nature reviews clinical oncology

https://doi.org/10.1038/s41571-025-00986-3

Perspective

Check for updates

Hiding in plain sight: NUT carcinoma is an unrecognized subtype of squamous cell carcinoma of the lungs and head and neck

Jia Luo[1,2] ✉, Justin A. Bishop[3], Steven G. DuBois[4], Glenn J. Hanna[1,2], Lynette M. Sholl[5], Edward B. Stelow[6], Lester D. R. Thompson[7], Geoffrey I. Shapiro[1,2] & Christopher A. French[5] ✉

Abstract

In the past two decades, treatment for non-small-cell lung cancers (NSCLCs) and head and neck squamous cell carcinoma (HNSCC) has advanced considerably, owing largely to the characterization of distinct oncological subtypes, the development of targeted therapies for each subtype and the advent of immunotherapy. Data emerging over the past two decades suggest that NUT carcinoma, a highly aggressive malignancy driven by a NUT fusion oncoprotein and arising in the lungs, head and neck, and rarely in other sites, is a squamous cell carcinoma (SCC) based on transcriptional, histopathological, cell-of-origin and molecular characteristics. NUT carcinoma has an estimated incidence of 1,400 cases per year in the United States, surpassing that of some rare NSCLC and HNSCC subtypes. However, NUT carcinoma is currently not recognized as an SCC of the lungs or head and neck. The orphan classification of NUT carcinoma as a distinct entity leads to a lack of awareness of this malignancy among oncologists and surgeons, despite early diagnosis being crucial for this cancer type with a median survival of only ~6.5 months. Consequently, NUT carcinoma is underdiagnosed and often misdiagnosed, resulting in limited research and progress in developing effective treatments in one of the most aggressive forms of lung and head and neck cancer. With a growing number of targeted agents that can potentially be used to treat NUT carcinoma, improved recognition through reclassification and inclusion of NUT carcinoma as a squamous NSCLC or an HNSCC when arising in these locations will accelerate the development of effective therapies for this disease. Thus, in the Perspective, we propose such a reclassification of NUT carcinoma as an SCC and discuss the supporting evidence.

Sections

A full list of affiliations appears at the end of the paper. ✉e-mail: jia_luo@dfci.harvard.edu; cfrench@bwh.harvard.edu

Introduction

The number of effective treatments for subsets of non-small-cell lung cancer (NSCLC) has increased rapidly over the past two decades owing to the identification of more than a dozen molecular drivers validated by rigorous preclinical and clinical studies[1,2]. However, among NSCLCs, the treatment of squamous cell carcinomas (SCCs) has remained comparatively stagnant owing to the identification of fewer targetable oncogenic drivers[3]. Data accumulated over the past two decades indicate that NUT carcinoma, a cancer driven by a nuclear protein in testis (NUT) fusion oncoprotein, is a subtype of SCC, based on transcriptional, histopathological, cell-of-origin and molecular oncogenic features[4–7]. These findings are important because NUT carcinoma is extremely aggressive[8,9] and constitutes a powerful, oncogene-driven model of SCC that can be leveraged to obtain biological insights and develop potential therapies for squamous NSCLCs and head and neck squamous cell carcinomas (HNSCCs).

NUT carcinoma is currently defined by the World Health Organization (WHO) as a poorly differentiated carcinoma with rearrangements involving *NUTM1* (ref. 10). The *NUTM1* fusion partners that characterize NUT carcinoma include *BRD4* (refs. 8,9,11), *BRD3* (refs. 8,9,12), *BRD2* (ref. 13), *NSD3* (refs. 8,9,14), *ZNF532*, *ZNF592* and *ZMYND8* (refs. 15–17). Less-common cancers with other *NUTM1* fusion partners (that is, *CIC*, *MXD4* and *YAP1*) are typically WHO-unclassified sarcomas or cutaneous adnexal tumours and are clinicopathologically distinct from NUT carcinoma[18–22]. Unfortunately, owing to poor understanding of these even rarer cancers, NUT carcinoma and non-NUT carcinoma *NUTM1*-rearranged tumours are often grouped together as *NUTM1*-rearranged tumours[23,24]. This classification adds to the confusion of defining NUT carcinoma, which should be defined as a group of carcinomas with similar oncogenic mechanisms driven by a subset of *NUTM1* rearrangements.

NUT carcinoma has only been shown to be a bona fide SCC in the past 2 years[4,25] and is not yet widely recognized as a form of squamous NSCLC or HNSCC by oncologists and pathologists. This non-recognition has resulted in a lack of awareness among otorhinolaryngologists (ear, nose and throat specialists), pulmonologists, thoracic surgeons and oncologists, leading to insufficient diagnostic testing. Consequently, although diagnosis of NUT carcinoma is rapid and straightforward, consisting of a single immunohistochemical stain for NUT protein expression[26], testing is often not performed. In addition, although NUT carcinoma can also be detected with DNA-based or preferably RNA-based sequencing for *NUTM1* fusions within a next-generation sequencing panel, the turnaround time is substantially slower than for immunohistochemistry (IHC). The challenge in diagnosing NUT carcinoma is not the test itself, but rather the clinical or pathological awareness to test for this cancer. Consequently, most patients miss opportunities to enrol in investigational trials (such as NCT05488548, NCT05372640 and NCT05019716) using NUT carcinoma-specific targeted agents, receive accurate prognostic information and/or benefit from standard-of-care treatment for SCCs. The continued orphan status of NUT carcinoma impedes research and effective therapeutic development for this cancer, and SCCs in general.

Advances in medicinal chemistry in the past 20 years or so have dramatically increased the number and range of targets and targeted therapies available to patients. With multiple clinical trials enrolling patients with NUT carcinoma at present, improved awareness and diagnosis of this cancer through inclusion within HNSCC and NSCLC classifications is urgently needed. In our experience, the under-recognition of NUT carcinoma has led to the perception among otorhinolaryngologists, thoracic surgeons, pulmonologists and oncologists that this cancer is so rare that it virtually does not exist. However, we estimate from an analysis of a large database of patients with squamous NSCLC or with HNSCCs who underwent DNA-based and RNA-based next-generation sequencing that the annual incidence of NUT carcinoma in the United States is 1,400 cases[27], which equals or surpasses that of well-known forms of oncogene-driven NSCLC (such as those driven by *NTRK*[28], *ROS1* or *RET*[29]) or non-squamous head and neck carcinomas (that is, *NTRK*-mutated salivary gland cancers[30]).

In this Perspective, we discuss the reasons for the current classification of NUT carcinoma as a non-squamous carcinoma, the rationale for its reclassification as a squamous NSCLC and HNSCC, and the immediate implications of this reclassification for clinical practice and research.

