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MINI REVIEW

The expanding universe of *NUTM1* fusions in pediatric cancer

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Abstract

NUT midline carcinoma family member 1 (*NUTM1*) fusions were originally identified in poorly differentiated and clinically aggressive carcinomas typically located in the midline structures of children and young adults, and collectively known as NUT (midline) carcinomas. Next-generation sequencing later uncovered *NUTM1* fusions in a variety of other pediatric and adult cancers of diverse location and type, including hematologic malignancies, cutaneous adnexal tumors, and sarcomas. A vast array of *NUTM1* fusions with bromodomain containing 4 (*BRD4)* or bromodomain containing 3 (*BRD3)*, which are characteristic of NUT carcinoma, and with several other fusion partners have been identified and associated with variable prognosis. These non-kinase fusions are thought to cause epigenetic reprogramming, thereby promoting proliferation, and hindering the differentiation of cancer cells. Many questions about both the function of the naïve NUTM1 protein, which is mostly restricted to the germ cells of the testis and is related to spermatogenesis and the oncogenic mechanisms of the various *NUTM1* fusions in both adult and pediatric cancer, are still unanswered. Moreover, whether there is a relationship defined by the presence of *NUTM1* fusions between conventional NUT carcinoma and other *NUTM1*-rearranged neoplasms remains to be elucidated. This review will focus on recent discoveries of *NUTM1* fusions found in pediatric cancers, their prognostic impact, and emergence as novel oncogenic drivers.

NUTM1: A PUZZLING PROTEIN

The NUT midline carcinoma family member 1 (*NUTM1*) gene on chromosome 15q14 encodes a poorly understood protein with nuclear expression restricted to germ cells of the testis and ovaries, and ciliary body. *NUTM1* has also been identified in germ cell tumors and *NUTM1*-rearranged neoplasms. Several aspects of *NUTM1* function were uncovered in the context of bromodomain containing 4 (*BRD4*)::*NUTM1* fusions in NUT carcinoma. As knowledge evolves, additional *NUTM1*-rearranged neoplasms are being identified in pediatric and adult patients, with *NUTM1* as the 3′ fusion partner fused to a variety of 5′ gene partners (Figures 1 and 2).[1–4]

NUTM1 fusions are increasingly recognized as capable of altering the epigenetic landscape. Not surprisingly, most identified *NUTM1* fusion partners are predicted to have at least transient nuclear localization and be involved

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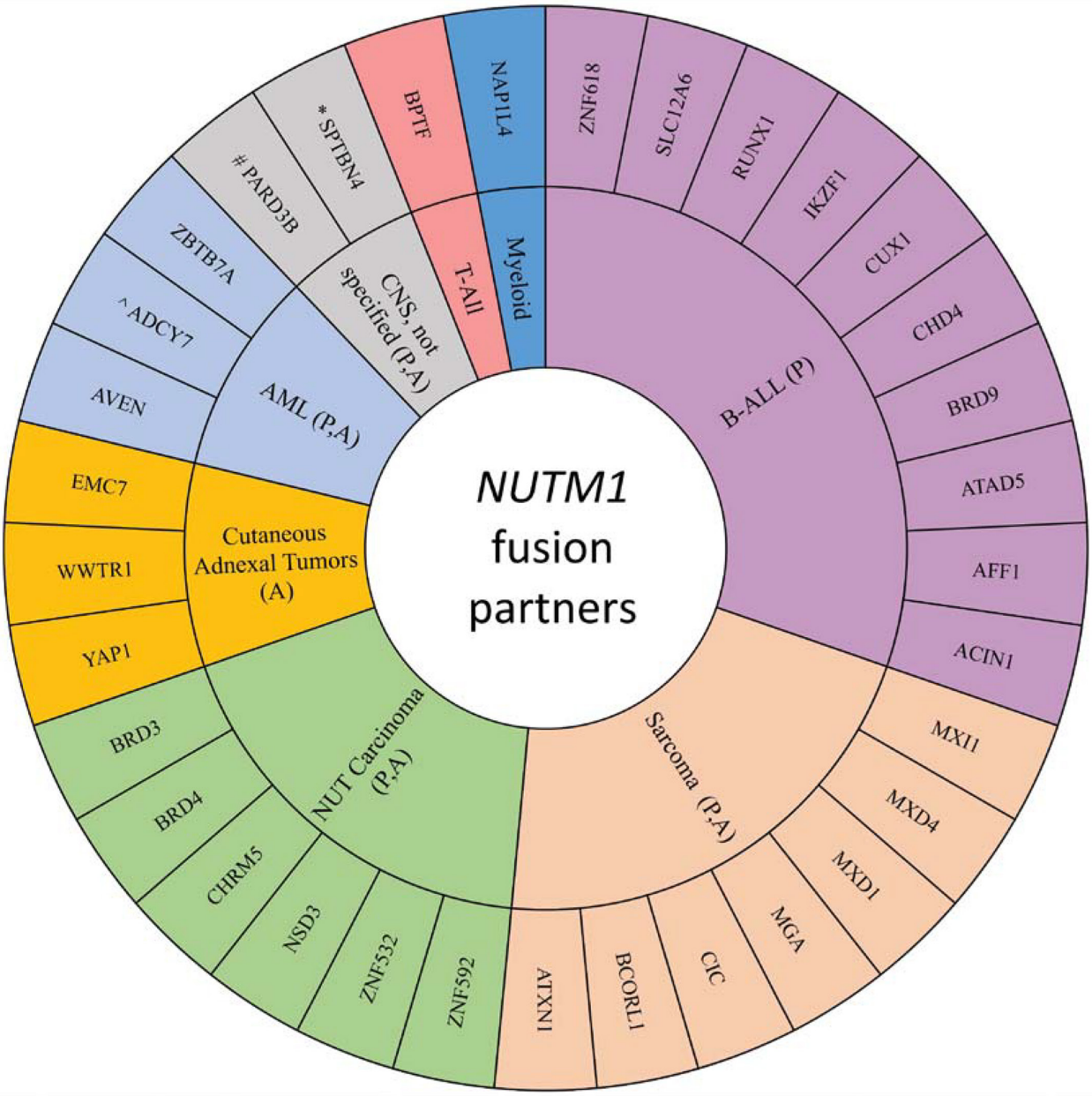

