NUT Carcinoma

22

Christopher A. French

22.1 Definition

NC (aka NUT midline carcinoma) is a poorly differentiated carcinoma with rearrangement of the *NUTM1* (aka *NUT*) gene (French and den Bakker 2015).

22.2 Clinical Features

NC is possibly the most aggressive solid tumor known, having a median survival of 6.7–10.1 months (Bauer et al. 2012; Jung et al. 2019). The majority of tumors arise centrally near the airways in the head and neck (39%) and thorax (50%) (Bauer et al. 2012), giving the tumor's original name NUT "midline" carcinoma; however, with the increasing diagnosis of this entity, cases are being reported in a broad range of non-midline sites, including kidney (Bishop et al. 2016; Sirohi et al. 2018; Zhu et al. 2019), thyroid (Landa et al. 2016), soft tissue (Dickson et al. 2018), salivary glands (Agaimy et al. 2018; Seim et al. 2017; Vulsteke et al. 2016; Ziai et al. 2010; den Bakker et al. 2009), pancreas (Shehata et al. 2010), and bladder (French et al. 2004). In fact, non-thoracic, non-head and neck primary sites comprise ~10% of NCs (Bauer et al. 2012; Chau et al. 2019). Radiologic (CT or MRI) features are not specific, typically revealing a large, invasive, heterogenous mass that often confluently involves local lymph nodes (Polsani et al. 2012; Sholl et al. 2015) (Fig. 22.1). Metastases are common at presentation (51%) (Bauer et al. 2012), including to bone and other solid organs (Sholl et al. 2015). The early presentation of hematogenous metastases and frequent origin within the lung or mediastinum can mimic that of small cell carcinoma; however, it progresses more rapidly and often effects younger individuals.

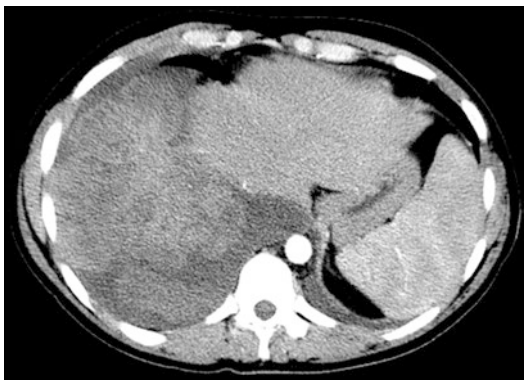

Fig. 22.1 Typical appearance of NC on thoracic CT scan reveals a large, heterogenous mass with extensive local invasion and lymph node involvement

C. A. French (✉)
Department of Pathology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA
e-mail: cfrench@bwh.harvard.edu

D. T. Schneider et al. (eds.), *Rare Tumors in Children and Adolescents*, Pediatric Oncology,
https://doi.org/10.1007/978-3-030-92071-5_22

22.3 Molecular Genetics

In all cases of NC, *NUTM1* is rearranged in a chromosomal translocation, most commonly (~70%) (Bauer et al. 2012; Chau et al. 2014) with *BRD4*, forming a *BRD4-NUTM1* fusion resulting from a t(15;19)(q14;p13.1). In almost every case, the breakpoint is upstream of exon 3 (transcript variant 1) of *NUTM1*, fusing this exon with the partner gene (Fig. 22.2). The most common alternative partner genes are *BRD3* (~15%) (Chau et al. 2019), which is highly homologous to *BRD4*, and *NSD3* (~6%) (Chau et al. 2019). Rare variant partners include ZNF532 and ZNF592. Interestingly, all of these encoded NUTM1 fusion partners (BRD3, BRD4, NSD3, ZNF532, ZNF592) interact with BRD4 and are critical components of the BRD4-NUT oncogenic complex (Alekseyenko et al. 2017). Thus, the fusion of any of these proteins to *NUTM1* leads to its association with BRD4 and is sufficient to recapitulate the function of the canonical BRD4-NUT oncogenic complex.

Molecular pathology utilizing next generation sequencing (NGS) has uncovered numerous novel *NUTM1*-fusions in a variety of tumors with uncertain relationship to NC. These *NUTM1*-fusion partners include *CIC* (Schaefer et al. 2018), *BCORL1* (Dickson et al. 2018), *MYXD1* (Dickson et al. 2018), *MYXD4* (Dickson et al. 2018; Tamura et al. 2018), and *MGA* (Stevens et al. 2019; Diolaiti et al. 2018) in solid tumors with various histologies, including some bearing resemblance to NC, and others with spindle cell sarcoma morphology. Remarkably, NUTM1-fusions (BRD9 (Andersson et al. 2015), *ACINI* (Andersson et al. 2015; Hormann et al. 2019; Gu et al. 2016; Liu et al. 2016), *SLC12A* (Gu et al. 2016), *ZNF618* (Gu et al. 2016), *IKZF1* (Gu et al. 2016; Lilljebjorn et al. 2016), *BPTF* (Liu et al. 2017), *CUX1*, and *IKZF1* (Hormann et al. 2019)) have also been discovered in a variety of leukemias, and CIC-NUTM1 was originally described as a variant of primitive neuroectodermal tumors (PNET) of the central nervous system (Sturm et al. 2016). The various *NUTM1*-fusions and corresponding pathology are summarized in Table 22.1.

Next-gen sequencing has also been key to characterizing exonic mutations other than the