Reasons for the current NUT carcinoma classification

History

The classification of NUT carcinoma as an orphan disease is a result of observations in small case series over the past three decades. NUT carcinoma was initially considered an aggressive thymic carcinoma of children and adolescents[31] on the basis of three case reports in the early 1990s, which purported a thymic origin of these tumours[32–34]. This classification persisted because the initial series of cases were identified through biased screens focused on patients under 40 years of age with poorly differentiated carcinomas[31]. Consequently, the WHO initially categorized NUT carcinoma, then known as carcinoma with t(15;19) translocation, as a type of thymic carcinoma[35]. Despite the evolving understanding of NUT carcinoma, this cancer remains primarily a subcategory of thymic neoplasms and has its own category within carcinomas of the head and neck in the WHO classification[10,36–38], rather than a subcategory of squamous NSCLC and HNSCC.

Tissue of origin

Until 2 years ago[4,25], the precise tissue of origin of NUT carcinomas was unknown. In contrast to conventional forms of SCC, no definite in situ lesions of NUT carcinoma have been described owing to its rapidly invasive growth, making it difficult to confirm a squamous origin and presenting an obstacle to correct classification of this entity.

Aetiology

The aetiology of NUT carcinoma differs considerably from that of typical SCCs. The only known risk factor for NUT carcinoma is the random occurrence of a chromosomal translocation resulting in a *NUTM1* fusion event, often resulting from chromoplexy (complex, large-scale chromosomal rearrangements)[39]. Unlike many other SCCs, NUT carcinoma is not associated with viral (predominantly human papillovirus (HPV)) or environmental exposures (that is, tobacco and/or excess alcohol consumption), which are common risk factors for other squamous NSCLC or head and neck cancers. However, NUT carcinoma can occur in individuals with a smoking history[9], and a third of known cases occur in patients over 40 years of age[8,9,40]; squamous or poorly differentiated carcinomas in these populations are unlikely to be evaluated for the presence of NUT carcinoma. Conversely, NUT carcinoma is more likely to be considered and diagnosed in young individuals without a smoking history and without risk factors for typical SCCs, thus perpetuating the misconception that NUT carcinoma is a standalone malignancy of adolescents and young adults.

Pathology

As a highly aggressive malignancy, the histopathology of NUT carcinoma is typically that of a poorly differentiated epithelial neoplasm[10],

Table 1 | Reasons for and against classification of NUT carcinoma as an SCC

Aspect	Classification	
	Against SCC (current)	**For SCC**
History	Initial disease-defining cases (n=3) led to biases in understanding and testing for NC that perpetuated these biases	Not applicable
Tissue of origin	Lack of precancerous lesions identified in people	In situ squamous carcinoma precursor lesions identified within squamous epithelium in GEMMs of NC (Supplementary Fig. 1)
Aetiology	Driven by chromosomal translocations Unrelated to tobacco exposure, viral infection or other environmental cause	A subset of SCCs of lung and/or head and neck are driven by fusion oncogenes, including *FGFR3–TACC3* (refs. 141,142) and *DEK–AFF2* (ref. 143)
Pathology	Poorly differentiated morphology can mask squamous origin.	Most human and mouse NCs demonstrate histological or immunohistochemical squamous differentiation (Fig. 2) NC falls within the poorly differentiated end of the morphological spectrum of SCC
Molecular drivers and pathogenetic mechanisms	Mutational landscape of NC is simpler than that of conventional SCC (Table 3) NC is driven by *NUTM1* fusions, similar to other non-NC *NUTM1*-rearranged cancers, perpetuating the idea that NC is different from SCC	NC and conventional SCC are driven by the same oncogenic factors (Table 3) The pathogenetic mechanism of NUT fusions in NC is distinct from that of other *NUTM1*-rearranged cancers
Epigenetics	NC is purely driven by epigenetic alterations (Fig. 1b and Table 3), whereas conventional SCCs are mutationally driven	Two epigenetic proteins key to NC, BRD4 and EZH2, play oncogenic roles in conventional SCCs The oncogenic drivers mutated in conventional SCCs are driven epigenetically in NC (Table 3) Aberrant epigenetics in NC is a shortcut to squamous carcinoma formation
Transcriptomics	None	Transcriptomes of human and mouse NCs cluster with conventional SCC of lung and head and neck
Clinical presentation (Table 2)	NC occurs in patients with a younger median age (23.6 years[8] versus 53–73 years[149]; Table 3) NC is more aggressive than conventional SCC NC can occur at anatomical sites that are atypical for conventional SCC	The majority of NCs present clinically like squamous lung or head and neck cancers (Fig. 2) SCC can also occur in the same atypical sites where NC arises NC has a metastatic organotropism similar to SCC
Experimental evidence	None	Genetic or pharmacological inhibition of BRD4–NUT induces squamous differentiation of NC, based on histology, IHC and transcriptomics

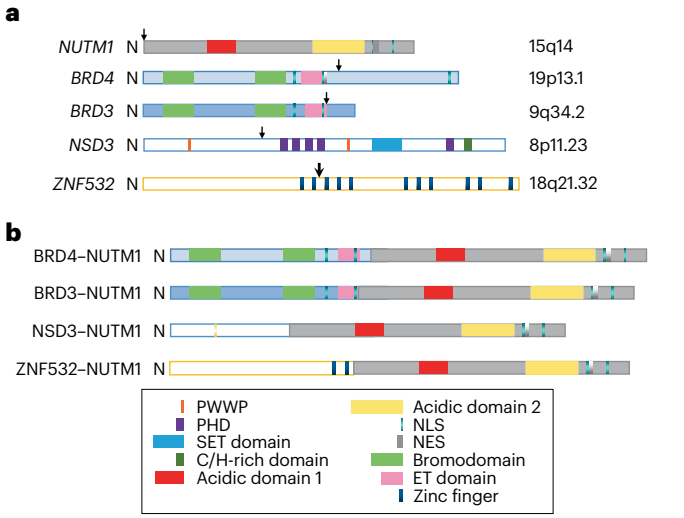
GEMMs, genetically engineered mouse models; IHC, immunohistochemistry; NC, NUT carcinoma; SCC, squamous cell carcinoma.

thus precluding histopathological classification. Although NUT carcinoma is often not recognizable as an SCC owing to its poorly differentiated appearance, most cases demonstrate both epithelial (that is, expression of common epithelial keratins detectable using AE1/AE3 and pan-keratin antibodies) and squamous differentiation markers (that is, expression of CK5, p63 and ΔNp63) by IHC[41,42]. Only a third of cases exhibit frank squamous differentiation, typically described as 'abrupt keratinization'[8]. The fairly nonspecific poorly differentiated morphology has led to frequent misdiagnosis of NUT carcinoma as a poorly differentiated carcinoma not otherwise specified, and more rarely as a small-cell carcinoma, Ewing sarcoma or leukaemia[43–46].

