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NUT midline carcinoma: Current concepts and future perspectives of a novel tumour entity

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ABSTRACT

NMC is a recently recognized cancer type hallmarked by chromosomal translocation involving the NUT gene, a catastrophic event leading to fusion oncoprotein responsible for malignant transformation and tumor progression. The aggressiveness of disease together with a poor response to conventional treatment make NMC one of the most lethal cancer. Moreover, although until recently NMC has been poorly understood and largely neglected, the number of reported cases is steadily rising. Recently, in addition to its pathogenetic and diagnostic role, NUT-fusion oncoprotein has been shown to be amenable to targeted inhibition using BET inhibitors. Future clinical trials are warranted with the aim of investigate the incorporation of targeted agent into multimodal therapeutic strategy. Since new promising NUT-targeting drugs are emerging that may affect the clinical course, the correct and prompt recognition of NMC is key to improve patients' outcome.

1. Introduction

Nuclear protein in testis (NUT) midline carcinoma (NMC) is a newly recognized, rare, and highly aggressive subtype of squamous cell carcinoma genetically driven by somatic chromosomal rearrangements involving the NUT gene. Following early sparse reports on midline pediatric tumours harboring the t(15;19) (Kubonishi et al., 1991; Kees et al., 1991; Lee et al., 1993; Vargas et al., 2001), in 2003 the group led by CA French in Boston first identified the fusion oncogene BRD4-NUT as result of such chromosomal abnormality therefore defining a novel disease entity since then termed NMC (French et al., 2003).

Given its rarity, non-unique histologic and immunophenotypic profile and the lack of awareness among clinicians, NMC has been largely underdiagnosed and misdiagnosed in the past decades. Only recently, the availability of an anti-NUT monoclonal antibody for immunohistochemical staining, has enabled an easier and accurate diagnostic work-up resulting in an increase in reported cases.

Although initially though to affect almost exclusively children and young-adults, it is increasingly being diagnosed in all age groups, with the primary involvement of midline anatomical sites such as chest or hand and neck. The aggressiveness of the disease coupled with a poor response to standard treatments made NMC one of the most lethal cancer with median overall survival ranging from 6 to 9 months (Bauer et al., 2012; Chau et al., 2016).Recent molecular advances have suggested that NUT fusion oncoproteins are disease-drivers that act by blocking squamous differentiation and maintaining tumor growth through aberrant histone acetylation (French et al., 2008). More importantly, these oncoproteins have shown to be druggable thereby opening new avenues for molecularly targeted therapeutic strategies. Bromodomain and Extra-Terminal (Bet) proteins inhibitors (BETi)and histone deacetylase inhibitors (HDACi)have shown promising preclinical and clinical activity and are currently being tested in trials enrolling NMC patients. In this article, we discuss clinicopathologic features, treatment approaches as well as future perspectives regarding

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NMC by reviewing the most updated and relevant literature.

2. Epidemiology and etiology

Although certainly uncommon tumours, the actual incidence and prevalence of NMCs are unknown.

The historical difficulty in distinguishing it from other undifferentiated or poorly differentiated malignancies along with a limited awareness of this disease entity likely contributed to underestimate the frequency of NMC in the past years.

Recently, NMCs have been reported to made up 7 % of poorly differentiated carcinomas occurring in a cohort of 98 patients younger than 40 years old (French et al., 2004) and up to 18 % of EBV-negative undifferentiated carcinomas of the upper aerodigestive tract in a cohort of 31 patients of any age (Stelow et al., 2008). NMCs exhibits no gender predilection and may affect people of any age, from the neonatal period through the elderly, albeit they have been traditionally reported to occur primarily in children and young-adults with a median age at diagnosis of 16 years (range 0.1–78) (Bauer et al., 2012). More recent reports suggest an increase in both overall incidence and age at presentation for NMC. Indeed, the diagnosis of NMC has increased annually since 2007, and since 2009 there has been an observed increase in the age at diagnosis from 14 to 29 years ($P < 0.05$) (Bauer et al., 2012). Accordingly, a 5-fold increase in the diagnosis of head and neck NMC from 2011 to 2014 has been reported with an increasing proportion of adult cases and a median age of onset of 21.9 years (range 0.1-81-7 years) (Chau et al., 2016). This pattern may be due to our improved diagnostic ability as well as the increased awareness of the disease within the scientific community, though an effect of a reporting bias related to the recent description of this entity has to be considered.

