tumors express e13a2-**BCR-ABL1** transcripts and 62.1% of tumors express e14a2-**BCR-ABL1** (Baccarani et al., 2019). Depending on the cohort, between ~2 and 19% of tumors co-express the e13a2 and e14a2-**BCR-ABL1** transcripts. Curiously, though many studies have defined the complex

![FIGURE 2](image-url) Schematic depictions of representative transcripts expressed from fusion genes generated by chromosomal rearrangements that disrupt (a) the **BCR** and **ABL** genes (t(9;22) (q34q11)). (Reprinted with permission from Deininger, Goldman, and Melo (2000). Copyright 2000 American Society of Hematology). (b) The **RUNX1** and **RUNX1T1** genes (ins(21;20)(q22; q11q11)). Genes and transcripts (not to scale) are shown 5 to 3. (Adapted with permission from Lam and Zhang (2012))
structure of the in-frame transcripts that express the different versions of the BCR-ABL1 oncoprotein, to date, the proteins that contribute to the biogenesis of these oncogenic transcripts are unknown.

Although the use of tyrosine kinase inhibitors (TKIs), such as imatinib, nilotinib, and dasatinib that target BCR-ABL1 have proven enormously successful, drug resistance occurs in 10–20% of patients with CML. In most cases, point mutations are responsible for TKI resistance, but the detection of new or altered levels of variant BCR-ABL1 transcripts in addition to the wild-type BCR-ABL1 in drug-treated CML samples has led to consideration of alternative splicing as another possible mechanism of resistance (Laudadio, Deininger, Mauro, Druker, & Press, 2008; Lee et al., 2008; Ma et al., 2009; Marin et al., 2008; Talpaz et al., 2006; White et al., 2006, 2010). Mutations in BCR-ABL1 that alter the processing of the transcript include a 35-nucleotide insertion of sequences between ABL1 exons 8 and 9, first identified in a patient with imatinib resistance (Ma et al., 2009) that results in the expression of a truncated protein. One follow-up study detected BCR-ABL1\textsuperscript{ins35bp} transcripts in only four of 20 patients, and the presence of this mutation did not track with treatment response (O’Hare et al., 2011). Furthermore, heterologous expression of BCR-ABL1\textsuperscript{ins35bp} mutant protein failed to induce resistance to imatinib (O’Hare et al., 2011). However, a second retrospective review of over 250 cases of CML that analyzed the presence of kinase mutations detected BCR-ABL1\textsuperscript{ins35bp} in 23% of samples, all of which came from patients that exhibited disease progression or treatment resistance (Berman et al., 2016). Using long-range sequencing of the BCR-ABL1 transcripts present at different phases of disease, the BCR-ABL1\textsuperscript{ins35bp} variant was also detected in one patient that represented 12% of total BCR-ABL1 mRNA 7 months after diagnosis and 38% after 13 months. Imatinib failed to mediate a clinical response in this patient, but nilotinib therapy proved efficacious. Other BCR-ABL1 isoforms detected by long-range sequencing of samples from this patient included isoforms that retained additional BCR-derived sequences or deletion of part of ABL1 exon 7. A previous study also observed alternative splicing events that remove ABL1-exon 7 sequences, as well as the presence of rare BCR-ABL1-e6a2 and e19a2 transcripts (Gruber et al., 2012). While the biological significance of these BCR-ABL1 variant molecules is unknown, the recurrent nature of some of these variant mRNAs suggests that changes in the processing of the BCR-ABL1 pre-mRNA during disease progression and treatment warrants further study.

### 3.2 | RUNX1-RUNX1T1 in acute myeloid leukemia

One of the earliest discovered chromosomal aberrations after BCR-ABL1 was a consistent nonhomologous balanced translocation between chromosomes 8 and 21 in leukemic patients. The t(8;21) translocation is one of the most common genetic defects in acute myeloid leukemia, and this chromosome abnormality results in the RUNX1-RUNX1T1 fusion gene (formerly called AML1-ETO) (Muller, Duque, Shizuru, & Lubbert, 2008; Figure 2b). The RUNX1-RUNX1T1 fusion protein (also known as RUNX1-ETO or RUNX1-CBFA2T1) is thought to interact with other proteins to repress transcription and cooperates with additional secondary events, including mutations in c-KIT, FLT3, or PDGFRα/β to trigger leukemogenesis in myeloid progenitor cells (Beghini et al., 2000; Hatlen, Wang, & Nimer, 2012; Higuchi et al., 2002; Yuan et al., 2001).