Molecular pathology

Another contributing factor to the misclassification of NUT carcinoma is conflation of this cancer with other *NUTM1*-rearranged neoplasms that are not carcinomas (Table 1). The emergence of non-carcinoma neoplasms with *NUTM1* rearrangements and a variable, mostly sarcomatous, histology has led some pathologists and oncologists to conflate these entities and thereby define *NUTM1*-rearranged neoplasms as a heterogeneous group with undefined histology[23,24]. Classifying NUT carcinoma with these other tumour types is problematic because NUT carcinomas have a different biology. Histologically, NUT carcinoma is a carcinoma, whereas the other *NUTM1*-rearranged neoplasms comprise sarcomas[19–21], cutaneous adnexal tumours[22], and lymphomas or leukaemias[47–49]. Molecularly, NUT carcinomas are a family of carcinomas harbouring fusions of *NUTM1* with a bromodomain and extraterminal domain (BET) gene (or a gene encoding a BET-associated-protein), including *BRD2* (ref. 13), *BRD3* (ref. 12), *BRD4* (refs. 11,50), *NSD3* (ref. 14), *ZNF592* (ref. 16) or *ZNF532* (refs. 15,16). All these *NUTM1* fusion partner genes encode proteins that are core members of oncogenic and/or wild-type BRD4-associated chromatin complexes[15,51,52] (Fig. 1). Thus, by analogy with MLL1 fusions (which commonly involve AF4, AF9 and ENL, among many other fusion partners)[53], all of these NUTM1 fusion partners can create the same oncogenic complex as the canonical BRD4–NUTM1 fusion. The BRD4–NUTM1 chromatin complex is fundamentally important to cell identity and growth as its function is to maintain transcription of target proto-oncogene transcription factors such as *SOX2*, *TP63* and *MYC*[5,7,54].

By contrast, none of the proteins encoded by the non-NUT carcinoma fusion partner genes (including *CIC*, *BCORL1*, *MXD1*, *MXD4*, *MGA*, *YAP1*, *BRD9*, *ACIN1*, *SLC12A6*, *ZNF618*, *BPTF*, *CUX1* and *IKZF1*) are



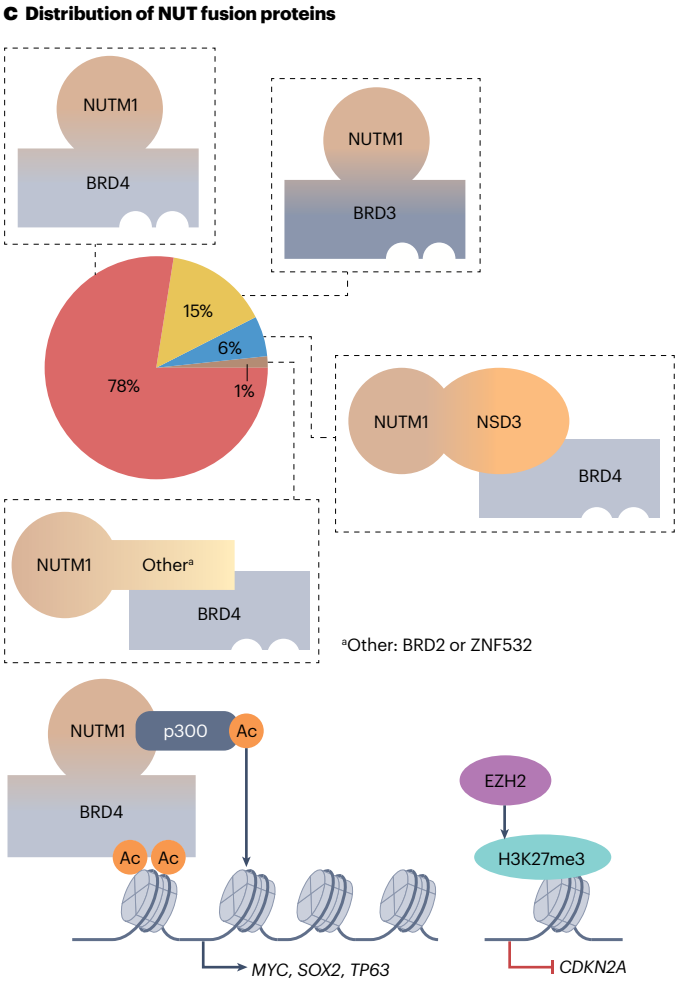


Fig. 1 | All NUT fusion partners in NUT carcinoma are interchangeable members of the BRD4-containing NUTM1 fusion core complex. a, Genes and common breakpoints involved in *NUTM1* fusions found in NUT carcinomas. The schematic depicts the domain structure of respective encoded wild-type proteins, with arrows denoting the canonical breakpoints resulting in *NUTM1* fusions, although these can vary in different patients. **b**, The encoded NUTM1 fusions that define NUT carcinomas. **c**, The NUTM1 fusion oncoprotein complex. Top, a variety of *NUTM1* fusion partners encode proteins that either contain BRD4, or that bind BRD4, thus the various NUT fusion proteins are interchangeable in that they all form an oncogenic complex containing the same protein constituents. The frequencies of each *NUTM1* fusion shown are based on the largest single series ($n = 127$)[8]. Bottom, this complex creates large super-enhancers via increased histone H3 lysine 27 acetyltransferase activity mediated by the p300–NUT complex interaction. Enrichment of super-enhancer regions with acetyl histone H3 lysine 27 drives expression of *MYC* and other oncogenic genes, such as *SOX2* and *TP63*, by increasing promoter–enhancer contacts that activate transcription. EZH2 expression is upregulated in NUT carcinomas, probably via upregulation of *EZH2* transcription by MYC. Increased expression of EZH2 results in transcriptional downregulation of the tumour-suppressor gene *CDKN2A* through trimethylation of histone H3 lysine 27 (H3K27me3). Ac, acetylated lysine residue; C/H, cysteine and histidine; ET domain, extraterminal domain; NES, nuclear export signal; NLS, nuclear localization signal; PHD, plant homeodomain (PHD) zinc finger domain; PWWP, Pro–Trp–Trp–Pro domain; SET, Su(var)3-9, Enhancer-of-zeste and Trithorax domain.