FIGURE 1 Landscape of *NUTM1* fusion partners in different pediatric and adult neoplasms. Commonly favored associations reported in the literature and/or public databases[3–5,12,15,24] between *NUTM1* fusion partners (outer circle) and neoplasm types (inner circle) are depicted; other associations may occur. *NUTM1*-rearrenged neoplasms with at least two reported fusion partners were categorized as typically occurring in pediatric (P), adult (A), or pediatric and adult (P, A) cases according to the references. The oncogenicity potential has not yet been fully characterized in the literature for most depicted fusion partners. The sarcoma category includes central nervous system (CNS) Ewing sarcoma family tumors with *CIC*::*NUTM1* fusions. Also refer to Table 1. ^*ADCY7*::*NUTM1*: truncated reading frame[12]; #*PARD3B*::*NUTM1*, CNS embryonal tumor, not otherwise specified[12]; **SPTBN4*::*NUTM1*, non-canonical[15]; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; *ACIN1*, apoptotic chromatin condensation inducer 1; *ADCY7*, adenylate cyclase 7; *AFF1*, ALF transcription elongation factor 1; *ATAD5*, ATPase family AAA domain containing 5; *ATXN1*, ataxin 1; *AVEN*, apoptosis and caspase activation inhibitor; *BCORL1*, BCL6 corepressor like 1; *BPTF*, bromodomain PHD finger transcription factor; *BRD3*, bromodomain containing 3; *BRD4*, bromodomain containing 4; *BRD9*, bromodomain containing 9; *CHD4*, chromodomain helicase DNA binding protein 4; *CHRM5*, cholinergic receptor muscarinic 5; *CIC*, capicua transcriptional repressor; *CUX1*, cut like homeobox 1; *EMC7*, ER membrane protein complex subunit 7; *IKZF1*, IKAROS family zinc finger 1; *MGA*, MAX dimerization protein MGA; *MXD1*, MAX dimerization protein 1; *MXD4*, MAX dimerization protein 4; *MXI1*, MAX interactor 1; *NAP1L4*, nucleosome assembly protein 1 like 4; *NSD3*, nuclear receptor binding SET domain protein 3; *NUTM1*, NUT midline carcinoma family member 1; *PARD3B*, par-3 family cell polarity regulator beta; *RUNX1*, RUNX family transcription factor 1; *SLC12A6*, solute carrier family 12 member 6; *SPTBN4*, spectrin beta, non-erythrocytic 4; *WWTR1*, WW domain containing transcription regulator 1; *YAP1*, Yes1 associated transcriptional regulator; *ZBTB7A*, zinc finger and BTB domain containing 7A; *ZNF532*, zinc finger protein 532; *ZNF592*, zinc finger protein 592; *ZNF618*, zinc finger protein 618.