Interestingly, several studies have demonstrated a wide range of variant protein-coding and noncoding transcripts generated from the RUNX1-RUNX1T1 gene related to the utilization of different RUNX1 promoters and alternative RUNX1T1 exons (Era et al., 1995; Erickson et al., 1992; Kozu, Fukuyama, Yamami, Akagi, & Kaneko, 2005; Kozu et al., 1993; LaFiura et al., 2008; Mannari, Gascoyne, Dunne, Chaplin, & Young, 2010; M. Yan et al., 2006). The breakpoints in the RUNX1 gene cluster in intron 5 and the breakpoints in RUNX1T1 cluster in intron 1. One RUNX1-RUNX1T1 transcript variant uses an alternative RUNX1T1 exon 9 (9a) and expresses a protein lacking the C-terminal domain that inhibits the transcriptional activation of RUNX1 (M. Yan et al., 2006). Expression of this variant fusion protein in a mouse model enhanced leukemogenesis (M. Yan et al., 2006). Another variant that occurs as a consequence of the alternative use RUNX1T1 exon 11 (11a) also results in the expression of a fusion protein lacking a C-terminal domain (Kozu et al., 2005). However, the impact of either variant on disease progression is unclear. Other rare variants detected in acute myeloid leukemia (AML) samples include transcripts containing a partial deletion of RUNX1T1-exon 3 with or without other changes (LaFiura et al., 2008; Mannari et al., 2010).

Similar to BCR-ABL, we know little about the proteins that facilitate the extensive alternative splicing of the RUNX1-RUNX1T1. One study attempted to begin to address this issue by examining the diversity of fusion transcripts present in AML cell lines and tumor samples and linking this to the recognition sequence motifs of different RNA processing proteins (Grinev et al., 2015). An analysis of over 100 possible RUNX1-RUNX1T1 variant transcripts revealed a diverse pool of full-length and truncated mRNA products formed from over 150 splicing events that map to 23 reference exons. Next, to identify cis-regulatory elements that could mediate the alternative splicing of RUNX1-RUNX1T1, Grinev and coworkers determined the enrichment for sequence motifs within each exon and flanking intron sequences. Their results highlighted the presence of RBFOX3 binding sites in the flanking regions of introns of some RUNX1-RUNX1T1 exons, and the study also showed AML
cell lines and tumor samples express RBFOX3 mRNA, whereas control samples do not. However, this analysis did not link any RNA processing factor to specific RUNX1-RUNX1T1 transcripts such as the 9a variant.

3.3 | EML4-ALK in non-small cell lung cancer

An alternative approach to the discovery of fusion oncogenes involves the use of cDNA expression libraries generated using RNA harvested from tumor samples. In 2007, this strategy identified EML4-ALK as a novel fusion oncogenic driver of a subset of non-small cell lung carcinoma (NSCLC) (Soda et al., 2007). Subsequent studies showed a chromosomal inversion on chromosome 2 (inv2) (p21:p23) fuses parts of the EML4 and ALK genes (Figure 3a). This inversion places the kinase domain of the receptor tyrosine kinase ALK under control of the constitutive promoter of EML4, enabling activated ALK signaling through well-established downstream pathways and malignant transformation (Rikova et al., 2007). The initial description of EML4-ALK fusion transcripts identified fusion of exon 13 or exon 20 of EML4 to exon 20 of ALK. The study of further NSCLC samples showed that the breakpoints in the ALK gene predominantly map to intron 19, but the breakpoints in EML4 can occur in several introns, including 6 (variant 3), 13 (variant 1), and 20 (variant 2) (Choi et al., 2008; Soda et al., 2007). Alternative splicing can generate two forms of variant 3; variant 3a contains EML4 exon 6a and variant 3b contains EML4 exons 6a and 6b. There are also reports of two variants (5a and b) involving breakpoints in EML4 intron 2. The variant EML4-ALK 5 fusions involve the same gene rearrangement in EML4 intron 2, but the processing of the variant 5b transcript includes an alternative splicing event that incorporates 117 nucleotides from the intron 2 into the fusion mRNA (Lin et al., 2018; Rikova et al., 2007; Soda et al., 2007; Yoshida et al., 2016).

Overall, 4–5% of NSCLC tumors contain ALK-rearrangements (Boland et al., 2009; Shaw et al., 2009; Soda et al., 2007; Takeuchi et al., 2008), which has enabled the clinical use of ALK TKI, such as crizotinib or alectinib, to treat this sub-group of patients. However, the clinical response to ALK TKI is variable, leading to speculation that ALK isoforms may exhibit different sensitivities to drug inhibition (Ali et al., 2016; Heuckmann et al., 2012; Woo et al., 2017; Yoshida et al., 2016). More recently though, an interim analysis of a Phase III clinical trial in ALK-positive NSCLC indicated no association between the clinical efficacy of alectinib or crizotinib and the EML4-ALK fusion type, although in some cases the sample numbers remain low and further analysis is ongoing to further investigate this question (Camidge et al., 2019). Also, while the availability of ALK inhibitors has proven a significant step forward for the treatment of at least a proportion of lung cancer patients (Kwak et al., 2010 and reviewed in Sasaki, Rodig, Chirieac, & Janne, 2010), resistance mechanisms are emerging. In the long-term, an improved understanding of how EML4-ALK fusion pre-mRNAs are processed could assist with the development of new treatment strategies for primary and refractory disease.