Currently, there are no known causative associations with exposures to environmental toxins, infectious agents, smoking, or oncogenic viruses such as human papillomavirus or Epstein-Barr virus (Stelow et al. and author's unpublished observations) (French, 2012).

Collaborative efforts to advance the knowledge about such a rare and challenging disease has led to the 2010 establishment of The International NUT Midline Carcinoma Registry (www.nmcregistry.org). The aims of this multinational registry include to gather clinical data and biological specimen, to provide pathologic review to assist in the diagnosis as well as dissemination of scientific information about NMC. With more than 100 cases so far included, this represents aprecious benchmark for informing both the medical community and citizenship about natural history, therapeutic interventions as well as research opportunities in this disease.

3. Pathogenesis and molecular pathology

Chromosomal aberration in the form of translocation of the NUT gene (also known as NUTM1 or *chr15orf55*) on chromosome 15q14 is the pathognomonic hallmarked of NMC. In roughly two-thirds of cases, NUT is involved in a balanced translocation with the BET family gene BRD4 on chromosome 19p13.1 [t(15;19)(q14;p13.1)], forming an in-frame BRD4-NUT fusion oncogene whose product is driven by the BRD4 promoter (French et al., 2003). In the remaining cases, termed NUT-variants, the partner genes are either BRD3 (25 %) on chromosome 9 [t(9;15) (q34.2;q14)] or other non–BRD containing genes such as the histone methyltransferase NSD3 on chromosome 8 [t(8;15)(p11.23;q14)] or ZNF532 on chromosome 18 t(15;18)(q14;q23) (French et al., 2014; Alekseyenko et al., 2017).Although a favorable prognostic role with longer survival has been suggested for the NUT-variants, conflicting results have been more recently reported. The resulting chimeric oncoproteins contain both bromodomains, an ET domain and a bipartite nuclear localization signal of the first half of BRD4/BRD3 and nearly the entire coding region of NUT (French, 2010). It has been postulated that BRD4 tethers NUT to acetylated chromatin where the NUT portion recruits the histone acetyltransferase p300, leading to acetylation of neighboring histones. In turn, this process drives a feed-forward expansion of acetylated chromatin and BRD4–NUT recruitment over massive genomic domains, forming the so-called "megadomains", often filling whole topologically associating domains (French, 2013). These are cell lineage-specific stretches of active chromatin measuring up to 2 megabases that drive the transcription of key cancer-associated genes such as MYC and TP63 (Alekseyenko et al., 2015; Grayson et al., 2014). BRD4 is a ubiquitously expressed coactivator that upon binding to acetylated histones of both mitotic and interphase chromatin, is thought to activate transcription of early G1 genes following mitosis via its interaction with the transcriptional elongation complex P-TEFb (Dey et al., 2003; Jang et al., 2005). On the other hand, NUT is largely unstructured and contains an acidic region responsible for binding to EP300 and under normal conditions it is known to shuttle between the nucleus and the cytoplasm, being restricted to almost exclusively to adult testis and ciliary ganglion (French, 2010). To date two non mutually exclusive models of BRD-NUT driven oncogenesis have been proposed: repression of pro differentiative genes through sequestration of p300 and p300-directed transcription of genes required for proliferation and arrest of differentiation (French, 2013).BRD-NUT oncoproteins have been shown to arrest squamous differentiation and favour cell growth both in in vitro and in vivo models, as evidenced by the rapid and terminal squamous differentiation that occurs following siRNA knockdown of the BRD4-NUT oncoproteins8. The exact cell of origin of NMC is unrecognized and most reliable hypotheses point toward an epithelial progenitor cell or a primitive neural crest-derived cell. The frequent involvement of midline structures, the lack of any in situ component when involved epithelium-lined organs and the similarity of their genomic profile to that of adult ciliary ganglion are in keeping with a neural crest-derive structure. A further distinctive feature of NMC is a remarkably simple karyotype, often with a single translocation as sole cytogenetic aberration, contrary to most carcinomas of adulthood which exhibit complex aneuploid karyotypes and high mutational burden. Thus, NUT rearrangement is supposed to be an early tumor-initiating event that acts as all-in-one driver in the oncogenesis of this cancer (French, 2013). Accordingly, a recent published integrative analysis of whole-genome sequencing, transcriptome sequencing and cytogenetic characterization of NMC, found complex genomic rearrangements (also known as chromoplexy) involving the BRD3/4–NUT oncogenic rearrangements likely attributable to single catastrophic events sufficient for neoplastic transformation (Lee et al., 2017)(Fig. 1).