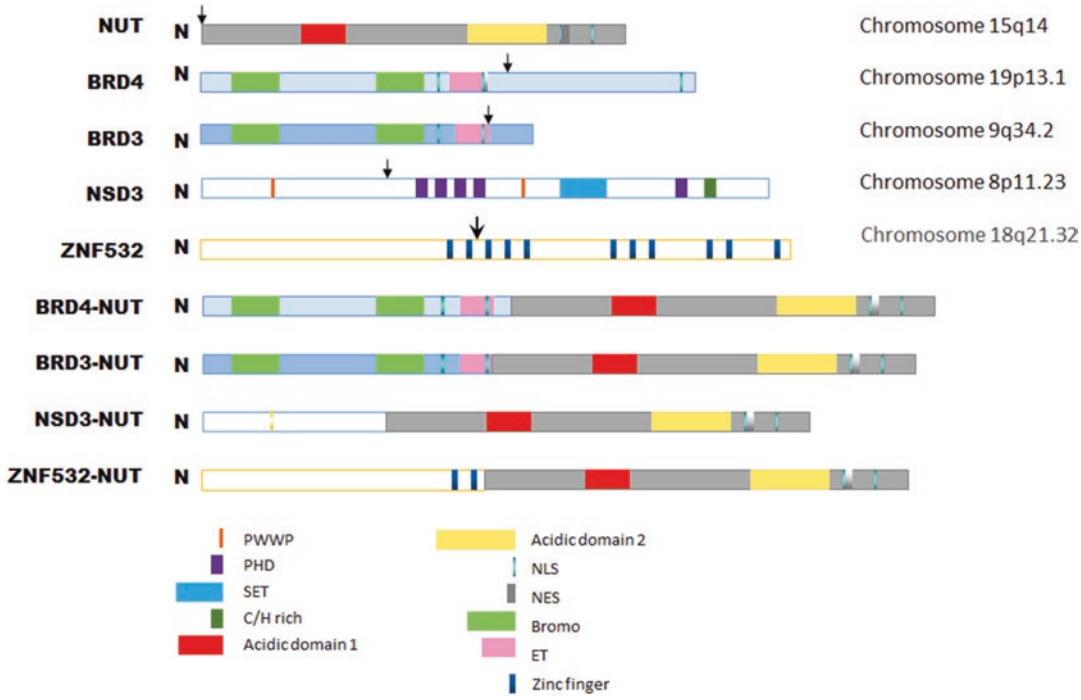

Fig. 22.2 Schematic of NUTM1 fusions reported in NC

Table 22.1 Spectrum of NUTM1-rearranged tumors

NUTM1 fusion partner	Primary site	Histology	References
BRD4	Airways, other organs	PD carcinoma	French et al. (2003); Dickson et al. (2018)
BRD3	Airways, bone and soft tissue	PD carcinoma	French et al. (2008); Dickson et al. (2018)
NSD3	Airways, bone and soft tissue	PD carcinoma	French (2014); Dickson et al. (2018)
ZNF532	Airways	PD carcinoma	Alekseyenko et al. (2017)
ZNF592	Bone and soft tissue	Undifferentiated epithelioid	Shiota et al. (2018)
CIC	CNS, bone and soft tissue	Undifferentiated epithelioid	Schaefer et al. (2018); Sturm et al. (2016)
BCOR1	Bone and soft tissue	Undifferentiated epithelioid	Dickson et al. (2018)
MYXD1	Soft tissue	Undifferentiated epithelioid	Dickson et al. (2018)
MYXD4	Colon	Undifferentiated epithelioid	Dickson et al. (2018); Tamura et al. (2018)
MGA	Lung, soft tissue, dura	Spindle cell sarcoma	Stevens et al. (2019); Diolaiti et al. (2018)
BRD9	Blood/bone marrow	Leukemia	Andersson et al. (2015)
ACINI	Blood/bone marrow	Leukemia	Andersson et al. (2015); Hormann et al. (2019); Gu et al. (2016); Liu et al. (2016)
SLC12A	Blood/bone marrow	Leukemia	Gu et al. (2016)
ZNF618	Blood/bone marrow	Leukemia	Gu et al. (2016)
IKZF1	Blood/bone marrow	Leukemia	Gu et al. (2016); Lilljebjorn et al. (2016)
BPTF	Blood/bone marrow	Leukemia	Liu et al. (2017)
CUX1	Blood/bone marrow	Leukemia	Hormann et al. (2019)
IKZF1	Blood/bone marrow	Leukemia	Hormann et al. (2019)

CNS central nervous system, *PD* poorly differentiated

NUTM1-fusion. Surprisingly, available data indicate that there are no additional oncogenic driver mutations or inactivating mutations of tumor suppressor genes, suggesting that the NUTM1 fusion may be the sole driver of NCs (Lee et al. 2017; Stathis et al. 2016).

22.4 Histology/ Immunohistochemistry (IHC)

NC has a characteristic, though not diagnostic, histologic appearance. It appears as an undifferentiated, malignant neoplasm, exhibiting a sheet-like growth pattern comprised of medium-sized, round- to oval-shaped cells. Its monomorphism is distinctive (Fig. 22.3a) and distinguishes it from garden variety poorly differentiated carcinomas, which are larger and exhibit more pleomorphism (Fig. 22.3b). Another common feature is the presence of clear spaces around cells and occasional "fried egg cells" that have abundant, clear cytoplasm (Fig. 22.3a). Consistent with its rapid growth, mitoses and single-cell or geographic necrosis are characteristic. The immune infiltrate cvaries, and is either neutrophilic or lymphocytic (Fig. 22.4). As a subtype of squamous carcinoma, NC often (33–40%) (Bauer et al. 2012; Chau et al. 2019) displays morphologic squamous differentiation, which is characteristically abrupt (Fig. 22.5), rather than displaying a gradient of poorly to well-differentiated cells as seen in conventional squamous cancers. This type of abrupt squamous differentiation can also be seen in basaloid HPV-associated squamous cancers of the head and neck (Table 22.2) (Chernock et al. 2010). In keeping with its squamous lineage, NCs are typically (~75%) (Tilson and Bishop 2014) positive for p63/p40 IHC and often also stain for CK5.

Unusual cases of NC, typically arising from salivary gland (den Bakker et al. 2009) or soft tissue (Dickson et al. 2018), can display morphologic features of myoepithelial differentiation, with rhabdoid-like cells and even formation of cartilage (Fig. 22.6); however, IHC markers do not support

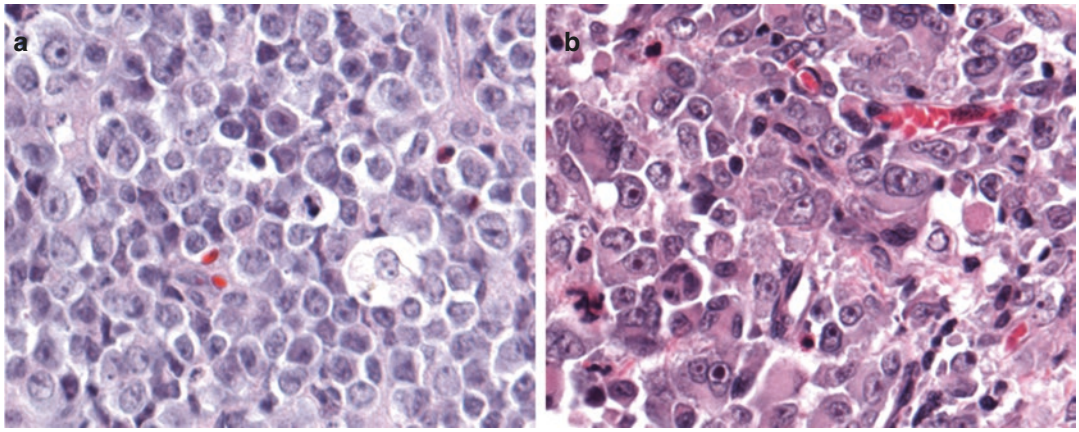

Fig. 22.3 Typical histologic appearance of (**a**) NC compared with (**b**) non-NUT poorly differentiated carcinoma. (**a**) NC is characteristically monomorphic with clear spaces separating cells and occasional "fried egg" cells. (**b**) Garden variety poorly differentiated carcinoma, by contrast, displays larger cell with greater pleomorphism. Both images are 400× magnification and H&E stained