3.4 | NUT-fusions in NUT-midline carcinoma

Nuclear protein in testis (NUT)-midline carcinoma is a rare and highly aggressive cancer typically caused by the translocation t(15;19). In most cases, the testis-specific nuclear gene NUTM1 is fused to the bromodomain-containing protein 4 (BRD4)
gene (French et al., 2003; Figure 3b). There are also rarer rearrangements involving BRD3, NSD3, ZNF532, or unidentified 5' partner genes (French, 2018). Unfortunately, patients with t(15;19)-positive carcinomas respond poorly to standard chemotherapeutic treatment and this cancer subtype rapidly metastasizes (French, 2012, 2018). The fusion protein BRD4-NUT drives this disease by disrupting cell differentiation and promoting the growth of carcinoma cells through several molecular mechanisms (French et al., 2008; R. Wang & You, 2015; J. Yan, Diaz, Jiao, Wang, & You, 2011). For instance, the fusion partner BRD4 can tether NUT to acetylated chromatin via the bromodomain and then the putative transcriptional domain in NUT can subsequently recruit p300 and activate its histone acetyltransferase activity (Alekseyenko et al., 2015; Reynoird et al., 2010).

Most BRD4-NUT fusion mRNAs involve alignment of exons 1–11 of BRD4 to NUTM1 exons 2–7, but recent studies have detected variants that include a dependency on particular splicing events to generate in-frame transcripts. For example, nested polymerase chain reaction (PCR)-based studies by Thompson-Wicking et al. (2013), identified fusion genomic breakpoints upstream of the first exon of NUT. Critically, this exon must be removed to generate the in-frame protein-coding transcripts. In another case, a rearrangement that fuses BRD4 exon 15 to the last part of NUTM1 exon 2 results in the use of a cryptic splice site that serves to maintain an open reading frame. To date, it is unknown which splicing factors contribute to the recognition or resolution of these splicing events.

3.5 \( \text{TMPRSS2-ERG} \) in prostate cancer

The fusion gene TMPRSS2-ERG is prevalent in prostate cancer and places the expression of the transcription factor ERG under the control of the androgen-regulated TMPRSS2 promoter (Figure 3c; Tomlins et al., 2005). Fusion-driven events result in increased aberrant expression of ERG that regulates diverse cellular process associated with cancer metastasis (for reviews, please see Adamo & Ladomery, 2016; Kumar-Sinha, Tomlins, & Chinnaiyan, 2008; Shah & Chinnaiyan, 2009). Other TMPRSS2 fusions include alignment of TMPRSS2 sequences with either ETV1 or ETV4 (Perner et al., 2006; Tomlins et al., 2005). TMPRSS2-ERG fusions can arise as a consequence of either an intrachromosomal translocation or deletion as the TMPRSS2 and ERG genes map to the same arm of chromosome 21. The position of the breakpoints in each fusion partner and alternative splicing of ERG exons both contribute to the diversity of TMPRSS2-ERG fusion transcripts observed in fusion gene positive prostate cancer. Distinct fusion transcripts include exons 1, 2, and 3 of TMPRSS2 spliced to ERG exons 2, 3, 4, or 6 that can generate either full-length ERG protein, N-terminal truncated ERG proteins or a TMPRSS2-ERG fusion protein (Clark et al., 2007; Hagen et al., 2014; Hu et al., 2008; Perner et al., 2006; Tomlins et al., 2005). A study of localized and invasive prostate cancers also showed an increase in the exclusion of two cassette exons (72- and 81-bp exon) within ERG (Hagen et al., 2014). The retention of the 72-bp exon in ERG leads to increased cell proliferation and invasion (J. Wang et al., 2008). Moreover, a recent study utilized splice-switching oligonucleotides to target the 3' splice site of the 72-bp exon in ERG and induce exon skipping in VCaP prostate cancer cells (Jumbe et al., 2019). Induction of exon skipping reduced both cell invasion and proliferation and stimulated apoptosis. Unfortunately, as with other fusion transcripts, we still know little about the proteins and other recognition sequences that regulate the processing of TMPRSS2-ERG pre-mRNAs.