4. Histopathology and diagnosis

The histopathology of NMC is characteristic but not diagnostic as its morphological features may overlap with those of various other high grade malignancies, including squamous cell carcinoma, sinonasal undifferentiated carcinoma, Ewing sarcoma, EBV-associated nasopharyngeal carcinoma, thymic carcinoma, neuroblastoma, pancreatoblastoma and even salivary gland carcinoma (Fig. 2).

NMCs usually showed sheets of poorly differentiated carcinoma with foci of abrupt squamous differentiation of varying degrees and extensive necrosis. NMC cells are monomorphic, small-to-medium sized, with round-to oval nuclei with clear cytoplasm and prominent nucleoli. Pronounced atypia and pleomorphism are uncharacteristic of NMCs (Stelow, 2011). On immunohistochemistry a limited staining pattern was observed, with virtually all NMCs positive for NUT protein and most of them for p63 (90 %), non specified pan-cytokeratin (77 %), and EMA (75 %); whereas positivity for CD34 and CD99 was only occasionally seen (Evans et al., 2012). Having said that, the demonstration of NUT rearrangement is the fundamental requirement for the definitive diagnosis of NMC. This can be made either upon demonstration of NUT gene rearrangement by fluorescence in situ hybridization (FISH) or of the BRD-NUT fusion transcript by reverse transcriptase–polymerase chain reaction (RT-PCR). In general, FISH is

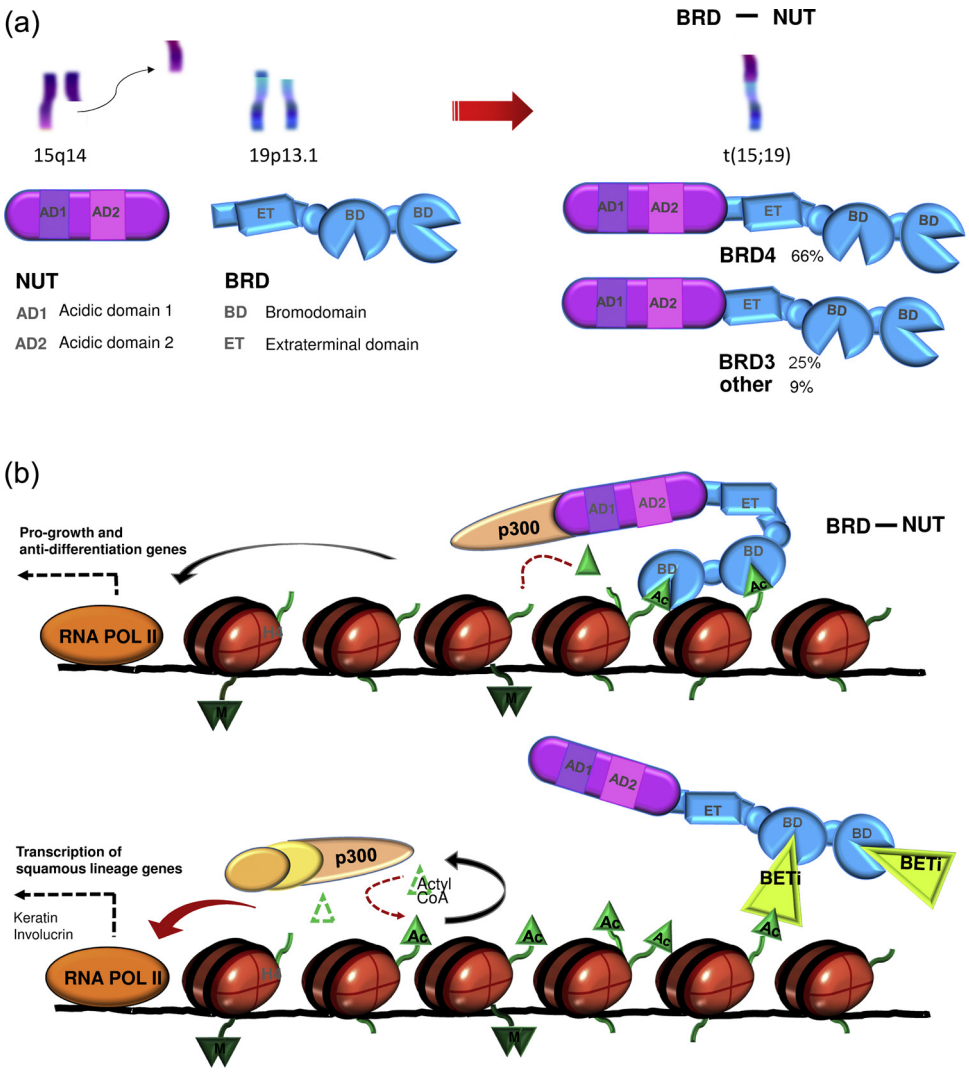

Fig. 1. a Schematic representation of NUT – BRD translocation. b Pathogenetic mechanism and reversal by drug targeting (with BETi).