detectable within BRD4-associated complexes[15]. The partner genes associated with a common non-NUT carcinoma *NUTM1*-rearranged histological group (*CIC*, *BCORL1*, *MXD1*, *MXD4* and *MGA*), sarcomas, all encode transcription co-repressors, which when fused to NUTM1 are predicted to switch to being activators of transcription that derepress expression of target genes[48]. Another group of non-NUT carcinoma *NUTM1* fusion cancers, poromas and porocarcinomas, most commonly harbour a *YAP1–NUTM1* fusion, which functions to bypass the HIPPO pathway to transactivate gene targets of transcriptional enhanced associate domain (TEAD) family transcription factors (nuclear effectors of the HIPPO pathway) to promote growth of cancer cells[22]. Thus, whereas NUTM1 fusion oncoproteins in NUT carcinomas all share an oncogenic mechanism (that is, enhanced expression of proto-oncogenes regulated by BRD4), non-NUT carcinoma NUTM1 fusions act by fundamentally different, BET-independent mechanisms to derepress tumour-suppressive pathways. This distinction is important because key efforts to target NUT carcinoma therapeutically have been centred on inhibition of BET proteins, a target class not known to have a role in the oncogenesis of non-NUT carcinoma *NUTM1*-rearranged neoplasms. Thus, *NUTM1*-rearranged tumours involve various fusion partners and oncogenic mechanisms (Table 2).

Comparison of the molecular pathology of NUT carcinomas and traditional SCCs of the lungs and head and neck reveals several differences linked to their distinct aetiologies. Non-NUT carcinoma SCCs of the lungs and head and neck are characterized by complex molecular aberrations that result from sustained exposure to mutagens, the antitumour suppressor effects of HPV or the oncogenic effects of Epstein–Barr virus. These aberrations include multiple activating mutations in or copy-number gains of proto-oncogenes, and inactivating mutations or deletions of tumour-suppressor genes (TSGs)[55,56]. SCCs, particularly smoking-related SCCs, are genetically complex[57], making it challenging to study them and to identify universal molecular therapeutic vulnerabilities for these cancers[58]. By contrast, NUT carcinoma is driven by a single genomic alteration, encoding a NUTM1 fusion oncoprotein on a low mutational background[39,59].

Epigenetics

Another difference between NUT carcinoma and conventional SCC is that the former is a cancer driven primarily by epigenetic alterations.

In NUT carcinoma, BRD4–NUTM1 drives proto-oncogene expression through epigenetic activation via histone hyperacetylation while also upregulating EZH2, which represses TSG expression through epigenetic silencing via H3K27 methylation. Specifically, BRD4–NUTM1 combines the chromatin reading activity of BRD4 with the writing activity of NUTM1, via recruitment of p300, to produce extremely large super-enhancers, termed megadomains, which upregulate and sustain expression of *MYC*, *SOX2* and other genes driving oncogenesis[15,54] (Fig. 1b). EZH2 expression is upregulated in NUT carcinoma by non-mutational mechanisms, probably through direct upregulation of *EZH2* transcription by MYC[60,61]. In non-BRD4–NUTM1-occupied regions of the genome, EZH2, presumably in complex with polycomb repressor complex 2 (PRC2), silences expression of target TSGs, including *CDKN2A*, through H3K27 methylation[6]. By contrast, tumorigenesis in conventional SCCs is thought to be driven primarily by mutations in oncogene and TSG signalling axes, leading to dysregulation of cellular proliferation and/or differentiation[3]. Nonetheless, epigenetic alterations also have an important role in conventional SCC tumorigenesis[62–64].

Clinical presentation

In contrast to the initial reported cases, most NUT carcinomas present as cancers originating in the lungs or head and neck. Indeed, the molecular and histological distinction of NUT carcinoma from other non-NUT carcinoma *NUTM1*-rearranged tumours is accompanied by different sites of origin. Whereas NUT carcinomas predominantly (90%)[8] arise within sites that overlap with those of conventional SCC (that is, the lungs and head and neck), *NUTM1*-rearranged sarcomas typically arise outside of these regions, most often in the abdomen, soft tissue or central nervous system[20,21,48,65–67]. Similarly, *NUTM1*-rearranged porocarcinomas arise within the skin[22].

For some pathologists and clinicians, the rare (8%) cases of NUT carcinoma arising in atypical sites[8], such as thyroid gland[68,69], salivary gland[70,71], kidney[23], pancreas[72] or bladder[31], have reinforced the idea that NUT carcinoma is not an SCC of the lungs and head and neck, as evidenced by its lack of classification as such by the WHO[10,37,38,73]. We argue that these exceptions should not define all NUT carcinomas. To this point, conventional SCCs have also been reported in all of these sites[74–77], yet these exceptions do not disqualify these SCCs as either an SCC or as a cancer of the lungs and head and neck.

The clinical features of NUT carcinoma differ somewhat from those of conventional SCCs, contributing further to its separate classification (Table 3). Whereas conventional SCCs typically affects older males, NUT carcinoma affects younger patients, is equally prevalent in both sexes and is more aggressive than conventional SCCs of the lungs and head and neck. Although conventional SCCs are a more aggressive form of NSCLC and HNSCCs than non-squamous cancers of

Table 2 | Characteristics of *NUTM1*-rearranged cancers

Cancer	Biological pathway	*NUTM1* fusion partner	Primary organ site	Histology	Refs.
NUT carcinoma	BRD4–NUTM1	*BRD4*	Lungs, head and neck, other tissues	Squamous or poorly differentiated carcinoma	8,11
		BRD3	Lungs, head and neck, other tissues	Squamous or poorly differentiated carcinoma	8,12
		NSD3	Lungs, head and neck, other tissues	Squamous or poorly differentiated carcinoma	8,14
		BRD2	Lungs	Squamous or poorly differentiated carcinoma	13
		ZNF532	Lungs; head and neck	Squamous or poorly differentiated carcinoma	15
NUTM1-rearranged sarcomas	Derepression of MYC targets (predicted)	*CIC*	Central nervous system, bone and soft tissue	Undifferentiated epithelioid sarcoma	150,151
		BCORL1	Bone and soft tissue	Undifferentiated epithelioid sarcoma	19
		MXD1	Soft tissue	Undifferentiated epithelioid sarcoma	19
		MXD4	Colon	Undifferentiated epithelioid sarcoma	19,65
		MGA	Lung, soft tissue, dura	Spindle cell sarcoma	20,66
NUTM1-rearranged skin cancers	Dysregulation of HIPPO pathway	*YAP1*	Skin adnexa	Porocarcinoma	22
NUTM1-rearranged leukaemias	Unknown	*BRD9*	Blood or bone marrow	Leukaemia	152
		ACIN1	Blood or bone marrow	Leukaemia	47,152–154
		SLC12A6	Blood or bone marrow	Leukaemia	153
		ZNF618	Blood or bone marrow	Leukaemia	153
		IKZF1	Blood or bone marrow	Leukaemia	153,155
		BPTF	Blood or bone marrow	Leukaemia	156
		CUX1	Blood or bone marrow	Leukaemia	47
		IKZF1	Blood or bone marrow	Leukaemia	47