3.6 \( \text{EWS-FLI1} \) and \( \text{EWS-ERG} \) in Ewing sarcoma

The primary oncogenic event in most Ewing sarcoma (EWS) tumors involves either a t(11;22) or t(21;22) translocation that fuses the 5' end of the EWSR1 gene to the 3' end of either the FLI1 (~85% of cases) or ERG (~10% of cases) genes, generating the fusion genes EWS-FLI1 or EWS-ERG. (Delattre et al., 1992; May et al., 1993; Sorensen et al., 1994). The most common EWS-FLI1 fusion transcripts involve alignment of the first seven exons of EWSR1 spliced to either exons 5–9 (type 2 fusion subtype) or 6–9 (type 1 fusion subtype) of FLI1, while the most EWS-ERG fusion transcripts align EWSR1 exons 1–7 to exons 8 or 11 of ERG (COSMIC, 2019 database; Figure 4a). The transcripts expressed by these fusion genes encode different variants of the chimeric transcription factor EWS-FLI1 or EWS-ERG. These transcription factors upregulate genes in cell cycle, invasion and proliferation pathways (Bailly et al., 1994; Braun, Frieden, Lessnick, May, & Denny, 1995; Dauphinot et al., 2001; Nagano et al., 2010), and repress the expression of tumor suppressor genes (Hahm et al., 1999). In some cases, expression of an in-frame EWS-FLI1 fusion transcript requires exon exclusion. For example, retention of EWSR1 exon 8 at the genomic level necessitates the removal of this exon during pre-mRNA processing to generate an in-frame fusion transcripts (Berger et al., 2013; Crompton et al., 2014; Patocs et al., 2013; Zoubek et al., 1994; Zucman et al., 1993). One study showed 15 of 42 EWS tumors and four cell lines harbor translocations in which the EWSR1 exon 8 is spliced out at a posttranscriptional level (Berger et al., 2013), consistent with an overall frequency of approximately one-third of EWS-FLI1 positive
tumors (Hawkins et al., 2011). Critically, study of this particular variant has offered one of the first opportunities to identify a protein required for the biogenesis of an oncogenic fusion transcript.

**4 | THE MAKING OF A FUSION TRANSCRIPT: INSIGHTS FROM A FUNCTIONAL GENETIC SCREEN**

Recently, our group performed a genome-wide RNAi screen in the TC32 EWS cell line modified to report a readout of EWS-FLI1 activity. The screen identified over 30 candidate genes that selectively decreased EWS-FLI1 activity and are associated with canonical or alternative splicing (Figure 4b; Grohar et al., 2016). One of the lead candidate genes identified by this screen was HNRNPH1, a member of the hnRNP family of RNA binding proteins that include H2 and F. The HNRNPH/F proteins are involved in pre-mRNA processing, particularly alternative splicing (Geuens, Bouhy, & Timmerman, 2016). The TC32 cell line used for the RNAi screen harbors a translocation in which the EWSR1 exon 8 is spliced out at a posttranscriptional level (Berger et al., 2013). We thus hypothesized that the exclusion of EWSR1 exon 8 in TC32 cells requires HNRNPH1. Analysis of HNRNPH1-silenced TC32 cells revealed that exclusion of EWSR1 exon 8 requires HNRNPH1 and loss of HNRNPH1 results in the retention of EWSR1 exon 8. These results were confirmed in a second EWS cell line, SKNMC that retains EWSR1 exon 8 at the genomic level. The failure to splice out EWSR1 exon 8 in these cell lines results in reduced expression of EWS-FLI1 mRNA and protein, and reversal of EWS-FLI1 driven expression. EWS cell lines harboring Chr. 22 breakpoints in EWSR1 intron 7 (TC71 and RD-ES) exhibited no dependence on the expression of HNRNPH1. Analysis of HNRNPH1-bound RNA indicated direct binding of HNRNPH1 to EWSR1 exon 8, and an in vitro binding assay showed HNRNPH1 binds G-rich RNA sequences at the 3′ end of EWSR1 exon 8 (Grohar et al., 2016). Together, our results suggest that the inhibition of the EWSR1 exon exclusion event regulated by HNRNPH1 could block the expression of the EWS-FLI1 fusion oncoprotein expressed in about a quarter to a third of cases of EWS.

Interestingly, the RNAi screen also identified many components of the spliceosome as selectively required for EWS-FLI1 activity including, SF3A1, SF3B1, SF3B2, SNRPD1, and SNRPD2 (Figure 4b). To understand the mechanistic basis for this finding, we focused our first study on the gene encoding the catalytic component of the spliceosome, SF3B1. We observed a decrease in EWS-FLI1 mRNA levels in SF3B1-silenced EWS cell lines representing different breakpoints and fusion types; however, we also detected at least one EWS-FLI1 protein variant in SF3B1-depleted ES cells. The detection of EWS-FLI1 protein variants in SF3B1-depleted ES cells led us to hypothesize that altered spliceosome activity results in reduced expression of full-length EWS-FLI1 and the expression of mis-spliced in-frame EWS-FLI1 variant transcripts. Using PCR-based analysis, we established that SF3B1-depleted EWS cells mis-splice EWS-FLI1 and that some of the mis-spliced products are in-frame, explaining the presence of at least one EWS-FLI1 protein variant. To further understand the consequences of
inhibiting spliceosome function on the splicing of *EWS-FLI1*, we next employed a pharmacological inhibitor of the SF3b spliceosome subunit, Pladienolide B (PlaB). In EWS cells, treatment with PlaB phenocopied the effect of silencing *SF3B1*, including changes in the splicing of the *EWS-FLI1* pre-mRNA, and altered expression of the EWS-FLI1 protein.