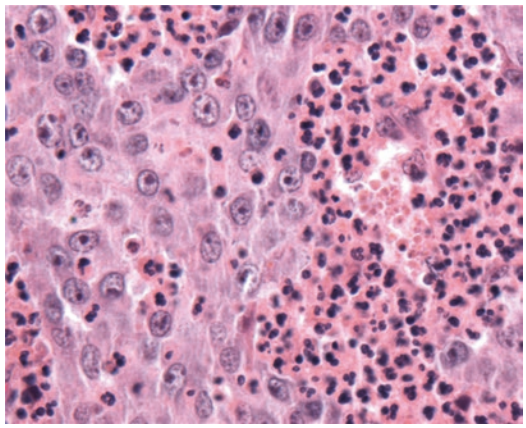

Fig. 22.4 NC showing a prominent neutrophilic infiltrate. Image is 400× magnification and H&E stained

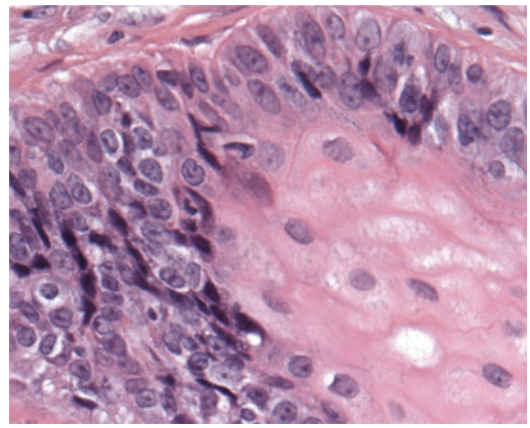

Fig. 22.5 Abrupt keratinization is a characteristic feature of NC. Image is 400× magnification and H&E stained

myoepithelial lineage; these tumors are negative for S100 and SMA and variably express p63 or GFAP (Dickson et al. 2018; Schaefer et al. 2018).

Due to the significant morphologic and immunophenotypic overlap of NC with other poorly differentiated malignancies, the diagnosis is often missed or mistaken for these other entities, including most commonly poorly differentiated carcinoma, poorly differentiated squamous carcinoma (Evans et al. 2012; Stelow et al. 2008; Stelow and French 2009; Chute and Stelow 2010), Ewing's sarcoma (Mertens et al. 2007), sino-nasal undifferentiated carcinoma (Stelow et al. 2008), and small cell carcinoma (Evans et al. 2012) (Table 22.2). Other entities that can be mistaken for NC include poorly differentiated transitional carcinoma of the genitourinary tract (Bishop et al. 2016; French et al. 2004), small-round-blue-cell tumors (blastomas) (Shehata et al. 2010), carcinoma ex pleomorphic adenoma (den Bakker et al. 2009), thymic carcinoma (Kubonishi et al. 1991; Toretsky et al. 2003; Petrini et al. 2012), and even thyroid carcinoma (Landa et al. 2016). The difficulty in distinguishing NC from these other entities by morphology alone has contributed to the vast underdiagnosis of NC (see Demographics/Prevalence, below).

The majority of NCs are carcinomas; however, a subset are so poorly differentiated that they lack

Table 22.2 Distinguishing features of tumors in the differential diagnosis of NC

Tumor	Monomorphic	Distinguishing marker	Abrupt squamous differentiation
Ewing/PNET	+		–
Extra-gonadal germ cell tumor	–	Oct3/4, CD30, β-HCG	–
Lymphoma/leukemia	+	LCA	–
Nasopharyngeal carcinoma	–	EBV	–
HPV-associated SQC	+/–	HPV	+
PD SQC	–		–
Olfactory neuroblastoma	+	S-100[a]	–
PD carcinoma	–		–
Small cell carcinoma	+	p63/p40 –	–
SNUC	+/–		–

Adapted from French CA. NUT Carcinoma: Clinicopathologic features, pathogenesis, and treatment. Pathol Int. 2018;68(11):583–595. https://doi.org/10.1111/pin.12727

EBV Epstein-Barr virus, *HMW* high molecular weight, *HPV* human papilloma virus, *LCA* leukocyte common antigen (CD45), *PNET* primitive neuroectodermal tumor, *PD* poorly differentiated, *SNUC* sino-nasal undifferentiated carcinoma, *SQC* squamous cell carcinoma

[a] Positive in sustentacular cells

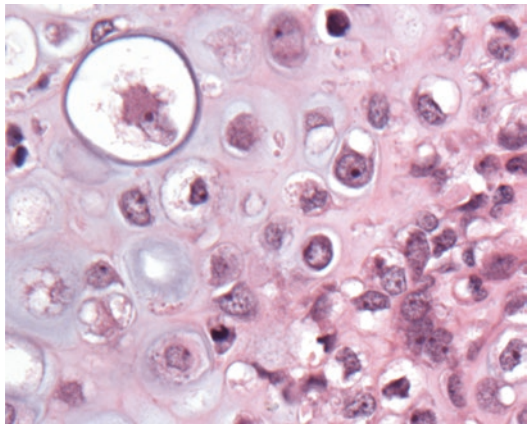

Fig. 22.6 Rare case of NC displays chondroid differentiation (left). Image is 400× magnification and H&E stained

epithelial markers (i.e., expression of keratin intermediate filaments). This has led to the misdiagnosis of leukemia or even lymphoma (unpublished observations), particularly when patient's initial biopsy is from bone marrow. Moreover, a small subset of NUTM1-rearranged malignancies, approximately 5–10% (Dickson et al. 2018; Chau et al. 2019; Stevens et al. 2019), arise from soft tissue and show variable expression of epithelial markers, raising the possibility that some of these are of different lineage from typical NC.

22.5 Diagnosis

NC is defined molecularly by *NUTM1*-rearrangement and as such historically could only be diagnosed by direct demonstration of *NUTM1* rearrangement, either by conventional cytogenetics, fluorescent in situ hybridization (FISH), or reverse-transcriptase PCR (RT-PCR). These methodologies are not widely available and thus hampered the diagnosis of NC during its early recognition. This changed in 2009 with the development of a NUT-specific antibody (Haack et al. 2009). Owing to the highly restricted expression of NUT protein to spermatids of the testis, expression of NUT is specific to NC and germ cell tumors (embryonal carcinoma, seminoma, and dysgerminoma) (Fig. 22.7); the rabbit anti-NUT monoclonal antibody clone C52B1 (Cell Signaling Technologies, Danvers, MA) exhibits a sensitivity of 87% and specificity of 100% when strict criteria are applied to interpretation: >50% of tumor nuclei positively stain (Haack et al. 2009). With this high specificity, positive NUT IHC is considered sufficient for the diagnosis of NC (French and den Bakker 2017).