Table 3 | Characteristics of NUT carcinoma and other squamous cancers

Characteristic		Cancer type			Refs.
		NUT carcinoma	Lung SCC[a]	Head and neck SCC[a]	
Median patient age at diagnosis		23.6 years (range 1–82 years)	73 years	53[b]–66 years	8,149,157
Primary organ site		Predominantly central lung or head and neck	Central lung	Head and neck	8,78,158
Aetiology		NUTM1 fusion	Tobacco exposure	Alcohol exposure, tobacco exposure, HPV infection	11,12,14–16,39
Female sex		52%	36%	27%	8,159
Median overall survival		6.7 months	10 months	20–130[b] months	8,149,160
Overall survival[c]	2-year	17%	–	–	8
	3-year	–	35.3%	–	SEER[161]
	5-year	–	24.7%	68.5%	SEER

HPV, human papillomavirus; SCC, squamous cell carcinoma; SEER, Surveillance, Epidemiology and End Results Program. [a]Based on SEER data[161]. [b]HPV-associated[157]. [c]Values for conventional SCC patients are for those with early stage disease.

these sites and currently lack targetable drivers, the natural history of NUT carcinoma is more aggressive, with a median overall survival (OS) of ~6.5 months[8,40]. Patients with NUT carcinoma who have relapsed or are refractory to treatment typically have a rapid decline in Eastern Cooperative Oncology Group performance status within a few weeks, with rapidly rising inflammatory markers and scans revealing tumours growing quickly or even invading into the bone marrow[59,78]. Whereas early palliative care is important in patients with advanced-stage lung or head and neck cancers, it is imperative in patients with NUT carcinoma to apply symptom-directed therapy and initiate important goals of care conversations at diagnosis. In the context of clinical trial eligibility, patients with NUT carcinoma need rapid access to the next line of therapy if the prior line is ineffective. If maximum flexibility is not utilized in trial design, strict metrics, including unnecessarily long washout periods from prior drug exposure or limitations on use of symptom-directed therapy or other measures to mitigate toxicity, lead to an impractical eligibility-screen failure rate and high rate of drug interruptions for patients with NUT carcinoma.

In summary, the current classification of NUT carcinoma as an entity distinct from SCC arose from a small number of early, non-representative observations and the distinct aetiology, single molecular driver, unique epigenetic features and somewhat unique clinical features of this malignancy. Despite some molecular and clinical distinctions between NUT carcinomas and conventional SCCs, most evidence argues in favour of classifying NUT carcinoma as an SCC.

Reasons for classifying NUT carcinoma as an SCC

Tissue of origin

NUT carcinoma and conventional SCCs of the lungs and head and neck arise from overlapping anatomical sites (Table 3), indicating similar tissues of origin. Studies with two genetically engineered mouse models (GEMMs) of NUT carcinoma (using either a *Krt14* or *Sox2* promoter to drive Cre expression) found that NUT carcinoma most commonly arises in the squamous epithelium of the oesophagus and head and neck[4,25]. In fact, in the *Sox2Cre* mouse, in situ NUT carcinoma precursor lesions were identified within the squamous epithelium of the oesophagus, providing histological evidence that NUT carcinoma can arise from keratinocyte precursors[4] (Supplementary Fig. 1). When the NUT carcinoma GEMM was crossed with the *NLSCre* mouse, thereby resulting in Cre expression in all tissues, NUT carcinomas arose in various tissue sites from all germ layers, but the majority arose within the epithelial lining of head and neck structures, including the oropharynx and sinonasal regions, mimicking the human disease[25]. Although none of these NUT carcinoma GEMMs developed lung SCC, the very long latency for tumour formation of mouse lung SCC, as observed in other GEMMs, probably means that tumours formed in organs such as the oesophagus and skin killed the mice before lung tumours could develop[58,79].

Pathology

Histologically, NUT carcinoma typically exhibits features of either a poorly differentiated carcinoma or poorly differentiated SCC[8–10,80] (Fig. 2). Keratinization, a hallmark of squamous differentiation, is detected in 33–42% of NUT carcinomas[8,9] (Fig. 2). Immunohistochemically, most NUT carcinomas (85%) express keratins[42], a marker of epithelial differentiation, and the squamous lineage markers CK5 (71–83%), p63 (87%) and, to a slightly lesser extent, the p63 isoform p40 (that is, ΔNp63; 65–86%)[42,78,81–83].

GEMMs of NUT carcinoma demonstrate that in tumours induced by BRD4–NUTM1, histological and immunohistochemical features of squamous differentiation are almost universal (Fig. 2), regardless of the site of origin. In the two known GEMMs of NUT carcinoma, most exhibit at least focal keratinization (Fig. 2) and immunohistochemical staining for keratin 14 and p63, consistent with a squamous origin[4,25]. Even tumours at atypical sites (that is, the stomach, pancreas or bone) exhibit both keratinization and immunohistochemical staining consistent with squamous differentiation[25]. Collectively, the pathological and immunohistochemical features of both human and mouse NUT carcinomas are consistent with this tumour being an SCC.

Transcriptomics

Although morphological and immunohistological features might suggest a cell of origin for NUT carcinomas, an unbiased, comprehensive analysis of the tumour transcriptome is a more rigorous and objective approach to correctly classify these tumours. Therefore, we performed whole-transcriptome RNA sequencing of human and mouse NUT carcinoma tumour tissue and tumour-derived cell lines and used an ensemble convolutional neural network called OTTER[84] to classify these samples. Almost all NUT carcinoma samples (89%) co-classified with human squamous NSCLC or HNSCC[4]. These findings, together with

the identification of in situ NUT carcinoma in squamous epithelium in GEMMs, provide compelling evidence that NUT carcinoma is an SCC.