One reason for the observed sensitivity to the inhibition of canonical splicing may relate to differences in the exon–intron architecture of the fusion pre-mRNA versus the wild-type counterpart genes. Most of the breakpoints in *EWSR1* occurring in either intron 7 or 8, which are 1,471 and 2,775 nucleotides in length, respectively. The breakpoints in the *FLI1* locus most commonly occur in introns 4 and 5, which are 8,972 and 23,342 nucleotides, respectively. The fusion intron that combines sequence elements from both *EWSR1* and *FLI1* genes are thus typically over 10,000 nts and includes splice site and branch site sequence derived from two genes (Figure 5a). There is also a complexity of overlapping putative splicing factor motifs identified within the exons surrounding the fusion junction that will affect splicing outcomes (Figure 5b; Desmet et al., 2009). Given the importance of the relative size of exons and introns as a determinant of splicing, the study of how this relationship is disrupted in many fusion pre-mRNAs may yield interesting information about the splicing process and, with the development of splicing inhibitors with in vivo efficacy, a potential therapeutic approach in some fusion-driven tumor types.

5 | SPLICING MODULATION AS A RISING THERAPEUTIC STRATEGY AGAINST FUSION-DRIVEN CANCERS?

There is a range of investigational or approved therapeutic modalities that could be employed to target fusion transcripts. Using the *EWS-FLI1* transcript as an exemplar fusion transcript, we have depicted some of these potential therapeutic approaches in Figure 6. Most of these approaches involve the application of nucleic acid analogues such as ASOs (Figure 6a),

**FIGURE 5** (a) Schematic depictions of the *EWSR1*, *FLI1*, and *EWS-FLI1* genes. Indicated are the relative size of each exon (upper) and each intron (lower part of each panel). Genes and transcripts (not to scale) are shown 5’ to 3’. (b) Schematic depiction of the putative splicing motifs in regions including and adjacent to *EWSR1* exons 7 and 8 and *FLI1* exon 6 that are involved in *EWS-FLI1* type 1 fusions. Representations are provided by Human Splicing Finder 3.1 (Desmet et al., 2009) for indicated regions within ENST00000406548 (*EWSR1*) or ENST00000527786 (*FLI1*)
small interfering RNAs (siRNAs) (Figure 6b), or microRNA (miRNA) mimics (Figure 6c). These nucleic acid analogues undergo complementary base-pairing with its target and modulate endogenous RNA processes. For example, splice-switching ASOs have been shown to base-pair with the pre-mRNA and alter splicing by blocking RNA:RNA base-pairing or protein–RNA interactions (Bauman, Jearawiriyapaisarn, & Kole, 2009; Havens & Hastings, 2016). Critical to the success of small RNA-based therapeutic approaches in recent years were the many breakthroughs in the chemical composition of the phosphate backbone and sugar components of oligonucleotides. Such modifications are needed to enhance the binding affinity, increase in vivo stability, and improve cellular uptake and release of ASOs, siRNAs, and miRNAs (reviewed in Evers, Toonen, & van Roon-Mom, 2015; Saleh, Arzumanov, & Gait, 2012; Sharma & Watts, 2015). Another nucleic acid-based therapeutic strategy involves the use of aptamers. Nucleic-based aptamers fold into distinct tertiary structures and bind to specific molecular targets with high affinity. One application of aptamer-based approaches for the targeting of a fusion transcript could involve the use of an aptamer to enhance the delivery and molecular recognition of an interfering RNA-based drug (Soldevilla, Meraviglia-Crivelli de Caso, Menon, & Pastor, 2018; Figure 6d). Alternatively, an aptamer-based strategy has the potential to act as an agonist or antagonist of an endogenous RNA process by targeting an RNA-binding protein. For more information about RNA-based drug design, we refer the reader to the reviews by Burnett and Rossi (2012) and Lieberman (2018).

Other promising approaches for targeting specific transcripts involves the application of small molecules that selectively interact with RNA (Figure 6e,f). As discussed above, some splicing factors interact with a pre-mRNA to antagonistically or
synergistically decide the fate of particular exon or introns. Studies have shown that the in vitro binding specificities of RNA-binding proteins can differ based on RNA sequence, structure, and contextual features (Dominguez et al., 2018). Thus, the identification of small molecules that bind specific RNA elements within a fusion pre-mRNA could displace the interaction of an RNA-binding protein required for the generation of the mature fusion transcript (Figure 6e, top). Although RNA structures are flexible and dynamic, one can identify RNA-binding small molecules through diverse structurally guided experimental approaches that trap thermodynamically favorable RNA conformations, blocking the recruitment of an RNA-binding protein at a specific sequence (reviewed by Connelly, Moon, & Schneekloth, 2016). Small molecules could also form ternary complexes with fusion pre-mRNAs and RNA-binding proteins, which may activate or inhibit alternative splicing (Figure 6e, bottom). For example, RG-7916 is currently in Phase II clinical trials for various types of spinal muscular atrophy and it functions by modulating pre-mRNA splicing of the survival motor neuron-2 (SMN2) gene (Poirier et al., 2018; Ratni et al., 2018; Sturm et al., 2019; and clinicaltrials.gov; NCT03779334). It was shown recently that binding of RG-7916 analogues to SMN2 pre-mRNA enhances the recruitment of two splicing activators (FUBP1 and KHSRP) and the formation of these ternary complexes contribute to its mechanism of action to enhance SMN2 splicing (J. Wang, Schultz, & Johnson, 2018). Another small molecule-based approach relevant to the targeting of fusion transcripts could involve the use of a bifunctional molecule that both selectively interacts with a transcript and facilitates the localized recruitment of an RNase, triggering the degradation of the targeted RNA (Figure 6f). This approach can be advantageous because linking a ribonuclease recruitment module to an RNA-binding small molecule may enhance selectivity and potency. Recent studies by Costales, Suresh, Vishnu, and Disney (2019) demonstrated the use of a bifunctional molecule as a chimeric recruitment strategy, in which their compound TGP-210-RL interacted with hypoxia-associated noncoding RNA and induced RNA degradation via the recruitment of RNase L. Overall, we anticipate a rise of RNA splicing modulators, both nucleic acid analogues and small molecules, that can selectively redirect alternative splicing as potential fusion-driven anti-cancer therapeutics.