Despite the high feasibility of diagnostic NUT IHC, many pathology laboratories still do not use it, due to the perceived rarity of NC; however, the

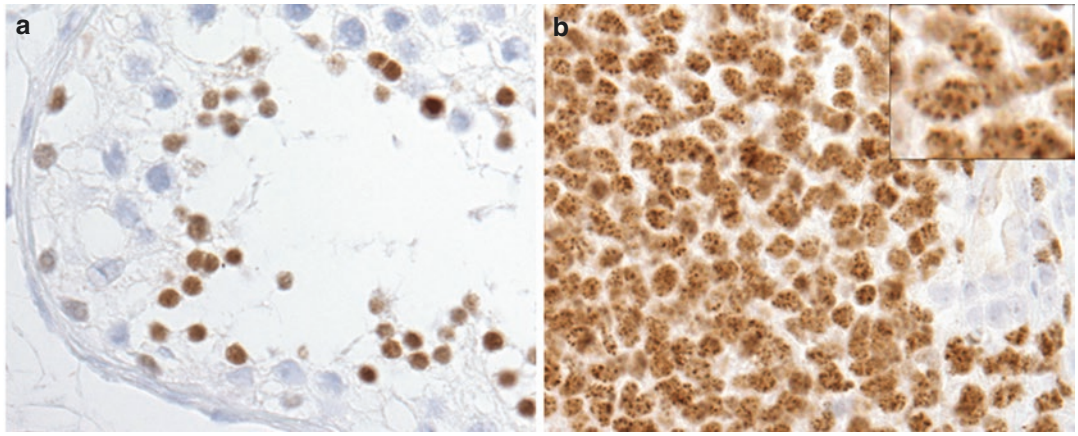

Fig. 22.7 Immunohistochemical staining of postmeiotic spermatids (**a**) and NC (**b**) nuclei using the anti-NUT, clone C52B1 antibody (Cell Signaling Technology, Inc.). The characteristic speckled nuclear staining pattern of BRD4-NUT can be seen in the inset (**b**). Images are 400× magnification

emergence of NGS is having a large impact on detecting cases not originally considered by the pathologist/oncologist. Targeted exome NGS platforms such as those provided by Foundation Medicine (Mangray et al. 2018) or Oncopanel (Stathis et al. 2016; Wagle et al. 2012) have led to the discovery of NCs, but their sensitivity is hampered by limited coverage and the large breakpoint region of *NUTM1* and *BRD4*. Rapid amplification of cDNA ends (RACE)-based NGS technology, however, is changing the landscape of *NUTM1*-rearranged tumors due to its unbiased use and high sensitivity. This test, which utilizes RNA from archival formalin-fixed paraffin-embedded material (FFPE), is able to detect the majority of *NUTM1*-rearrangements, because it can detect any fusion to *NUTM1*, including novel fusion partners, as long as the canonical exon 3 (transcript variant 1) of *NUTM1* is involved, which it is in the majority of cases (Lee et al. 2017; Thompson-Wicking et al. 2013; Stirnweiss et al. 2015, 2017; French et al. 2003; Haruki et al. 2005). Companies that offer this assay include, but are not limited to, ArcherDx (Boulder, CO) (Dickson et al. 2018; Shiota et al. 2018), Caris Life Sciences (Phoenix, AZ) (Stevens et al. 2019), and Foundation Medicine (Cambridge, MA).

22.6 When to Consider the Diagnosis of NC

Because testing and diagnosis of NC is increasing, the disease spectrum is becoming broader, with cases arising from a large variety of non-midline organs including pancreas (Shehata et al. 2010), kidney (Bishop et al. 2016), thyroid (Landa et al. 2016), bladder (French et al. 2004), salivary glands (Ziai et al. 2010; den Bakker et al. 2009; Chau et al. 2014), bone (Mertens et al. 2007), and soft tissues (Dickson et al. 2018; Stevens et al. 2019). For this reason, we recommend performing NUT IHC to rule out NC in all poorly differentiated non-cutaneous carcinomas, with or without squamous differentiation, that have a monomorphic appearance. It is important to not exclude NC on the basis of some lineage-associated markers, such as those of neuroendocrine (chromogranin or synaptophysin), pulmonary (TTF-1), or stem cell (CD34), because NCs can exhibit positive staining for any of these (French et al. 2004; Tanaka et al. 2012; Raza et al. 2015; Bishop and Westra 2012). Moreover, advanced patient age or history of smoking should not be criteria that exclude NC, because it can affect patients of all ages, including elderly patients (Bauer et al. 2012; Stelow et al. 2008),

and numerous patients with a smoking history have been diagnosed.

When should one *not consider* NC? Gland-forming NCs are extremely rare, if nonexistent; thus, NC need not be considered in the differential diagnosis of adenocarcinomas. Moreover, known viral etiology, such as HPV or EBV, has never been detected in NC and can be used as a basis to exclude NC. This being said, expression of the HPV-associated marker, p16, is frequently seen in NC (Salles et al. 2014) and should not be used to exclude it.

22.7 Demographics/Prevalence

NC affects patients of all, but predominantly young, ages, with a median age of 16–22 (range 01.–81.7 years) (Bauer et al. 2012; Chau et al. 2014) and with an equal predilection for males and females (Bauer et al. 2012; Chau et al. 2019). NC is not associated with smoking, but a history of smoking is not uncommon (unpublished observations). The prevalence of NC is not precisely known; however, a recent study using the Caris RACE-NGS platform to identify *NUTM1*-fusions in a cohort of 14,107 tumors revealed 9 *NUTM1*-fusion-positive tumors, suggesting a rough incidence of ~0.06% among all tumors (Stevens et al. 2019). Extrapolating this data based on the estimated 1.7 million new cases in the USA in 2019 (American Cancer Society) would suggest an incidence of over 1000 new cases of NC in the USA per year. This estimate confirms that NC is vastly underdiagnosed and that testing must increase.

22.8 Pathogenesis (BRD4-NUT Function)

NGS indicates that BRD4-NUT is the sole oncogenic driver of NC (Lee et al. 2017; Stathis et al. 2016). The fusion protein is known to bind to acetylated histones via the dual bromodomains of BRD4 (Grayson et al. 2014), which tether NUT, an unstructured protein, to chromatin. NUT recruits the histone acetyltransferase (HAT), p300, to BRD4-NUT (Alekseyenko et al. 2017; Reynoird et al. 2010) where it is presumed to acetylate the chromatin and recruit further BRD4-NUT in an iterative process that leads to massive contiguous regions of chromatin enriched with BRD4-NUT (Alekseyenko et al. 2015). These regions are termed megadomains and function essentially to upregulate transcription of underlying coding and noncoding genes (Alekseyenko et al. 2015). A critical target of BRD4-NUT in all NCs is *MYC*, whose upregulation drives growth and arrests NC cell differentiation (Grayson et al. 2014; Alekseyenko et al. 2015). Knockdown of either MYC or BRD4-NUT results in rapid differentiation of these cells, indicating that targeting either of these proteins could be effective strategies to treat this cancer (Grayson et al. 2014; French et al. 2008).