preferred over RT-PCR because it enables the detection of potential NUT-variants, while RT-PCR at present time can only detect BRD4- or BRD3-NUT tumours. Over recent years, a specific monoclonal antibody to NUT (C52; Cell Signaling) has become commercially available for diagnostic purpose, proving to be 100 % specific and 87 % sensitive (Haack et al., 2009). This has made cheaper and easier the screening for NMC. Hence, current recommendations suggest immunohistochemical testing for NUT expression in all poorly differentiated carcinomas

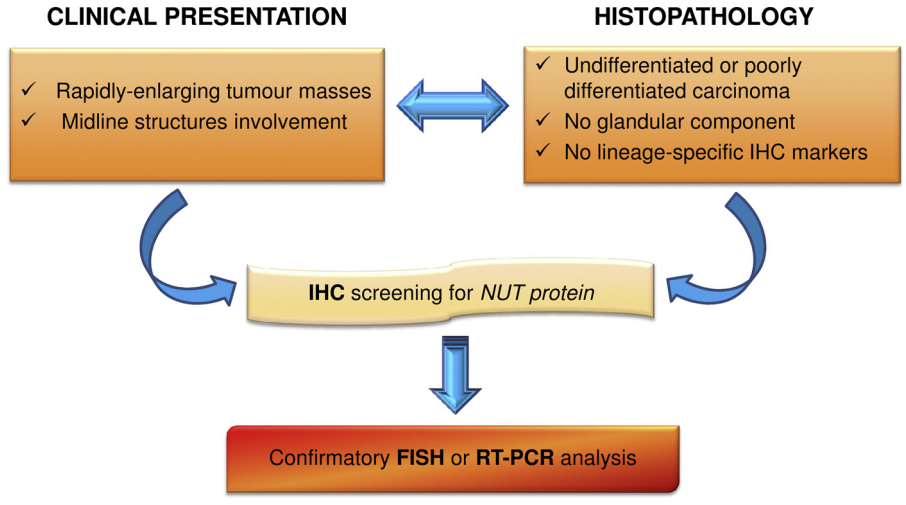

Fig. 2. Suggested/recommended diagnostic work-flow for suspected NUT cases.

without glandular differentiation, with or without squamous differentiation, arising in the chest, head and neck. Characterization of the fusion gene is not mandatory for the diagnosis but is recommended by FISH or RT-PCR. NMC should be considered in the differential diagnosis of any aggressive non gland forming poorly differentiated carcinoma in the absence of a viral or other known etiology (French, 2010).

5. Clinicopathologic features

NMC owes its name to its predilection for arising from supradiaphragmatic midline anatomic structures, with the primary involvement of the mediastinum (56 %) and the head and neck area (21 %) 6. In the latter case, sinonasal is the most commonly affected site accounting for more than half of all head and neck cases (57 %), followed by nasopharynx, oropharynx, hypopharynx, the larynx, and salivary gland (Chau et al., 2016).Despite this tendency, rarer cases are being increasingly diagnosed either below the diaphragm such as in the bladder or outside the midline axis such as in the parotic gland, iliac bone, adrenal gland and pancreas (Shehata et al., 2009; den Bakker et al., 2009; Ziai et al., 2010). NMC displays a devastating clinical course usually presenting with rapidly enlarging masses and at an advanced stage at diagnosis. Up to two-thirds of patients are diagnosed with advanced unresectable disease and more than 50 % of them have metastastic disease (Bauer et al., 2012; Chau et al., 2016). Most common site of metastatic spread are distant lymph nodes, bone, lung and pleura, while the liver and the brain are only unfrequently affected (Lemelle et al., 2017).Unlike the whole NMC population, the subgroup of head and neck NMC presents different clinicopathologic features as they mostly present with localized or locally advanced disease with regional nodal metastases (26 %), whereas distant metastases (6 %) are less common at diagnosis, arguably owing to an early onset of symptoms compared to other primary locations (Chau et al., 2016).