## 6 Conclusion

Some of the first studies that assessed the feasibility of RNA-based therapeutic strategies targeted the mRNAs expressed by fusion oncogenes. Unfortunately, no active drug development programs emerged from these early studies. The recent approval of the first RNA-based molecules that target transcripts for degradation suggests that this may be the time to rethink onco- genic fusion transcripts as viable candidate targets. However, before embarking on such efforts, we need to more fully appreciate the inter- and intra-tumoral diversity of oncogenic fusion transcripts. In this study, we highlighted the complexity of the fusion transcripts expressed in six cancer subtypes, showing how variations in the position of chromosomal breakpoints and alternative splicing can contribute to the generation of a more heterogeneous population of fusion transcripts than many diagnostic-based assays will detect. We also highlighted our limited knowledge of the proteins that regulate the expression of fusion transcripts and how we overcome this in one case by using a functional genetic approach. We hope our work will stimulate the employment of similar strategies to probe the biogenesis of other fusion mRNAs. With a clearer appreciation of the unusual RNA-related vulnerabilities present in fusion-driven cancers, we consider that this class of tumors will once again be a focus of efforts that will target the lethal transcripts they express.

## Acknowledgments

We thank Tamara Jones, Allison Cross, and Katelyn Ludwig, Genetics Branch, CCR, NCI for helpful discussion and for assistance editing the manuscript.

## Conflict of Interest

The authors have declared no conflicts of interest for this article.

## Related WIREs Articles

- Targeting RNA in mammalian systems with small molecules
- Alternative-splicing defects in cancer: Splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics
- Splicing and cancer: Challenges and opportunities
REFERENCES

Adamo, P., & Ladomery, M. R. (2016). The oncogene ERG: A key factor in prostate cancer. Oncogene, 35(4), 403–414. https://doi.org/10.1038/onc.2015.109

Alekseyenko, A. A., Walsh, E. M., Wang, X., Grayson, A. R., Hsi, P. T., Kharchenko, P. V., … French, C. A. (2015). The oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains. Genes & Development, 29(14), 1507–1523. https://doi.org/10.1101/gad.267583.115

Ali, S. M., Hensing, T., Schrock, A. B., Allen, J., Sanford, E., Gowen, K., … Salgia, R. (2016). Comprehensive genomic profiling identifies a subset of crizotinib-responsive ALK-rearranged non-small cell lung cancer not detected by fluorescence in situ hybridization. The Oncologist, 21(6), 762–770. https://doi.org/10.1634/theoncologist.2015-0497

Baccarani, M., Castagnetti, F., Gugliotta, G., Rosti, G., Soverini, S., Albea, A., … International BCR-ABL Study Group. (2019). The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia, 33, 1173–1183. https://doi.org/10.1038/s41375-018-0341-4

Bailly, R. A., Bosselut, R., Zucman, J., Cormier, F., Delattre, O., Roussel, M., … Ghysdael, J. (1994). DNA-binding and transcriptional activation properties of the EWS-FLI1 fusion protein resulting from the (t(11;22) translocation in Ewing sarcoma. Molecular and Cellular Biology, 14(5), 3230–3241.

Barnes, D. J., & Melo, J. V. (2002). Cytogenetic and molecular genetic aspects of chronic myeloid leukaemia. Acta Haematologica, 108(4), 180–202. https://doi.org/10.1159/000065655

Bauman, J., Jearawiriypaisarn, N., & Kole, R. (2009). Therapeutic potential of splice-switching oligonucleotides. Oligonucleotides, 19(1), 1–13. https://doi.org/10.1089/oli.2008.0161

Beghini, A., Peterlongo, P., Ripamonti, C. B., Larizza, L., Cairoli, R., Morra, E., & Mecucci, C. (2000). C-kit mutations in core binding factor leukemias. Blood, 95(2), 726–727.

Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N., & Baltimore, D. (1986). The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science, 233(4760), 212–214.

Berger, M., Dirksen, U., Braeuninger, A., Koehler, G., Juergens, H., Krumbholz, M., & Metzler, M. (2013). Genomic EWS-FLI1 fusion sequences in Ewing sarcoma resemble breakpoint characteristics of immature lymphoid malignancies. PLoS One, 8(2), e56408. https://doi.org/10.1371/journal.pone.0056408

Berget, S. M. (1995). Exon recognition in vertebrate splicing. Journal of Biological Chemistry, 270(6), 2411–2414.