Molecular drivers and pathogenetic mechanism

Most key mutationally activated molecular drivers of SCC are also deregulated in NUT carcinoma (Table 4). The proto-oncogenes *MYC*, *TP63* and *SOX2* are implicated as key factors driving tumour growth in conventional SCCs[85–88] and are frequently amplified in these cancers[3,89–93]. In NUT carcinomas, the expression of these same proto-oncogenes are directly upregulated as a result of epigenetic changes in BRD4–NUTM1-associated megadomains[54]. BRD4–NUTM1-associated megadomains arise from pre-existing enhancers[54]; thus, the presence of these aberrant transcriptional activating domains at known squamous-lineage transcription factor genes is further evidence that NUT carcinoma arises within a squamous precursor cell. The tumour-suppressor p53 is frequently inactivated by missense *TP53* mutations in SCCs[94], or by the E6 protein in HPV-associated SCCs[95]. In NUT carcinoma, evidence indicates that p53 is inactivated through sequestration by BRD4–NUTM1 (ref. 96). Another TSG, *CDKN2A*, encodes both $p16^{INK4A}$ and $p14^{ARF}$; $p16^{INK4A}$ blocks cell-cycle progression via inhibition of CDK4 and CDK6, whereas $p14^{ARF}$ inhibits MDM2-mediated degradation of p53. *CDKN2A* is frequently mutationally inactivated in SCC. Similarly, cyclin D1 (encoded by *CCND1*), which phosphorylates and inactivates the tumour-suppressor RB1, a function that depends on cyclin D1 binding to CDK4 and CDK6, is often amplified in conventional SCC, attesting to the importance of these components in cell-cycle progression. In NUT carcinoma, the CDK4/CDK6–cyclin D1–RB1 axis is also perturbed, but via epigenetic silencing (H3K27 methylation) by EZH2, which represses transcription of *CDKN2A* and thereby reducing the levels of the CDK4/CDK6 inhibitor $p16^{INK4A}$ and the MDM2 inhibitor $p14^{ARF}$ (ref. 6). EZH2 also maintains keratinocyte stemness and proliferation via repression of *CDKN2A*[97]. Moreover, *EZH2* is often mutated in SCCs[3] and EZH2 upregulation is associated with malignant progression in lung and cutaneous SCC[98,99].

The other major driving genetic alterations in SCC for which a role in NUT carcinoma remains an open question involve the amplification or mutational activation of *EGFR*, *FGFR* and *PI3K*, which results in the activation of receptor tyrosine kinase (RTK) signalling[100–102]. Although the role of RTK signalling in the growth of NUT carcinoma remains to be clarified, activating RTK signalling by transgenic

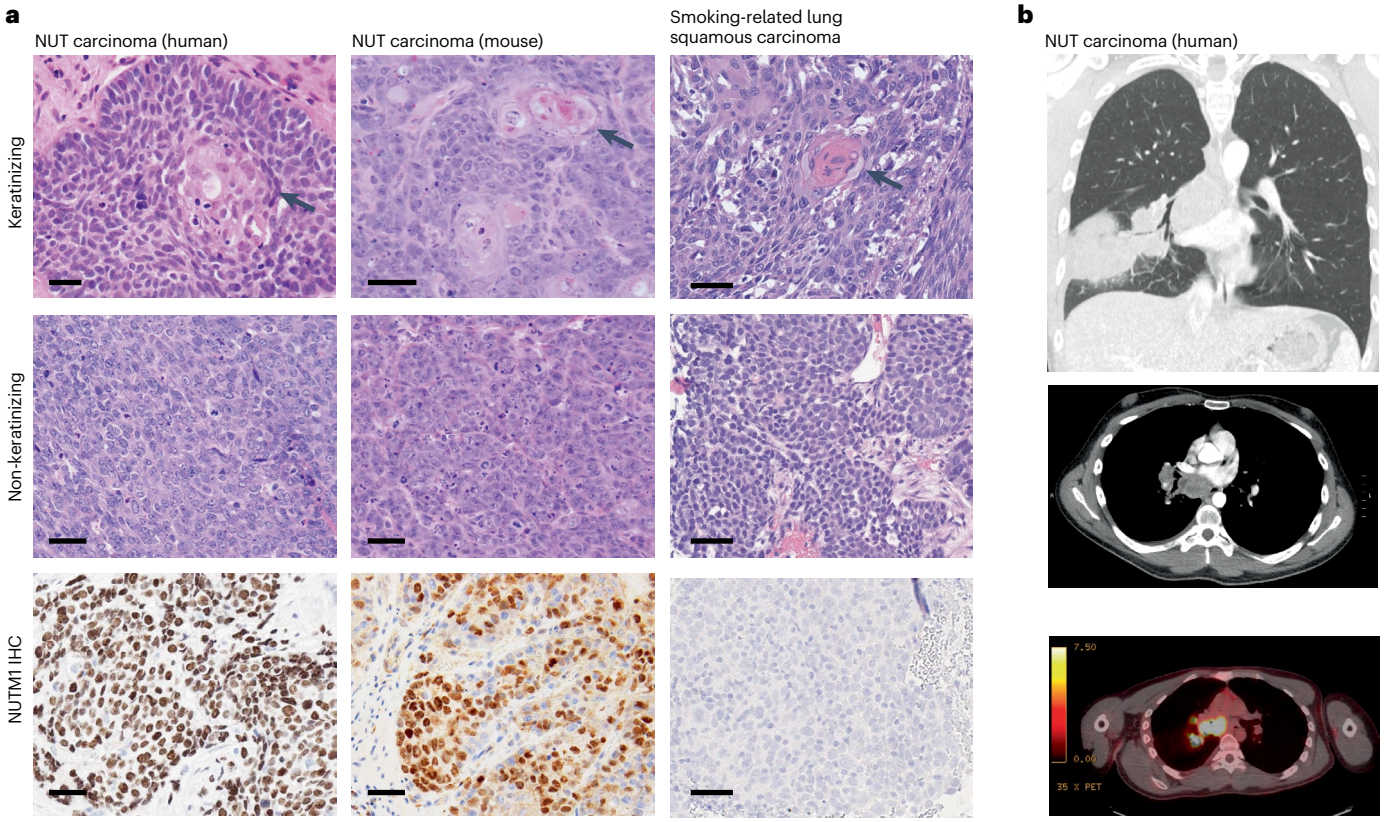


Fig. 2 | Overlapping histopathological and imaging features of NUT carcinoma and other SCCs. a, Keratinizing squamous cell carcinomas (SCCs; top row) demonstrate focal areas of keratinocytic differentiation characterized by accumulation of eosinophilic cytoplasmic keratin (arrows). Non-keratinizing SCCs (middle row) lack such areas of keratinocytic differentiation. The tissue sections in the top and middle rows are stained with haematoxylin and eosin. Immunohistochemical staining for NUT (NUT immunohistochemistry (IHC); bottom row) highlights BRD4–NUT protein within nuclei of NUT carcinoma cells. In the context of a tumour with a carcinoma histology, diffuse (>50% of nuclei) positive for nuclear staining by NUT IHC is 100% specific for the diagnosis of NUT carcinoma. Scale bars, 50 μm. **b**, Chest CT (top and middle) and PET (bottom) scans of NUT carcinoma demonstrating fluorodeoxyglucose-avid disease (bottom) consisting of a lung mass with associated bronchial narrowing, post-obstructive atelectasis and bulky thoracic adenopathy. This presentation appears similar to that of a conventional SCC of the lungs or a poorly differentiated non-small-cell lung cancer. Part **a**, photomicrographs of keratinizing and non-keratinizing mouse and human NUT carcinoma, adapted with permission from ref. 4, American Association for Cancer Research.