22.9 Treatment of NC

Currently, there is no established treatment strategy for NC; however, for children, the Scandinavian Ewing SSG IX regimen has led to cure of NC in a small number ($n = 3$) of reported cases (Mertens et al. 2007; Storck et al. 2017). Thus, for pediatric patients with localized or disseminated NC, this regimen is often recommended; however, it is not effective in all patients. In addition, complete resection has been shown repeatedly to lead to significantly ($p = 0.0003–0.01$) improved overall survival (Bauer et al. 2012; Chau et al. 2014), and so whenever possible, surgical resection with clean margins should be performed as soon as possible after diagnosis. Often, however, the patient has disseminated or unresectable disease at the time of diagnosis, due to its rapid growth, and chemoradiation is the only available option. More often than not, chemoradiation leads to a transient response, followed by rapid progression and death; thus, there is an urgent need for novel approaches to treat NC.

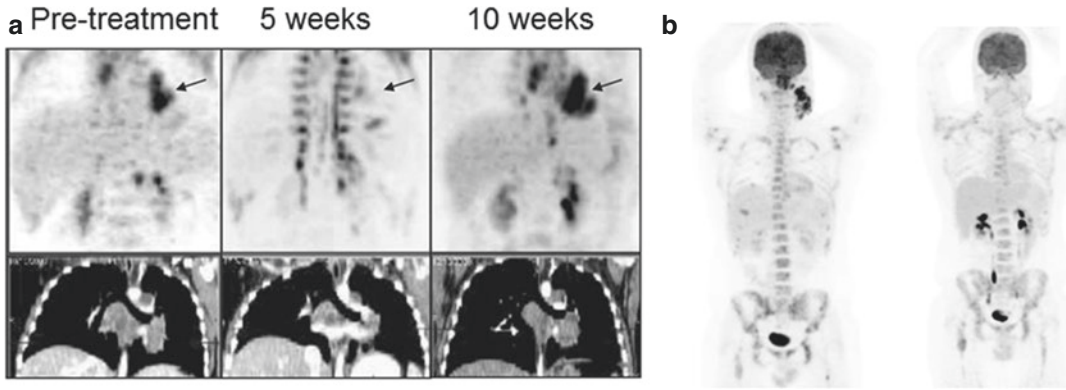

Fig. 22.8 Response of NC patients to targeted inhibitors. (**a**) PET/CT of a 10-year-old patient to single agent HDAC inhibitor, vorinostat. Recurrence at 10 weeks was due to treatment interruption secondary to gastrointestinal toxicity. (Reproduced from Schwartz BE, Hofer MD, Lemieux ME, DE, Cameron MJ, West NH, Agoston ES, Reynoird N, Khochbin S, Ince TA, Christie A, Janeway KA, Vargas SO, Perez-Atayde AR, Aster JC, Sallan SE, Kung AL, Bradner JE, French CA. Differentiation of NUT midline carcinoma by epigenomic reprogramming. Cancer Res 2011;71(7):2686–96. https://doi.org/10.1158/0008.5472.CAN-10-3513. (**b**) PET scan of patient treated with the BET inhibitor, MK-8628/OTX015 comparing baseline (left) with two cycles of treatment (right). (Reproduced from Stathis A, Zucca E, Bekradda M, Gomez-Roca C, Delord JP, de La Motte Rouge T, Uro-Coste E, de Braud F, Pelosi G, French CA. Clinical Response of Carcinomas Harboring the BRD4-NUT Oncoprotein to the Targeted Bromodomain Inhibitor OTX015/MK-8628. Cancer Discov 2016; 6(5):492–500. https://doi.org/10.1158/2159-8290.CD-151335

22.10 New Treatment Strategies

NC cells are extremely sensitive to histone deacetylase (HDAC) inhibitors in cell culture experiments (Schwartz et al. 2011). The effect of HDAC inhibitors was originally ascribed to reversal of global chromatin hypoacetylation imparted by BRD4-NUT. HDAC inhibitors cause NC cells to differentiate and arrest proliferation at low doses, and activity has been seen in mouse models (Schwartz et al. 2011). Anecdotally, HDAC inhibitors, alone or in combination with chemotherapy, do show activity against NC in humans (Fig. 22.8a), though the response is transient and not curative (Schwartz et al. 2011; Maher et al. 2015).

A more precise, though still generally nonselective, approach to treating NC came about in 2010 using BET inhibitor compounds, typified by the molecule, JQ1, which are acetyl-lysine mimetic compounds that competitively inhibit the binding of BET (BRD2, BRD3, BRD4, BRDT) protein dual bromodomains to chromatin (Filippakopoulos et al. 2010; Filippakopoulos and Knapp 2012). BET inhibitors evict BET proteins, including BRD4-NUT, from chromatin, resulting in loss of function and differentiation of NC cells in vitro and in vivo (Grayson et al. 2014; Filippakopoulos et al. 2010). Multiple trials were conducted evaluating the efficacy of BET inhibitors in human cancers, including NC. On-target activity has been shown (Fig. 22.8b); however, dose-limiting toxicity has precluded cure with these drugs (Stathis et al. 2016; Lewin et al. 2018; O'Dwyer et al. 2016). Likely a combination strategy with BET and/or other targeted inhibitors will be required for the ultimate cure of this aggressive cancer. A recent CRISPR-based screen suggests that combination of BET inhibitors with a CDK4/6 inhibitor, such as palbociclib, is synergistic in inhibiting NC (Liao et al. 2018).

22.11 Conclusions

NC is a recently described predominantly pediatric and young adult cancer that remains poorly recognized and underdiagnosed. The distinctly poor prognosis and need for alternative approaches to treat NC provide a strong rationale

to make the diagnosis. The longer this disease remains undiagnosed, the longer it will take to better understand it and explore novel treatment approaches.