Berman, E., Jhanwar, S., Hedvat, C., Arcila, M. E., Wahab, O. A., Levine, R., … Albitar, M. (2016). Resistance to imatinib in patients with chronic myelogenous leukemia and the splice variant BCR-ABL1(35INS). Leukemia Research, 49, 108–112. https://doi.org/10.1016/j.leukres.2016.08.006

Boland, J. M., Erdogan, S., Vasmatzis, G., Yang, P., Tillmans, L. S., Johnson, M. R., … Yi, E. S. (2009). Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. Human Pathology, 40(8), 1152–1158. https://doi.org/10.1016/j.humpath.2009.01.012

Branford, S., Rudzki, Z., & Hughes, T. P. (2000). A novel BCR-ABL transcript (e8a2) with the insertion of an inverted sequence of ABL intron 1b in a patient with Philadelphia-positive chronic myeloid leukaemia. British Journal of Haematology, 109(3), 635–637.

Braunschweig, U., Gueroussov, S., Plocik, A. M., Graveley, B. R., & Blencowe, B. J. (2013). Dynamic integration of splicing within gene regulatory pathways. Cell, 152(6), 1252–1269. https://doi.org/10.1016/j.cell.2013.02.034

Burnett, J. C., & Rossi, J. J. (2012). RNA-based therapeutics: Current progress and future prospects. Chemistry & Biology, 19(1), 60–71. https://doi.org/10.1016/j.chembiol.2011.12.008

Camidge, D. R., Dzidziuszko, R., Peters, S., Mok, T., Noe, J., Nowicka, M., … Shaw, A. T. (2019). Updated efficacy and safety data and impact of the EML4-ALK fusion variant on the efficacy of Alectinib in untreated ALK-positive advanced non-small-cell lung cancer in the global phase III ALEX study. Journal of Thoracic Oncology, 14, 1233–1243. https://doi.org/10.1016/j.jtho.2019.03.007

Chan, L. C., Karhi, K. K., Rayter, S. I., Heisterkamp, N., Erdani, S., Powles, R., … Wiedemann, L. M. (1987). A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. Nature, 325(605), 635–637. https://doi.org/10.1038/325635a0

Choi, Y. L., Takeuchi, K., Soda, M., Inamura, K., Togashi, Y., Hatano, S., … Mano, H. (2008). Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Research, 68(13), 4971–4976. https://doi.org/10.1158/0008-5472.CAN-07-6158

Clark, J., Merson, S., Jhavar, S., Flohr, P., Edwards, S., Foster, C. S., … Cooper, C. S. (2007). Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. Oncogene, 26(18), 2667–2673. https://doi.org/10.1089/onc.2007.120070

Connolly, C. M., Moon, M. H., & Schneekloth, J. S., Jr. (2016). The emerging role of RNA as a therapeutic target for small molecules. Cell Chemical Biology, 23(9), 1077–1090. https://doi.org/10.1016/j.chembiol.2016.05.021

COSMIC. (2019). Catalog of somatic mutations in cancer. Retrieved from https://cancer.sanger.ac.uk/cosmic
Talpaz, M., Shah, N. P., Kantarjian, H., Donato, N., Nicoll, J., Paquette, R., ... Sawyers, C. L. (2006). Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. The New England Journal of Medicine, 354(24), 2531–2541. https://doi.org/10.1056/NEJMoA055229

Thomas, X., & Heiblig, M. (2016). The development of agents targeting the BCR-ABL tyrosine kinase as Philadelphia chromosome-positive acute lymphoblastic leukemia treatment. Expert Opinion on Drug Discovery, 11(11), 1061–1070. https://doi.org/10.1080/17460441.2016.1227318

Thompson-Wicking, K., Francis, R. W., Stirnweiss, A., Ferrari, E., Welch, M. D., Baker, E., ... Beesley, A. H. (2013). Novel BRD4-NUT fusion isoforms increase the pathogenic complexity in NUT midline carcinoma. Oncogene, 32(39), 4664–4674. https://doi.org/10.1038/onc.2012.487

Tomlins, S. A., Rhodes, D. R., Perner, S., Dhanasekaran, S. M., Mehra, R., Sun, X. W., ... Chinnaiyan, A. M. (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science, 310(5748), 644–648. https://doi.org/10.1126/science.1117679

Varley, K. E., Gertz, J., Roberts, B. S., Davis, N. S., Browning, K. M., Kirby, M. K., ... Myers, R. M. (2014). Recurrent read-through fusion transcripts in breast cancer. Breast Cancer Research and Treatment, 146(2), 287–297. https://doi.org/10.1007/s10549-014-3019-2

Wang, J., Cai, Y., Yu, W., Ren, C., Spencer, D. M., & Ittmann, M. (2008). Pleiotropic biological activities of alternatively spliced TMPRSS2/ERG fusion gene transcripts. Cancer Research, 68(20), 8516–8524. https://doi.org/10.1158/0008-5472.CAN-11-1417