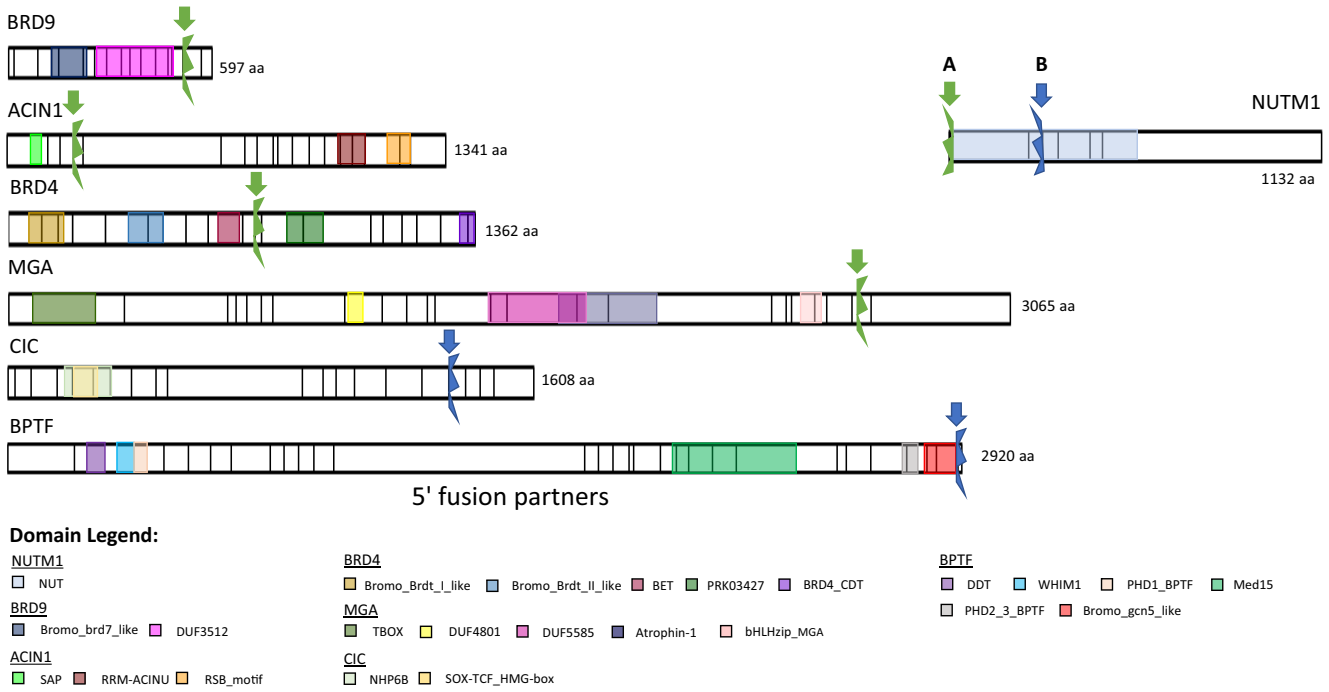

FIGURE 2 Schematic structure of NUTM1 fusion proteins. Arrows and marks (🡇) denote representative breakpoints; other variants may occur.[12,15,16] For 5′ fusion partners, green and blue arrows and marks represent examples of fusions involving *NUTM1* exons 3–8 or exons 5–8, respectively, as the 3′ partner (Reference Sequence (RefSeq) Accession No: NM_175741.3). Displayed protein domains were retrieved from each respective NCBI Entrez Gene page.[25] Translation length is indicated for each of the represented fusion partner protein isoforms. For additional information on reference sequences, refer to Table S2. *NUTM1*, NUT midline carcinoma family member 1 [NUT, NUT protein]; *BRD9*, bromodomain containing 9 [Bromo_brd7_like, Bromodomain, brd7_like subgroup; DUF3512, domain of unknown function (DUF3512)]; *ACIN1*, apoptotic chromatin condensation inducer 1 [SAP, Putative DNA-binding (bihelical); RRM_ACINU, RNA recognition motif in apoptotic chromatin condensation inducer in the nucleus (acinus) and similar proteins; RSB_motif, RNSP1-SAP18 binding (RSB) motif]; *BRD4*, bromodomain containing 4 [Bromo_Brdt_I_like, bromodomain, Brdt_like subfamily, repeat I; Bromo_Brdt_II_like, bromodomain, Brdt_like subfamily, repeat II; PRK03427, cell division protein ZipA; BET, bromodomain extra-terminal – transcription regulation; BRD4_CDT, C-terminal domain of bromodomain protein 4]; *MGA*, MAX dimerization protein MGA [TBOX, T-box DNA binding domain of the T-box family of transcriptional regulators; Atrophin-1, Atrophin-1 family; DUF4801, domain of unknown function (DUF4801); DUF5585, family of unknown function (DUF5585); bHLHzip_MGA, basic Helix–Loop–Helix-zipper (bHLHzip) domain]; *CIC*, capicua transcriptional repressor [NHP6B, chromatin-associated proteins containing the HMG domain; SOX-TCF_HMG-box, SOX-TCF_HMG-box, class I member of the HMG-box superfamily of DNA-binding proteins]; *BPTF*, bromodomain PHD finger transcription factor [Bromo_gcn5_like, bromodomain Gcn5_like subfamily; Med15, ARC105 or Med15 subunit of mediator complex non-fungal; PHD1_BPTF, PHD finger 1 found in bromodomain and PHD finger-containing transcription factor (BPTF); PHD2_3_BPTF, PHD finger 2 and 3 found in bromodomain and PHD finger-containing transcription factor (BPTF); DDT, DDT (DNA binding homeobox and different transcription factors) domain; WHIM1, WSTF, HB1, Itc1p, MBD9 motif 1]; aa, amino acids.

in transcriptional control, chromatin remodeling, and epigenetic regulation (Figure S1, Table S1).[4] Along with that, the tissue of origin and context signaling provide unique features contributing to the reported heterogeneity of *NUTM1*-rearranged neoplasms; however, the essential components of this group of gene fusions are yet to be fully characterized.