References

Agaimy A, Fonseca I, Martins C, Thway K, Barrette R, Harrington KJ, Hartmann A, French CA, Fisher C (2018) NUT carcinoma of the salivary glands: clinicopathologic and molecular analysis of 3 cases and a survey of NUT expression in salivary gland carcinomas. Am J Surg Pathol 42(7):877–884

Alekseyenko AA, Walsh EM, Wang X, Grayson AR, Hsi PT, Kharchenko PV, Kuroda MI, French CA (2015) The oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains. Genes Dev 29(14):1507–1523

Alekseyenko AA, Walsh EM, Zee BM, Pakozdi T, Hsi P, Lemieux ME, Dal Cin P, Ince TA, Kharchenko PV, Kuroda MI, French CA (2017) Ectopic protein interactions within BRD4 chromatin complexes drive oncogenic megadomain formation in NUT midline carcinoma. Proc Natl Acad Sci U S A 114(21):E4184–E4E92

Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, Nakitandwe J, Holmfeldt L, Parker M, Easton J, Huether R, Kriwacki R, Rusch M, Wu G, Li Y, Mulder H, Raimondi S, Pounds S, Kang G, Shi L, Becksfort J, Gupta P, Payne-Turner D, Vadodaria B, Boggs K et al (2015) The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet 47(4):330–337

Bauer DE, Mitchell CM, Strait KM, Lathan CS, Stelow EB, Luer SC, Muhammed S, Evans AG, Sholl LM, Rosai J, Giraldi E, Oakley RP, Rodriguez-Galindo C, London WB, Sallan SE, Bradner JE, French CA (2012) Clinicopathologic features and long-term outcomes of NUT midline carcinoma. Clin Cancer Res 18(20):5773–5779

Bishop JA, Westra WH (2012) NUT midline carcinomas of the sinonasal tract. Am J Surg Pathol 36(8):1216–1221

Bishop JA, French CA, Ali SZ (2016) Cytopathologic features of NUT midline carcinoma: a series of 26 specimens from 13 patients. Cancer Cytopathol 124(12):901–908

Chau NG, Mitchell CM, Aserlind A, Grunfeld N, Kaplan L, Bauer DE, Lathan CS, Rodriguez-Galindo C, Hurwitz S, Tishler RB, Haddad RI, Sallan SE, Bradner JE, French CA (2014) Aggressive treatment and survival outcomes in NUT midline carcinoma (NMC) of the head and neck (HN). J Clin Oncol, 2014 ASCO Annual Meeting Abstracts 32(15_suppl)

Chau NG, Ma C, Danga K, Al-Sayegh H, Nardi V, Barrette R, Lathan CS, DuBois SG, Haddad RI, Shapiro GI, Sallan SE, Dhar A, Nelson JJ, French CA (2019) An anatomical site and genetic-based prognostic model for patients with nuclear protein in testis (NUT) midline carcinoma: analysis of 124 patients. JNCI Cancer Spectr 4(2):pkz094. https://doi.org/10.1093/jncics/pkz094. PMID: 32328562; PMCID: PMC7165803

Chernock RD, Lewis JS Jr, Zhang Q, El-Mofty SK (2010) Human papillomavirus-positive basaloid squamous cell carcinomas of the upper aerodigestive tract: a distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. Hum Pathol 41(7):1016–1023

Chute DJ, Stelow EB (2010) Cytology of head and neck squamous cell carcinoma variants. Diagn Cytopathol 38(1):65–80

den Bakker MA, Beverloo BH, van den Heuvel-Eibrink MM, Meeuwis CA, Tan LM, Johnson LA, French CA, van Leenders GJ (2009) NUT midline carcinoma of the parotid gland with mesenchymal differentiation. Am J Surg Pathol 33(8):1253–1258

Dickson BC, Sung YS, Rosenblum MK, Reuter VE, Harb M, Wunder JS, Swanson D, Antonescu CR (2018) NUTM1 gene fusions characterize a subset of undifferentiated soft tissue and visceral tumors. Am J Surg Pathol 42(5):636–645

Diolaiti D, Dela Cruz FS, Gundem G, Bouvier N, Boulad M, Zhang Y, Chou AJ, Dunkel IJ, Sanghvi R, Shah M, Geiger H, Rahman S, Felice V, Wrzeszczynski KO, Darnell RB, Antonescu CR, French CA, Papaemmanuil E, Kung AL, Shukla N (2018) A recurrent novel MGA-NUTM1 fusion identifies a new subtype of high-grade spindle cell sarcoma. Cold Spring Harb Mol Case Stud 4(6):a003194

Evans AG, French CA, Cameron MJ, Fletcher CD, Jackman DM, Lathan CS, Sholl LM (2012) Pathologic characteristics of NUT midline carcinoma arising in the mediastinum. Am J Surg Pathol 36(8):1222–1227

Filippakopoulos P, Knapp S (2012) The bromodomain interaction module. FEBS Lett 586(17):2692–2704

Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE (2010) Selective inhibition of BET bromodomains. Nature 468(7327):1067–1073

French CA, Rahman S, Walsh EM, Kühnle S, Grayson AR, Lemieux ME, Grunfeld N, Rubin BP, Antonescu CR, Zhang S, Venkatramani R, Dal Cin P, Howley PM (2014) NSD3-NUT fusion oncoprotein in NUT midline carcinoma: implications for a novel oncogenic mechanism. Cancer Discov 4(8):928–941. https://doi.org/10.1158/2159-8290. CD-14-0014. Epub 2014 May 29. PMID: 24875858; PMCID: PMC4125436

French CA, den Bakker MA (2015) In: Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG (eds)