Wang, J., Schultz, P. G., & Johnson, K. A. (2018). Mechanistic studies of a small-molecule modulator of SMN2 splicing. Proceedings of the National Academy of Sciences of the United States of America, 115(20), E4604–E4612. https://doi.org/10.1073/pnas.1800261115

Wang, R., & You, J. (2015). Mechanistic analysis of the role of bromodomain-containing protein 4 (BRD4) in BRD4-NUT oncoprotein-induced transcriptional activation. Journal of Biological Chemistry, 290(5), 2744–2758. https://doi.org/10.1074/jbc.M114.600759

Weerkamp, F., Dekking, E., Ng, Y. Y., van der Velden, V. H., Wai, H., Bottcher, S., ... EuroFlow, C. (2009). Flow cytometric immunobead assay for the detection of BCR-ABL fusion proteins in leukemia patients. Leukemia, 23(6), 1106–1117. https://doi.org/10.1038/leu.2009.93

White, D. L., Dang, P., Engler, J., Frede, A., Zrim, S., Osborn, M., ... Hughes, T. P. (2010). Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. Journal of Clinical Oncology, 28(16), 2761–2767. https://doi.org/10.1200/JCO.2009.26.5819

White, D. L., Saunders, V. A., Dang, P., Engler, J., Zannettino, A. C., Cambarelli, A. C., ... Hughes, T. P. (2006). OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): Reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood, 108(2), 697–704. https://doi.org/10.1182/blood-2005-11-4687

Woo, C. G., Seo, S., Kim, S. W., Jang, S. J., Park, K. S., Song, J. Y., ... Choi, J. (2017). Differential protein stability and clinical responses of EML4-ALK fusion variants to various ALK inhibitors in advanced ALK-rearranged non-small cell lung cancer. Annals of Oncology, 28(4), 791–797. https://doi.org/10.1093/annonc/mdw693

Wright, R. L., & Vaughan, A. T. (2014). A systematic description of MLL fusion gene formation. Critical Reviews in Oncology/Hematology, 91(3), 283–291. https://doi.org/10.1016/j.critrevonc.2014.03.004

Yan, J., Diaz, J., Jiao, J., Wang, R., & You, J. (2011). Perturbation of BRD4 protein function by BRD4-NUT protein abrogates cellular differentiation in NUT midline carcinoma. Journal of Biological Chemistry, 286(31), 27663–27675. https://doi.org/10.1074/jbc.M111.246975

Yan, M., Kanbe, E., Peterson, L. F., Boyapati, A., Miao, Y., Wang, Y., ... Zhang, D. E. (2006). A previously unidentified alternatively spliced isoform of t(8;21) transcript promotes leukemogenesis. Nature Medicine, 12(8), 945–949. https://doi.org/10.1038/nm1443

Yoshida, T., Oya, Y., Tanaka, K., Shimizu, J., Horio, Y., Kuroda, H., ... Yatabe, Y. (2016). Differential crizotinib response duration among ALK fusion variants in ALK-positive non-small-cell lung cancer. Journal of Clinical Oncology, 34(28), 3383–3389. https://doi.org/10.1200/JCO.2015.65.8732

Yuan, Y., Zhou, L., Miyamoto, T., Iwasaki, H., Harakawa, N., Hetherington, C. J., ... Zhang, D. E. (2001). AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. Proceedings of the National Academy of Sciences of the United States of America, 98(18), 10398–10403. https://doi.org/10.1073/pnas.171321298

Zhang, Y., Gong, M., Yuan, H., Park, H. G., Frierson, H. F., & Li, H. (2012). Chimeric transcript generated by cis-splicing of adjacent genes regulates prostate cancer cell proliferation. Cancer Discovery, 2(7), 598–607. https://doi.org/10.1158/2159-8290.CD-12-0042

Zhu, L., Zhang, Y., Zhang, W., Yang, S., Chen, J. Q., & Tian, D. (2009). Patterns of exon–intron architecture variation of genes in eukaryotic genomes. BMC Genomics, 10, 47. https://doi.org/10.1186/1471-2164-10-47

Zoubek, A., Pfeiferer, C., Salzer-Kuntschik, M., Aman, G., Windhager, R., Fink, F. M., ... Kovar, H. (1994). Variability of EWS chimaeric transcripts in Ewing tumours: A comparison of clinical and molecular data. British Journal of Cancer, 70(5), 908–913.

Zucman, J., Melot, T., Desmaze, C., Ghysdael, J., Plougastel, B., Peter, M., ... Ture-Carell, C. (1993). Combinatorial generation of variable fusion proteins in the Ewing family of tumours. The EMBO Journal, 12(12), 4481–4487.

How to cite this article: Neckles C, Sundara Rajan S, Caplen NJ. Fusion transcripts: Unexploited vulnerabilities in cancer? WIREs RNA. 2020;11:e1562. https://doi.org/10.1002/wrna.1562