Several approaches may be used in isolation or in combination to identify *NUTM1* fusions in tumor samples, including detection of *NUTM1* nuclear expression by immunohistochemistry (IHC) or by detection of the fusion presence by fluorescence in situ hybridization, reverse transcriptase polymerase chain reaction, or by using a sequencing method.[2–5]

NUTM1 fusions are considered relevant targets to pediatric cancer and are included in the Relevant Molecular Target List developed by the US Food and Drug Administration (FDA), with input from the National Cancer Institute and the pediatric cancer research community, to comply with the amended provisions of the Pediatric Research Equity Act (PREA).[6] This review will focus on this emergent group of non-kinase fusions in pediatric cancer, particularly in NUT carcinoma, hematologic malignancies, and sarcomas, and the challenges

posed by their rarity, evolving knowledge, and yet unraveled oncogenic mechanisms.

NUT CARCINOMA: THE PROTOTYPE

The presence of *NUTM1* gene fusions characterizes a heterogeneous group of neoplasms, of which NUT carcinoma, a rare and aggressive subtype of squamous cell carcinoma with variable responses to aggressive multimodality management, is the prototype.[3,4,7–9] The initial discovery of the *BRD4*::*NUTM1* fusion in NUT carcinoma provided for genetic causality, which was later substantiated by characterization of additional *NUTM1* fusions with different partners, a low tumor mutational burden, and the lack of other identified oncogenic drivers.[3]

The cell of origin of NUT carcinoma is not established. It has been suggested that NUT carcinoma originates from primitive, undifferentiated, or poorly differentiated cells. Although initially described as occurring in midline epithelial tissues of the head, neck, or thorax, NUT carcinoma can also occur in non-midline locations.[2,3] NUT carcinoma has been reported in a broad range of ages from newborn to patients over 80 years old. No sex preference, geographic location, or risk factors have been clearly determined. Some of the NUT carcinoma features overlap with those of other poorly differentiated neoplasms, creating diagnostic challenges and leading to a wide range of differential diagnoses. Moreover, in pediatric patients, NUT carcinoma morphology also overlaps with that of blastomas.[3] Because of its rarity, heterogeneity of presentations and potential for incorrect diagnosis, the actual prevalence of NUT carcinoma is unknown; it is reported to account for 7% of poorly differentiated or undifferentiated carcinomas in children and young adults.[2,3]

BRD4::*NUTM1* fusions, megadomains, and the road to epigenetic reprograming

The *BRD4*::*NUTM1* fusion is found in approximately two-thirds of NUT carcinoma cases, and thus most data have been generated in the context of this fusion. Other *NUTM1* fusion partners identified in the remaining cases include bromodomain containing 3 (*BRD3*), the nuclear receptor binding SET domain protein 3 (*NSD3*), which is a histone-lysine N-methyltransferase, and zinc finger (*ZNF*) proteins (e.g., *ZNF532* and *ZNF592*).[2,3] For a landscape of fusion partners found in pediatric and adult cases of NUT carcinoma, and in other *NUTM1*-rearrenged neoplasms, refer to Figure 1. Despite most partners being associated with transcriptional processes (Table S1, Figure S1), it is notable that the spectrum of partners typically found in pediatric and adult NUT carcinoma differs from that found in other *NUTM1*-rearranged neoplasms (Figure 1). These differences will be highlighted in the next sections.

Different *BRD4*::*NUTM1* fusion variants have been reported; the predominant variant appears to involve the in-frame fusion between *BRD4* exon 11 and *NUTM1* exon 3 (exon number may vary with the splice variant). The NUTM1 protein has an unstructured domain architecture and includes two putative acidic transcriptional activation domains, a bipartite nuclear localization sequence (NLS), and a nuclear export signal. Nearly the whole *NUTM1* coding region is preserved and joined to three *BRD4* domains (two bromodomains and the extra-terminal domain) and the bipartite NLS. The *BRD4* carboxyl-terminal domain, known to interact with the core positive transcription elongation factor b and regulate RNA polymerase II elongation, is lost[2] (Figure 2).

Both *BRD4* and *NUTM1* partners appear to have distinct and cooperative roles in the fusion, potentially combining the epigenetic reader capabilities of the bromodomain and extra-terminal domain (BET) protein, *BRD4*, with *NUTM1*. *BRD4* acts as a histone acetylation reader, and its ability to recognize and bind to acetylated lysines of histones provides a scaffold for key chromatin and transcriptional regulatory factors. In the context of spermatogenesis, the naïve *NUTM1* cooperates with bromodomain testis associated *(BRDT*), a testis-specific BET protein family member, and the histone acetyltransferase E1A Binding Protein P300 (*EP300*) to obtain a state of histone hyperacetylation and genomewide chromatin remodeling within condensing spermatids, that includes histone eviction and replacement by protamines. *NUTM1* recruits *EP300*, which catalyzes the acetylation of histone H4 at lysine 5 and lysine 8 residues. This specific histone acetylation pattern is recognized by *BRDT*, which binds to and facilitates histone displacement, ultimately leading to histone-to-protamine transition, and the final compaction of the haploid male genome in mature sperm cells. Disruptions in the transition process can affect male fertility. This functional loop appears to be recapitulated in somatic tumor cells through the *BRD4*::*NUTM1* fusion.[1] The BET protein fusion partner, *BRD4*, leads *NUTM1* to acetylated chromatin via its bromodomains, and *NUTM1* recruits and activates *EP300*, leading to increased local histone acetylation. In a feed-forward loop, acetylated histones lead to the recruitment of additional *BRD4*::*NUTM1* through *BRD4* (Figure 3). This self-perpetuating recruitment mechanism leads to the formation of hyperacetylated nuclear foci ("megadomains") and has the potential to cover a broad genomic area and dysregulate the gene output of transcription factors commonly implicated in