NUT carcinoma, 4th edn. International Agency for Research on Cancer (IARC), Lyon, pp 229–230
French CA, den Bakker MA (2017) In: El-Naggar A, Chan JKC, Grandis JR, Takata T, Slootweg P (eds) WHO classification of head and neck tumours, 4th edn. International Agency for Research on Cancer (IARC), Lyon, pp 20–21
French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA (2003) BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. Cancer Res 63(2):304–307
French CA, Kutok JL, Faquin WC, Toretsky JA, Antonescu CR, Griffin CA, Nose V, Vargas SO, Moschovi M, Tzortzatou-Stathopoulou F, Miyoshi I, Perez-Atayde AR, Aster JC, Fletcher JA (2004) Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 22(20):4135–4139
French CA, Ramirez CL, Kolmakova J, Hickman TT, Cameron MJ, Thyne ME, Kutok JL, Toretsky JA, Tadavarthy AK, Kees UR, Fletcher JA, Aster JC (2008) BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. Oncogene 27(15):2237–2242
Grayson AR, Walsh EM, Cameron MJ, Godec J, Ashworth T, Ambrose JM, Aserlind AB, Wang H, Evan GI, Kluk MJ, Bradner JE, Aster JC, French CA (2014) MYC, a downstream target of BRD-NUT, is necessary and sufficient for the blockade of differentiation in NUT midline carcinoma. Oncogene 33(13):1736–1742
Gu Z, Churchman M, Roberts K, Li Y, Liu Y, Harvey RC, McCastlain K, Reshmi SC, Payne-Turner D, Iacobucci I, Shao Y, Chen IM, Valentine M, Pei D, Mungall KL, Mungall AJ, Ma Y, Moore R, Marra M, Stonerock E, Gastier-Foster JM, Devidas M, Dai Y, Wood B, Borowitz M et al (2016) Genomic analyses identify recurrent MEF2D fusions in acute lymphoblastic leukaemia. Nat Commun 7:13331
Haack H, Johnson LA, Fry CJ, Crosby K, Polakiewicz RD, Stelow EB, Hong SM, Schwartz BE, Cameron MJ, Rubin MA, Chang MC, Aster JC, French CA (2009) Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. Am J Surg Pathol 33(7):984–991
Haruki N, Kawaguchi KS, Eichenberger S, Massion PP, Gonzalez A, Gazdar AF, Minna JD, Carbone DP, Dang TP (2005) Cloned fusion product from a rare t(15;19)(q13.2;p13.1) inhibit S phase in vitro. J Med Genet 42(7):558–564
Hormann FM, Hoogkamer AQ, Beverloo HB, Boeree A, Dingjan I, Wattel MM, Stam RW, Escherich G, Pieters R, den Boer ML, Boer JM (2019) NUTM1 is a recurrent fusion gene partner in B cell precursor acute lymphoblastic leukemia associated with increased expression of genes on chromosome band 10p12.31-12.2. Haematologica 104(10):e455–e459. https://doi.org/10.3324/haematol.2018.20696
Jung M, Kim S, Lee JK, Yoon SO, Park HS, Hong SW, Park WS, Kim JE, Kim J, Keam B, Kim HJ, Kang HJ, Kim DW, Jung KC, Kim YT, Heo DS, Kim TM, Jeon YK (2019) Clinicopathological and preclinical findings of NUT carcinoma: a multicenter study. Oncologist 24(8):e740–e748
Kubonishi I, Takehara N, Iwata J, Sonobe H, Ohtsuki Y, Abe T, Miyoshi I (1991) Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. Cancer Res 51(12):3327–3328
Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, Dogan S, Ricarte-Filho JC, Krishnamoorthy GP, Xu B, Schultz N, Berger MF, Sander C, Taylor BS, Ghossein R, Ganly I, Fagin JA (2016) Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest 126(3):1052–1066
Lee JK, Louzada S, An Y, Kim SY, Kim S, Youk J, Park S, Koo SH, Keam B, Jeon YK, Ku JL, Yang F, Kim TM, Ju YS (2017) Complex chromosomal rearrangements by single catastrophic pathogenesis in NUT midline carcinoma. Ann Oncol 28(4):890–897
Lewin J, Soria JC, Stathis A, Delord JP, Peters S, Awada A, Aftimos PG, Bekradda M, Rezai K, Zeng Z, Hussain A, Perez S, Siu LL, Massard C (2018) Phase Ib trial with Birabresib, a small-molecule inhibitor of bromodomain and extraterminal proteins, in patients with selected advanced solid tumors. J Clin Oncol 36(30):3007–3014
Liao S, Maertens O, Cichowski K, Elledge SJ (2018) Genetic modifiers of the BRD4-NUT dependency of NUT midline carcinoma uncovers a synergism between BETis and CDK4/6is. Genes Dev 32(17–18):1188–1200
Lilljebjorn H, Henningsson R, Hyrenius-Wittsten A, Olsson L, Orsmark-Pietras C, von Palffy S, Askmyr M, Rissler M, Schrappe M, Cario G, Castor A, Pronk CJ, Behrendtz M, Mitelman F, Johansson B, Paulsson K, Andersson AK, Fontes M, Fioretos T (2016) Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukaemia. Nat Commun 7:11790
Liu YF, Wang BY, Zhang WN, Huang JY, Li BS, Zhang M, Jiang L, Li JF, Wang MJ, Dai YJ, Zhang ZG, Wang Q, Kong J, Chen B, Zhu YM, Weng XQ, Shen ZX, Li JM, Wang J, Yan XJ, Li Y, Liang YM, Liu L, Chen XQ, Zhang WG et al (2016) Genomic profiling of adult and pediatric B-cell acute lymphoblastic leukemia. EBioMedicine 8:173–183
Liu Y, Easton J, Shao Y, Maciaszek J, Wang Z, Wilkinson MR, McCastlain K, Edmonson M, Pounds SB, Shi L, Zhou X, Ma X, Sioson E, Li Y, Rusch M, Gupta P, Pei D, Cheng C, Smith MA, Auvil JG, Gerhard DS, Relling MV, Winick NJ, Carroll AJ, Heerema NA et al (2017) The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet 49(8):1211–1218
Maher OM, Christensen AM, Yedururi S, Bell D, Tarek N (2015) Histone deacetylase inhibitor for NUT midline carcinoma. Pediatr Blood Cancer 62(4):715–717
Mangray S, Kelly DR, LeGuellec S, Fridman E, Aggarwal S, Shago M, Matoso A, Madison R, Pramanik S, Zhong S, Li R, Lombardo KA, Cramer S, Pressey

J, Ross JS, Corona RJ, Bratslavsky G, Argani P, Coindre JM, Somers GR, Ali SM, Yakirevich E (2018) Clinicopathologic features of a series of primary renal CIC-rearranged sarcomas with comprehensive molecular analysis. Am J Surg Pathol 42(10):1360–1369

Mertens F, Wiebe T, Adlercreutz C, Mandahl N, French CA (2007) Successful treatment of a child with t(15;19)-positive tumor. Pediatr Blood Cancer 49(7):1015–1017

O'Dwyer PJ, Piha-Paul SA, French CA, Harward S, Ferron-Brady G, Wu Y, Barbash O, Wyce A, Annan M, Horner T, Parr NJ, Prinjha RK, Carpenter C, Shapiro G, Dhar A, Hann C (2016) Abstract CT014: GSK525762, a selective bromodomain (BRD) and extra terminal protein (BET) inhibitor: results from part 1 of a phase I/II open-label single-agent study in patients with NUT midline carcinoma (NMC) and other cancers. Cancer Res 76(14 supplement):CT014

Petrini P, French CA, Rajan A, Cameron MJ, Jaffe ES, Zucali PA, Xie J, Wang Y, Giaccone G (2012) NUT rearrangement is uncommon in human thymic epithelial tumors. J Thorac Oncol 7(4):744–750

Polsani A, Braithwaite KA, Alazraki AL, Abramowsky C, Shehata BM (2012) NUT midline carcinoma: an imaging case series and review of literature. Pediatr Radiol 42(2):205–210

Raza A, Cao H, Conrad R, Cobb C, Castelino-Prabhu S, Mirshahidi S, Shiraz P, Mirshahidi HR (2015) Nuclear protein in testis midline carcinoma with unusual elevation of alpha-fetoprotein and synaptophysin positivity: a case report and review of the literature. Expert Rev Anticancer Ther 15(10):1199–1213

Reynoird N, Schwartz BE, Delvecchio M, Sadoul K, Meyers D, Mukherjee C, Caron C, Kimura H, Rousseaux S, Cole PA, Panne D, French CA, Khochbin S (2010) Oncogenesis by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chromatin domains. EMBO J 29(17):2943–2952

Salles PG, Moura Rde D, Menezes LM, Bacchi CE (2014) Expression of P16 in NUT carcinomas with no association with human papillomavirus (HPV). Appl Immunohistochem Mol Morphol 22(4):262–265

Schaefer IM, Dal Cin P, Fletcher CDM, Hanna GJ, French CA (2018) CIC-NUTM1 fusion: a case which expands the spectrum of NUT-rearranged epithelioid malignancies. Genes Chromosomes Cancer 57(9):446–451