The outcome of patients with NMC is extremely poor despite intensive multimodality treatment. In the largest series so far reported, the median overall survival was 6.7 months and the overall survival at 2 years was 19 %, with more than 80 % of patients who died within 1 year of their diagnosis (Bauer et al., 2012). Interestingly, NMC arising in the head and neck showed slightly better survival outcome (median survival of 9.7 months) than thoracic NMC according to a historical comparison, probably reflecting an earlier diagnosis (Chau et al., 2016). Until now, only an isolated anecdotal case of a sustained complete remission from a BRD4-NUT rearranged NMC of the right iliac wing treated with chemotherapy has been described, albeit the exact diagnosis has been questioned (Mertens et al., 2007).

6. Conventional therapeutic management

Heterogenous treatment approaches have been employed over time and only case reports or small case series are available in the literature to inform oncologists regarding pattern of care and treatment outcome of NMC. As a result, no guidelines exist and the optimal management of NMC is yet to be established. When feasible, an intensive local treatment including gross surgical resection and radiotherapy has been shown to produce the best long-term results. In the largest series so far reported which enrolled 63 NMC patients, gross total surgical resection (R0 and R1) and initial radiotherapy were independently associated with improved 2-year PFS (60 % vs 10 %, P = 0.001) and 2-year OS (80 % vs 44 %, P = 0.002) (Bauer et al., 2012). Likewise, in a series of 40 HNNMC, both initial surgery with or without adjuvant chemoradiation or radiation (P = 0.04) and complete resection with negative margins (P = 0.01)have shown to favorably impact on progression-free survival (PFS) and overall survival (OS) (Chau et al., 2016). This arguably reflects the earlier diagnosis of HNNMC resulting in a greater proportion of patients amenable to potentially curative treatment. Unfortunately, this can apply only to a proportion of cases since the majority of NMC arises outside the HN area and presents with advanced-stage disease. Notably, chemotherapy and radiotherapy alone turned out to be inadequate as to date they have no proved impact on survival. In addition, no specific chemotherapy regimen has shown to be superior over another. Despite the lack of evidence, cytotoxic chemotherapy is an obliged choice in the vast majority of cases. Platinum-containing regimens and Ewing sarcoma protocols (vincristine, doxorubicin and cyclophosphamide, alternating with ifosfamide and etoposide) are among the most commonly adopted treatment combinations (Bauer et al., 2012; Chau et al., 2016). In patients not candidate to receive combination chemotherapy, single agent taxane has shown to be an active alternative (Engleson et al., 2006).Response to chemotherapy can occur in 30–40% of cases though they are usually transient and virtually all patients ultimately progressed. No salvage chemotherapy have been shown to be active.

Given the largely unsatisfying results achieved with conventional approaches, there is a urgent need for novel therapeutic strategies against this challenging disease.

7. Targeted therapeutics and future perspectives

The almost uniformly refractoriness to conventional treatments along with the discovery of the oncogenic driver, have rapidly made NMC an excellent candidate for molecularly targeted therapy. In this view, the BET inhibitors are acetyl-histone mimetics compounds that target BRD4-NUT by competitively inhibiting its binding to chromatin (Filippakopoulos et al., 2010). The first-in-classJQ1 caused rapid dissolution of BRD4-NUT nuclear speckles resulting in rapid differentiation and growth arrest in either cultured NMC cells or NMC xenograft models (Alekseyenko et al., 2015). More recently, the novel BETi OTX015/MK-8628 has shown an impressive antitumor activity in patients with confirmed BRD4-NUT fusions (Stathis et al., 2016).OTX015/MK-8628 was given to four advanced-stage chemo-refractory NMC at a dose of 80 mg once daily in a compassionate-use context. Two patients experienced partial responses with rapid tumor regression and symptomatic relief, while another one achieved a meaningful stable disease with a minor metabolic response. More interestingly, the two responding patients achieved an OS of 18 and 19 months which is the longest so far described in the literature. OTX015/MK-8628 was generally well tolerated with mild to moderate gastrointestinal toxicity, fatigue and reversible grade 3 thrombocytopenia. This represented the first proof-of-concept evidence of clinical activity of a BETi in targeting BRD4-NUT. On the basis of these encouraging results, several phase I trials are currently evaluating BETi in NMC both in the United States (NCT01587703, NCT01987362, and NCT02431260) and Europe (NCT02259114 and NCT01587703). One of this study reported that Birabresib achieved partial responses in 3 of 10 patients with NUT midline carcinoma (Massard et al., 2016), while GSK525762 is another pan-BET inhibitor that achieved, among 10 response-evaluable patients with NUT carcinoma, 2 patients achieved partial response and 4 had stable disease (O'Dwyer et al., 2016) (Table 1).