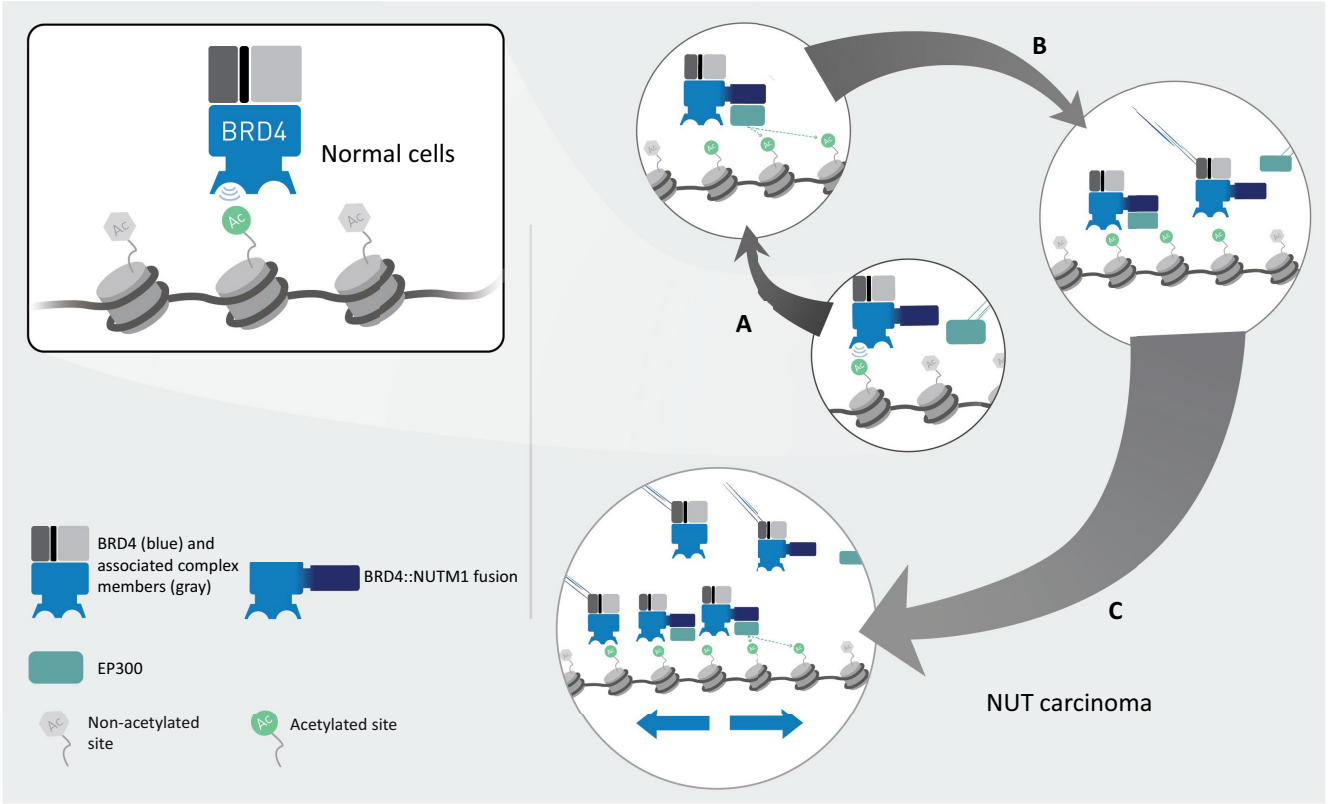

FIGURE 3 Current view of *BRD4*::*NUTM1* fusion feed-forward oncogenic mechanism. *BRD4* recognizes and binds to specific histone acetylation sites providing a scaffold for key chromatin and transcriptional regulatory factors. In normal cells, *BRD4* complexes are involved in transcription at gene-specific locations. (a) In *BRD4*::*NUTM1* driven-NUT carcinoma, *BRD4*, through its bromodomains, directs *NUTM1* to acetylated chromatin. (b) *NUTM1* recruits and activates the histone acetyltransferase *EP300*, leading to increased local histone acetylation. (c) In a feed-forward loop, acetylated histones lead to the recruitment of additional *BRD4*::*NUTM1* and interacting complex proteins through *BRD4*. This self-perpetuating recruitment mechanism has the potential to cover a broad genomic area and dysregulate the gene output of transcription factors implicated in cancer.[1,2,4,10] For simplification, other histone modifications are not described. *BRD4*, bromodomain containing 4; *NUTM1*, NUT midline carcinoma family member 1; *EP300*, E1A binding protein p300.