Schwartz BE, Hofer MD, Lemieux ME, Bauer DE, Cameron MJ, West NH, Agoston ES, Reynoird N, Khochbin S, Ince TA, Christie A, Janeway KA, Vargas SO, Perez-Atayde AR, Aster JC, Sallan SE, Kung AL, Bradner JE, French CA (2011) Differentiation of NUT midline carcinoma by epigenomic reprogramming. Cancer Res 71(7):2686–2696

Seim NB, Philips RHW, Schoenfield L, Teknos TN, Rocco JW, Agrawal A, Ozer E, Carrau RL, Kang SY, Old MO (2017) NUT midline carcinoma of the sublingual gland: clinical presentation and review. Head Neck Pathol 11(4):460–468

Shehata BM, Steelman CK, Abramowsky CR, Olson TA, French CA, Saxe DF, Ricketts RR, Katzenstein HM (2010) NUT midline carcinoma in a newborn with multiorgan disseminated tumor and a 2-year-old with a pancreatic/hepatic primary. Pediatr Dev Pathol 13(6):481–485

Shiota H, Elya JE, Alekseyenko A, Chou PM, Gorman SA, Barbash O, Becht K, Danga K, Kuroda MI, Nardi V, French CA (2018) 'Z4' complex member fusions in NUT carcinoma: implications for a novel oncogenic mechanism. Mol Cancer Res 16(12):1826–1833

Sholl LM, Nishino M, Pokharel S, Mino-Kenudson M, French CA, Janne PA, Lathan C (2015) Primary pulmonary NUT midline carcinoma: clinical, radiographic, and pathologic characterizations. J Thorac Oncol 10(6):951–959

Sirohi D, Garg K, Simko JP, Grenert JP (2018) Renal NUT carcinoma: a case report. Histopathology 72(3):528–530

Stathis A, Zucca E, Bekradda M, Gomez-Roca C, Delord JP, de La Motte Rouge T, Uro-Coste E, de Braud F, Pelosi G, French CA (2016) Clinical response of carcinomas harboring the BRD4-NUT oncoprotein to the targeted bromodomain inhibitor OTX015/MK-8628. Cancer Discov 6(5):492–500

Stelow EB, French CA (2009) Carcinomas of the upper aerodigestive tract with rearrangement of the nuclear protein of the testis (NUT) gene (NUT midline carcinomas). Adv Anat Pathol 16(2):92–96

Stelow EB, Bellizzi AM, Taneja K, Mills SE, Legallo RD, Kutok JL, Aster JC, French CA (2008) NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. Am J Surg Pathol 32(6):828–834

Stevens TM, Morlote D, Xiu J, Swensen J, Brandwein-Weber M, Miettinen MM, Gatalica Z, Bridge JA (2019) NUTM1-rearranged neoplasia: a multi-institution experience yields novel fusion partners and expands the histologic spectrum. Modern Pathol 32:764–773. https://doi.org/10.1038/s41379-019-0206

Stirnweiss A, McCarthy K, Oommen J, Crook ML, Hardy K, Kees UR, Wilton SD, Anazodo A, Beesley AH (2015) A novel BRD4-NUT fusion in an undifferentiated sinonasal tumor highlights alternative splicing as a contributing oncogenic factor in NUT midline carcinoma. Oncogenesis 4:e174

Stirnweiss A, Oommen J, Kotecha RS, Kees UR, Beesley AH (2017) Molecular-genetic profiling and high-throughput in vitro drug screening in NUT midline carcinoma-an aggressive and fatal disease. Oncotarget 8(68):112313–112329

Storck S, Kennedy AL, Marcus KJ, Teot L, Vaughn J, Gnekow AK, Markl B, Leuschner I, DuBois SG, French CA, Fruhwald MC (2017) Pediatric NUT-midline carcinoma: therapeutic success employing a sarcoma based multimodal approach. Pediatr Hematol Oncol 34(4):231–237

Sturm D, Orr BA, Toprak UH, Hovestadt V, Jones DTW, Capper D, Sill M, Buchhalter I, Northcott PA, Leis I, Ryzhova M, Koelsche C, Pfaff E, Allen SJ, Balasubramanian G, Worst BC, Pajtler KW, Brabetz S, Johann PD, Sahm F, Reimand J, Mackay A, Carvalho

DM, Remke M, Phillips JJ et al (2016) New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell 164(5):1060–1072

Tamura R, Nakaoka H, Yoshihara K, Mori Y, Yachida N, Nishikawa N, Motoyama T, Okuda S, Inoue I, Enomoto T (2018) Novel MXD4-NUTM1 fusion transcript identified in primary ovarian undifferentiated small round cell sarcoma. Genes Chromosomes Cancer 57(11):557–563

Tanaka M, Kato K, Gomi K, Yoshida M, Niwa T, Aida N, Kigasawa H, Ohama Y, Tanaka Y (2012) NUT midline carcinoma: report of 2 cases suggestive of pulmonary origin. Am J Surg Pathol 36(3):381–388

Thompson-Wicking K, Francis RW, Stirnweiss A, Ferrari E, Welch MD, Baker E, Murch AR, Gout AM, Carter KW, Charles AK, Phillips MB, Kees UR, Beesley AH (2013) Novel BRD4-NUT fusion isoforms increase the pathogenic complexity in NUT midline carcinoma. Oncogene 32(39):4664–4674

Tilson MP, Bishop JA (2014) Utility of p40 in the differential diagnosis of small round blue cell tumors of the sinonasal tract. Head Neck Pathol 8(2):141–145

Toretsky JA, Jenson J, Sun CC, Eskenazi AE, Campbell A, Hunger SP, Caires A, Frantz C, Hill JL, Stamberg J (2003) Translocation (11;15;19): a highly specific chromosome rearrangement associated with poorly differentiated thymic carcinoma in young patients. Am J Clin Oncol 26(3):300–306

Vulsteke C, Lurquin E, Debiec-Rychter M, Gheysens O, Nuyts S, Schoenaers J, Politis C, Mebis J, Hauben E, Clement PM (2016) First evidence of treatment efficacy in metastatic carcinoma of the parotid gland with BRD4/NUT translocation. J Chemother 28(3):242–246

Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, Ducar M, Van Hummelen P, Macconaill LE, Hahn WC, Meyerson M, Gabriel SB, Garraway LA (2012) High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov 2(1):82–93

Zhu Y, Liu YF, Zhao J, Yu YW (2019) [BRD4-NUT fusion oncogene carcinoma in the kidney]. Zhonghua Bing Li Xue Za Zhi 48(3):237–9

Ziai J, French CA, Zambrano E (2010) NUT gene rearrangement in a poorly-differentiated carcinoma of the submandibular gland. Head Neck Pathol 4(2):163–168