Table 4 | Common molecular aberrations in conventional squamous cell carcinomas[a] and NUT carcinoma

Gene	Aberration in non-NC squamous cell carcinomas	Aberration in NC	Refs.
TP53	Missense mutations, inhibition by E6 and E7 viral proteins	Sequestration in BRD4–NUTM1 megadomains, epigenetic downregulation of $p14^{ARF}$ by EZH2	6,94–96
CDKN2A	Loss-of-function mutations	Epigenetic silencing by EZH2	3,6,98,99
MYC	Amplified	Epigenetic upregulation by BRD4–NUT	5,54,90,162,163
TP63	Amplified	Epigenetic upregulation by BRD4–NUT	54,92,164,165
SOX2	Amplified	Epigenetic upregulation by BRD4–NUT	7,54,89,93,166,167
CCND1	Amplified	Epigenetic downregulation of $p16^{INK4A}$ by EZH2	6,90,168
EGFR, *FGFR* and *PI3K*	Amplified and/or activating mutations	Unknown	102,168–170
EZH2	Upregulated	Upregulated	6,62,98,99

[a]Reviewed elsewhere[3].

expression of *ERBB2* (encoding the EGFR family member HER2) or *PIK3CA* (encoding the catalytic subunit of PI3K) confers a considerable growth advantage to NUT carcinoma cells in the presence of BET inhibitors[103].

Therefore, apart from the presence of a NUT fusion oncoprotein, the oncogenic pathways (that is, oncogene activation and TSG inactivation) in NUT carcinoma completely overlap with those described in conventional SCC (Supplementary Fig. 2). The striking convergence in molecular alterations in NUT carcinoma and conventional SCC suggests that in NUT carcinoma, alternative epigenetic mechanisms activate and inactivate major pro-growth and tumour-suppressive factors implicated in SCC pathogenesis. In essence, BRD–NUTM1 fusions provide a shortcut to SCC without the need for years of accumulated mutations or infection by HPV.

Experimental evidence

Long before transcriptomic profiling provided evidence for classifying NUT carcinoma as an SCC, it was established that BRD–NUTM1 fusion oncoproteins maintain tumour growth by preventing differentiation of NUT carcinoma cells. In vitro experiments using human NUT carcinoma-derived cell lines showed that small interfering RNA mediated knockdown of any of the major BRD–NUTM1 fusions, including *NUTM1* fusions with *BRD4* (refs. 5,12), *BRD3* (ref. 12), *NSD3* (ref. 14) or *ZNF532* (ref. 15), induces squamous differentiation of these cell lines. The same observation has also been made in GEMM-derived NUT carcinoma cell lines[4]. In fact, direct inhibition of BRD–NUTM1 fusions with BET inhibitors[5,104,105], or pharmacological or genetic perturbation of key pathway components (that is, MYC[5], SOX2 (ref. 7), NSD3 (ref. 14), PVT1 (ref. 54), MED24 (ref. 54) or p300 (refs. 105,106)) induces squamous differentiation of NUT carcinoma cell lines. In all these studies, the keratinocyte differentiation was determined based on morphological changes (flattening, stratification and enlargement of cells), immunohistochemical changes (expression of the terminal squamous differentiation marker involucrin or keratin 14) and transcriptomic changes (induction of epidermal development programmes). Similarly, small-molecule inhibition of EZH1 and/or EZH2 methyltransferase activity also leads to squamous differentiation of NUT carcinoma cells, which is greatly augmented when both BRD–NUT and EZH1/EZH2 are co-inhibited[6].

The induction of squamous differentiation of NUT carcinoma cells through BRD–NUTM1 or PRC2/EZH pathway inhibition supports the idea that this cancer is of squamous lineage, but alone does not provide definitive evidence because lineage commitment during differentiation does not define the lineage of the undifferentiated progenitor cells. For example, depending on growth conditions, mouse embryonic stem cell lines can differentiate along a single cell lineage[107,108], even if the progenitor cells are pluripotent and not committed to a specific lineage. Moreover, numerous real-world examples exist of non-squamous-origin tumours in humans that frequently display squamous differentiation, including, among others, endometrial adenocarcinomas, adamantinoma-like Ewing sarcomas and small-cell carcinomas. However, although the final differentiated cell type does not confirm the cell of origin, the preponderance of data together with the ability to induce squamous differentiation by inhibiting NUTM1 fusions are further evidence that strongly imply that NUT carcinoma is an SCC.

Clinical features

Although NUT carcinoma can be markedly more aggressive than conventional SCC, NUT carcinoma still shares several important similarities with squamous NSCLC and HNSCC. Radiographically, NUT carcinomas seem indistinguishable from squamous NSCLC and HNSCCs[46,78]. Pulmonary NUT carcinoma often presents as a bulky mediastinal and/or hilar mass with associated lymphadenopathy and effusions[78,109] (Fig. 2). The clinical presentation of NUT carcinomas is also similar to that of conventional squamous NSCLC and HNSCCs. Patients often present with a cough or post-obstructive pneumonia. NUT carcinoma of the head and neck typically presents with a mass in the sinonasal tract[110,111] with accompanying swelling, congestion and sometimes even epistaxis[112].

Common sites of metastasis of NUT carcinoma include bone and liver[59], whereas metastases are rarely seen in the central nervous system at diagnosis. This pattern of organotropism is similar to that of SCCs of the lung and head and neck.

In addition, NUT carcinoma, when responsive to chemotherapy, tends to respond to SCC-targeted regimens such as platinum-based chemotherapy and concurrent chemotherapy with radiotherapy[9,113,114]. These clinical parallels further justify classifying NUT carcinoma as an SCC.

Treatment

Conventional treatment approaches. As NUT carcinoma has been classified as a standalone entity, and not as a subset of SCC or even sometimes not as a lung or head and neck cancer, treatments recommended by oncologists in academic and non-academic institutions

have been heterogeneous. Chemotherapy combinations vary from sarcoma regimens (for example, the Scandinavian Sarcoma Group (SSG) IX regimen for Ewing sarcoma or other ifosfamide-based regimens)[43,113,115,116], to small-cell carcinoma regimens (etoposide and platinum)[9,117], to lymphoma regimens (ifosfamide, carboplatin and etoposide)[118], to combinations typically used in conventional SCCs (that is, taxane and platinum)[9,113,114]. The wide variation in treatment for NUT carcinoma and the lack of ownership by a subset of the oncology community (for example, thoracic and head and neck oncologists) has made rigorous retrospective statistical comparisons between different regimens challenging, posing a major obstacle to understanding which specific treatments are most effective for NUT carcinoma, thereby limiting the development of treatment guidelines for this rare disease.