cancer, such as the MYC proto-oncogene, bHLH transcription factor (*MYC*). A *BRD4*::*NUTM1* chromatin complex has been identified in which members that associate with *BRD4*, such as *BRD3*, *NSD3*, and certain zinc finger proteins, also exist as rare *NUTM1* fusion partners, pointing to a mechanistic parallel.[2,3,10] However, the normal *BRD4* function goes beyond a passive scaffolding factor role. *BRD4* has also intrinsic kinase and histone acetyltransferase enzymatic activities, supporting a more dynamic regulatory role toward its interaction partners and the local chromatin architecture than initially predicted. Moreover, *BRD4* functions as a regulatory factor at active enhancers and super-enhancers, including the *MYC* super-enhancer.[11] Therefore, the dysregulation of *BRD4* and its associated complex has the potential to dramatically affect transcriptional and epigenetic regulation in the tumor, which may underlie the aggressive behavior of NUT carcinomas.

Like with *BRD4*, other *NUTM1* fusions identified in NUT carcinoma are reported to preserve almost the entire *NUTM1* structure. In addition, different splice isoforms of partner genes, some with variable or even opposing roles in oncogenesis, have been described, illustrating the complex interplay of genes involved in these fusions.[11]

NUTM1 fusions, prognosis, and uncertainties

A recent analysis with a total of 124 patients from the NUT midline carcinoma registry (38% <18 years old) indicated that tumor location and the *NUTM1* fusion partner appear to affect prognosis, with *BRD4*::*NUTM1* conferring a poorer overall survival (OS) compared to *BRD3*::*NUTM1* or *NSD3*::*NUTM1*. Thoracic tumors had the worst prognosis regardless of the fusion, with a median OS of 4.4 months. Non-thoracic tumors with *BRD3*::*NUTM1* and *NSD3*::*NUTM1* had a median OS of 36.5 months versus 10 months in those with a *BRD4*::*NUTM1* fusion.[7]

TABLE 1 Diagnosis of *NUTM1*-rearrenged neoplasms pre- and post-molecular analysis.

Initial diagnosis	Age at diagnosis (y)	Primary tumor location	Proposed molecular diagnosis [post-molecular analysis]	References
Cerebral rhabdoid tumor (AT/RT)	2.2	Interventricular foramen	*CIC*::*NUTM1* sarcoma	[13]
Ewing-like sarcoma	3.2	Temporal area	*CIC*::*NUTM1* sarcoma	[13]
Ewing-like sarcoma	6.6	Paravertebral muscles	*CIC*::*NUTM1* sarcoma	[13]
Ewing sarcoma	50.3	Nasolabial area	NUT carcinoma (*BRD4*::*NUTM1*)	[13]
SMARCA4-deficient thoracic sarcoma[a]	27	Left lung	*CIC*::*NUTM1* sarcoma	[20]
Undifferentiated carcinoma	60	Soft tissue of the head	NUT carcinoma spectrum (*CIC*::*NUTM1*)	[21]
Unclassified small round cell sarcoma	5.7	Occipital area	*CIC*::*NUTM1* sarcoma	[13]
Unclassified small round cell sarcoma	12.3	Eye socket	NUT carcinoma (*BRD3*::*NUTM1*)	[13]

Note: In addition to immunohistochemical findings, molecular analyses included RNA sequencing (RNAseq),[13] fluorescence in situ hybridization (FISH) and targeted next-generation sequencing[21] or FISH, array comparative genomic hybridization (aCGH) tumor profiles and RNAseq.[20]

[a]*SMARCA4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; *NUTM1*, NUT midline carcinoma family member 1; AT/RT, atypical teratoid rhabdoid tumors; *CIC*, capicua transcriptional repressor; *BRD3*, bromodomain containing 3; *BRD4*, bromodomain containing 4.

Moreover, a spectrum of morphologies has been attributed in the literature to cancers with *NUTM1* fusions under the umbrella of NUT carcinoma, along with sarcomas and hematologic malignancies.[12] Due to phenotypic heterogeneity and uncertainty of histogenesis, pathology can differ from molecular diagnosis. This is exemplified by a case of a *BRD3*::*NUTM1*-rearranged tumor in the eye socket of a 12-year-old patient, which initially received a pre-molecular pathological diagnosis of unclassified round cell sarcoma, and was then reclassified to NUT carcinoma post-molecular diagnosis based on RNA sequencing (RNAseq) fusion and clustering results[13] (Table 1).

NUTM1 fusions with multiple other partners have been reported in a subset of pediatric and/or adult cancers from soft tissue and other organs with uncertain relationship to NUT carcinoma and with varying prognosis. Most of these cases appear to occur within the context of histologically defined high-grade sarcomas, which may have a distinct pathogenetic pathway.[2,3,12] Some of these fusions will be discussed below in the context of pediatric cancer.