Since these are uncontrolled data it is not possible to estimate the real impact of BETi on NUT. Moreover, though active these compounds yield a response rate is in the range of 20%–30% and all responding patients invariably progress during treatment. Thus, a better molecular characterization of NMC treated with BETi is warranted together with new combinatorial approaches.

On a different approach, HDACi have exhibited preclinical and clinical activity in NMC. The demonstration by Schwartz et al. that the expression of BRD4-NUT in NMC cell lines is associated with a global decrease in histone acetylation and overall repression ofgene expression, provide a strong rationale to evaluate HDACi in this disease (Schwartz et al., 2011). The restoration of acetylation using HDACi induced squamous differentiation of NMC cells, and abrogated their growth in vitro and in vivo. A pediatric patient was treated with the US FDA-approved HDACi vorinostat at a dose of 400 mg daily with the achievement of a PR after 5 weeks of treatment. A similar case was

Table 1
Selected targeted therapeutics in clinical development for NMC patients. From ClinicalTrials.gov (https://clinicaltrials.gov/), last accessed on September 10, 2018.

Name	Drug Class	Phase	ClinicalTrial.gov identifier	Starting date	Status	Results
GSK525762	BET inhibitor	I/II	NCT01587703	April 30, 2012	Active/not recruiting	Not reported
CUDC-907	PI3K/HDAC inhibitor	I	NCT02307240	December 4, 2014	Recruiting	Not reported
MK-8628-006	BET inhibitor	IB	NCT02698176	March 3, 2016	Terminated	Not reported
OTX105/MK-8628	BET inhibitor	I	NCT02259114	October 8, 2014	Completed	Abstract, case series
INCB054329	BET inhibitor	I/II	NCT02431260	April 30, 2015	Completed	Not reported
BAY1238097	BET inhibitor	I	NCT02369029	February 23, 2015	Terminated	Abstract

reported by the MD Anderson Cancer Center. After disease progression with three cycles of cisplatin, docetaxel, and 5-fluorouracil, the patient received vorinostat 300 mg daily concomitantly with radiation therapy followed by maintenance vorinostat. A response to vorinostat was documented by CT scan both within and outside of radiation field (Maher et al., 2015). Currently, there is an ongoing Phase I trial for CUDC-907, an orally bioavailable HDAC and PI3K inhibitor, in patients with NMC and there is a clinical trial in the United States enrolling NMC patients for whom bromodomain inhibitors have failed (NCT02307240).

Very recently, through function-based miRNA library screening containing 1090 miRNAs, Tonouchi et al. were able to identify miR-3140 as a novel tumor suppressive miRNA, that directly suppresses BRD4 by binding to its coding sequence (Tonouchi et al., 2018).Interestingly, miR-3140 inhibited tumor cell growth in vitro in various cancer cell lines, including a NMC cell line, Ty-82 cells, via down-regulation of the BRD4-NUT fusion protein in JQ1-resistant NMC cells. These preclinical findings provide new insights into a miRNA-based targeted therapeutic strategy with potential of overcome resistance to BET inhibitors.

Still, CDK inhibition emerged as a promising molecularly targeted approach to be pursued in NMC. In a large-scale kinase inhibitor screening, the CDK9 inhibitor LDC67 resulted in impaired viability mediated by robust induction of apoptosis and DNA damage response in NMC cells, thus validating CDK9 as a druggable target that deserves further investigation in NMC (Brägelmann et al., 2017).

8. Concluding remarks

NUT midline carcinoma is an emerging epigenetic-driven cancer hallmarked by translocation involving the NUT gene. The poor response to conventional treatments and the devastating clinical course of the disease resulted in a very dismal survival. With the availability of accurate diagnostic tools and the growing awareness among physicians, the cases of NMC are expected to increase in the next years. Recently, the identification of the NUT-fused oncoproteins as target amenable to small molecule inhibition has hold great promisewith several agents in clinical development in this rare patient population. Future clinical trials are warranted with the aim of investigate the incorporation of targeted agent into multimodal therapeutic strategy. Since new promising NUT-targeting drugs are emerging that may affect the clinical course, the correct and prompt recognition of NMC is key to improve patients' outcome.

Declaration of Competing Interest

The authors have no competing interests to declare.

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