HEMATOLOGIC MALIGNANCIES: NOVEL *NUTM1* FUSION PARTNERS UNCOVERED

NUTM1 fusions as a potential novel entity in B-cell acute lymphoblastic leukemia

NUTM1 fusions with various partners have been identified in ~3–5% of infant and in less than 1% of children with B-cell acute lymphoblastic leukemia (B-ALL). The *NUTM1*-fusion positive B-ALL molecular subset is typically pediatric and appears to be more prevalent in patients with lysine methyltransferase 2A (*KMT2A*)-wildtype status (i.e., without *KMT2A* rearrangements). The fusion partners appear to be different from those predominantly found in NUT carcinoma (Figure 1), suggesting cancer type preference based on partner gene. Moreover, differences between partners occurring in infants compared to older children were also observed. In a series of 71 cases of pediatric *NUTM1*-rearranged B-ALL with known partners, 10 different fusion partners of *NUTM1* were identified.[5] Among infants, apoptotic chromatin condensation inducer 1 (*ACIN1*) (44%), bromodomain containing 9 (*BRD9*, 26%), and cut like homeobox 1 (*CUX1*, 15%) were the most frequently observed, whereas *CUX1* (28%), *ZNF618* (28%), and *ACIN1* (22%) were the most found among children. In contrast to NUT carcinoma and infant B-ALL with *KMT2A* rearrangements, *NUTM1*-fusion positive B-ALL has been linked to a more favorable prognosis.[3,5] However, two high-risk cases of *NUTM1*-rearranged B-ALL characterized by early relapse were recently reported, an infant patient with co-occurring *BRD9*::*NUTM1* and P2Y receptor family member 8 (*P2RY8*)::cytokine receptor like factor 2 (*CRLF2*) fusions and an adult patient with an *ACIN1*::*NUTM1* fusion,[14] suggesting that factors affecting prognosis are not fully known.

The *BRD9*::*NUTM1* fusion identified in infant B-ALL with *KMT2A*-wildtype may be analogous to the *BRD3/4*::*NUTM1* fusions found in NUT carcinoma. Although not a BET family member, *BRD9* contains a bromodomain and binds to acetylated lysine residues of histones[4] (Figure 2). It also has been postulated that the *NUTM1*-rearranged B-ALL can be further subdivided in homeobox A9 (*HOXA9*)-positive and *HOXA9*-negative subgroups characterized by differences in age of onset and distribution of fusion partners.[5] *NUTM1* fusions leading

to *HOXA9* upregulation might bind to gene promoters in the *HOXA* cluster through a DNA binding domain provided by the fusion partner, affecting thereby local DNA methylation, histone modification, and gene expression.

NUTM1 fusions and other hematologic malignancies

Rarely, *NUTM1* fusions have also been reported in samples of pediatric patients with hematologic malignancies aside from B-ALL. A *NUTM1* fusion with the bromodomain PHD finger transcription factor (*BPTF*), a non-BET bromodomain-containing protein acting as an epigenetic reader, was identified in pediatric T-cell acute lymphoblastic leukemia.[12,15] Moreover, a *NUTM1* fusion with the zinc finger and BTB domain containing 7A (*ZBTB7A*) was identified in an unclassified acute myeloid leukemia (AML) sample (Figure 1) from the Children's Oncology Group.[15] Through RNAseq clustering analysis, the authors found that this sample clustered with other AML samples that did not have *NUTM1* fusions, rather than with *NUTM1*-rearranged ALL, suggesting that, in this case, the cellular lineage rather than the molecular alteration was the basis for the clustering.

SARCOMAS OF VARIOUS SITES, INCLUDING IN THE CENTRAL NERVOUS SYSTEM

NUTM1 fusions and the MYC/MAX/MXD network

The incidence and clinical course of *NUTM1*-rearranged sarcomas are presently unclear due to the small number of described cases.[3] Two recent pediatric cases of the MAX dimerization protein MGA (*MGA*) fused to *NUTM1* were reported in high-grade spindle cell sarcoma (occurring in the thigh and intracranially, respectively) with no epithelial differentiation and with a favorable prognosis, suggesting these tumors are different from NUT carcinoma, despite having *NUTM1* fusions and overlapping histopathological features.[16] Fusions between other MAX dimerization (*MXD*) family members (e.g., MAX dimerization protein 1 [*MXD1*], *MXD4*) and *NUTM1* were also identified in sarcoma tissue samples of adult patients[4] (Figure 1). Different from *BRD4*::*NUTM1* proposed mechanism of action, the fusion of MXD proteins, which antagonize *MYC* transcriptional activity, to *NUTM1*, is hypothesized to convert these into *MYC*-phenocopies, potentially decreasing their tumor repressor effect.[17] Although through a potentially different mechanism of action compared to a *BRD4*::*NUTM1* fusion, the dysregulation of *MYC* function is attained in both cases, suggesting potential convergence of oncogenic mechanisms.

Capicua transcriptional repressor-fused sarcoma or NUT carcinoma: When the boundaries are blurred

Fusions with a common partner often define categories with distinct molecular and morphological characteristics. Aside from *NUTM1* fusions, other fusion types occurring in sarcomas include the group of capicua transcriptional repressor (*CIC*) fusions. Sarcomas with *CIC* fusions represent an emerging family of tumors with distinct 3′ fusion partners, more often fused to double homeobox 4 (*DUX4*) and less frequently to other partners including *NUTM1*. In pediatric brain tumors, *NUTM1* was initially reported as a fusion partner in central nervous system (CNS) Ewing sarcoma family tumors with *CIC* alterations (CNS EFT-CIC)[18,19] and lacking EWS RNA binding protein 1 (*EWSR1*) rearrangement. What could represent a case of ambiguous lineage, *CIC*::*NUTM1* sarcomas are considered a new molecular subset within *CIC*-fused sarcomas with a CNS preference and younger pediatric population. This is in contrast with the reported mean age in *CIC*-fused sarcomas in the young adult population.[20] Although *NUTM1* breakpoints can occur early in *NUTM1*-rearranged neoplasms, breakpoints at more distal exons have also been found, including in *CIC*::*NUTM1* sarcomas (Figure 2).[2,20]

As with NUT carcinomas, the diagnosis presents challenges reflected by differences between the pre- and post-molecular analysis.[13] At gene expression level, *CIC*::*NUTM1* tumors resemble *CIC*-fused sarcomas rather than NUT carcinomas, favoring thus the former classification, even though at the microscopic level some features may overlap. However, this is not universal and may rely on a range of characteristics and marker expression for a differential diagnosis, as for a case of a tumor with a *CIC*::*NUTM1* fusion in an adult patient, where NUT carcinoma was favored over *CIC*-rearranged sarcoma (Table 1).[3,21] Of note, *NUTM1* IHC is positive for both NUT carcinoma and *CIC*::*NUTM1* sarcoma, and therefore cannot longer be considered a hallmark of only NUT carcinoma.

As for *BRD4*::*NUTM1* fusion proposed oncogenic mechanism, *CIC* target genes would be transcriptionally activated by *NUTM1* via *EP300* recruitment.[18] The *CIC*::*NUTM1* fusion is an example where each partner is considered to be a defining characteristic of a different group of tumors (i.e., *CIC*-fused sarcomas or NUT carcinoma), reiterating the importance of the cellular context in shaping different tumor types, and underscoring challenges of tumor classification and diagnosis when

boundaries between tumors of different lineages (mesenchymal vs. epithelial) may not be clear.[4,20,21]

Recently, three cases with novel ataxin 1 (*ATXN1*)/ataxin 1 like (*ATXN1L*)-associated fusions with NUT family member 2A (*NUTM2A*) and features of *CIC*-fused sarcomas without *CIC* fusions were reported, and which may also be associated with younger age and more aggressive disease, underscoring the complexity and diagnostic challenges of non-kinase fusions.[22]

TREATMENT

Differently from kinase fusions, where the shared architecture of an activated kinase creates a vulnerability that can be exploited by drug development, non-kinase fusions are more heterogenous and challenging to counteract directly. NUT carcinoma has a dismal prognosis and is known to be generally resistant to standard therapeutic interventions, including multimodality therapy. BET inhibitors have shown promise against NUT carcinoma where *BRD4* or one of its interaction partners, such as *BRD3* or *NSD3*, are fused to *NUTM1*. Histone deacetylase inhibitors have also shown potential in a pediatric case of a 10-year-old patient diagnosed with an NUT carcinoma positive for *BRD4*::*NUTM1*.[23] However, options such as BET inhibitors may not be effective in tumors with fusions not expected to be directly associated to the *BRD4* chromatin complex. This highlights the importance of uncovering the oncogenic mechanisms of the various fusions and of an accurate molecular diagnosis. Other *NUTM1*-rearrenged neoplasms appear to have a more favorable prognosis and a better response to the standard therapies.[4] Discoveries are still unfolding and fusions are rare, adding to the challenges presented by this group of neoplasms in terms of potential therapeutic interventions, including the development of rational combination therapies.

FINAL THOUGHTS

NUTM1-rearrenged neoplasms constitute a rare and heterogenous group with variable prognosis affecting children and adults. NUT carcinomas and *NUTM1*-rearranged sarcomas have been reported in pediatric and adult patients, whereas *NUTM1*-rearranged B-ALL has been typically reported in pediatric patients. Although through different ways, *NUTM1* fusions with various partners appear to share a mode of action directed to a profound reprogramming of transcriptional networks, finding different solutions to a common goal. Shared rules of *NUTM1* fusion architecture, tissue preference and potential for differential oncogenic activity, and associated risk based on partner gene or fusion variant, however, are not yet clearly understood. Uncovering the mechanism of action of *NUTM1* fusions in different settings is critical to inform the design of treatment modalities. Moreover, because of diagnostic challenges in part due to non-specificity of NUT carcinoma histology, the adoption of an unbiased sequencing approach to identify *NUTM1* fusion partners as part of the diagnostic process may help to establish differential diagnosis and envision future modes of treatment.

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CONFLICT OF INTEREST STATEMENT

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DISCLAIMER

The opinions expressed in this article are those of the authors and should not be interpreted as the position of the US Food and Drug Administration.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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