We believe that effective and expeditious development of best practices for treating NUT carcinoma begins with reclassifying it as an SCC, a cancer type with which oncologists are very familiar. This reclassification will provide a starting point for oncologists to begin treating NUT carcinoma consistently using SCC regimens. Several case reports and series demonstrating partial or complete responses of NUT carcinoma to SCC-type chemotherapy regimens suggest that this approach is a reasonable first-line treatment for most cases of NUT carcinoma[9,113,114,119] (Box 1). Although several case reports demonstrate that ifosfamide-based regimens similar to those used to treat Ewing sarcoma can lead to durable complete responses in patients with NUT carcinoma, in all these cases the patients were younger than 18 years old and most had non-metastatic disease[43,115,116]. In addition, a retrospective analysis comparing ifosfamide-based and platinum-based regimens in patients with NUT carcinoma found that the objective response rate and disease-free survival were only slightly better in patients with non-metastatic NUT carcinoma who received ifosfamide-based therapy than those who received platinum-based therapy, with no significant difference in OS between the two groups[9]. Moreover, no difference was seen in progression-free survival or OS in patients presenting with metastatic disease[9]. Patients who were alive longer than 3 years also had a mix of platinum-based and ifosfamide-based treatment[9].

Ifosfamide has a less-favourable toxicity profile than platinum-based chemotherapy, with adverse effects unique to ifosfamide including neurotoxicity and haemorrhagic cystitis. Indeed, ifosfamide-based therapy is not generally administered to patients older than 40 years of age with any cancer, which encompasses most patients with conventional SCC[120], owing to adverse effects and unclear efficacy. Therefore, treating clinicians typically consider ifosfamide-based therapy only for younger and healthier individuals, which introduces a bias in favour of good outcomes with ifosfamide regimens in retrospective analyses[121]. Despite this advantage, the aforementioned retrospective study[9] did not find a signal of superiority of ifosfamide-based therapy over platinum-based regimens in adults with metastatic disease. Although prospective studies testing different chemotherapy regimens in NUT carcinoma are yet to be performed, owing to the difficulty of randomizing patients in a highly aggressive disease with a considerable screen failure rate, this retrospective analysis suggests that ifosfamide is not more effective than platinum-based therapy in adult patients. Taken together, these data strongly suggest that patients with advanced-stage disease should be spared from the toxicities associated with ifosfamide-based therapy. In summary, by classifying NUT carcinoma as an SCC, adult patients are unlikely to be missing out on any benefit from receiving ifosfamide-based regimens.

As for SCC, immune-checkpoint inhibitors (ICIs) might also have a role in the treatment of a subset of NUT carcinomas. A quarter of NUT carcinomas are positive for PD-L1 expression, and a few case reports indicate a potential clinical benefit using ICIs alone or combined with chemotherapy in select patients[27,59,113,122–125] (Box 1). Platinum-based chemotherapy combined with ICIs is already approved for squamous NSCLC and HNSCC, so classifying NUT carcinoma as an SCC of these sites would provide patients with more consistent upfront access to ICI-based regimens, which might be important considering many patients with this highly aggressive cancer are ultimately unable to receive second-line therapy owing to rapid functional decline.

Treatment of NUT carcinoma should therefore be designed to address its squamous biology. Although treatment based on histology

Box 1 | Treatment approaches for NUT carcinoma

Conventional approaches

Initial treatment of NUT carcinoma in adults should be similar to that of conventional squamous cell carcinomas (SCCs), including combined taxane and platinum-based chemotherapy (such as paclitaxel and carboplatin) with or without an immune-checkpoint inhibitor (such as an anti-PD-1 antibody)

- No difference in overall survival (OS) when comparing platinum-based regimens with ifosfamide-based regimens in patients with metastatic or non-metastatic NUT carcinoma[9,59]
- No difference in progression-free survival in metastatic NUT carcinoma when comparing platinum-based with ifosfamide-based regimens[9]
- Initial treatment of adults with non-metastatic NUT carcinoma with ifosfamide-based regimens might improve progression-free survival but not OS[9,59]
- Initial treatment of paediatric patients with non-metastatic NUT carcinoma with ifosfamide-based regimens might improve OS in some patients
- Several case reports have described durable complete responses using Ewing SSG IX regimen[43,115,116] in paediatric patients with non-metastatic NUT carcinoma
- Role of immune-checkpoint inhibitors is not established, but case reports suggest this approach might benefit some patients[59]

Approaches under investigation

- Monotherapy using agents directly targeting BRD4–NUTM1 (that is, bromodomain and extraterminal domain (BET) inhibitors) showed modest activity
- Clinical experience indicates that monotherapy with BET inhibitors has limited efficacy, probably owing to a narrow therapeutic window[127–132]
- Combination of BET inhibitors with other targeted or chemotherapeutic agents (for example, EZH2 or CDK4/6 inhibitors) will improve efficacy by co-targeting other NUT carcinoma dependencies[6,103]
- Histone deacetylase inhibitors, alone and combined with chemotherapy, have demonstrated activity in patients with NUT carcinoma[171,172]
- NUT carcinoma might be a paradigm that can aid in identification of targets for therapy in conventional SCC

rather than the unique molecular biology of this malignancy seems counterintuitive, small-molecule inhibitors that are being evaluated for the treatment of NUT carcinoma are in early clinical development (phase I or II trials), and therefore the trials are not designed to assess these inhibitors as first-line treatments. Consistent use of SCC treatment regimens in the context of existing, rational medical oncology diagnostic and treatment frameworks for thoracic and head and neck cancers will generate greater recognition, familiarity and consistency in treatment of NUT carcinomas, which will facilitate meaningful analysis of outcomes data to identify the most effective regimens. This important starting point of situating NUT carcinoma within a highly active, robust drug development oncology community is a key initial step to further refine and optimize treatments for this cancer.

Novel treatment approaches under investigation. Preclinical studies in vivo and in vitro have identified molecular vulnerabilities of NUT carcinoma, beginning with the BET oncoproteins encoded by NUT carcinoma fusions. NUT carcinoma is considered the index cancer for BET inhibition (Box 1); BET inhibitors competitively inhibit binding of BET protein bromodomains to chromatin[104]. As all *NUTM1* fusion partners encode proteins that normally bind to BRD4 and are core members of the BRD4–NUTM1 complex, BET inhibitors target NUT carcinoma fusions of all types, including BRD4–NUTM1, BRD3–NUTM1, NSD3–NUTM1, BRD2–NUTM1, ZNF532–NUTM1 and ZNF592–NUTM1 (refs. 14,15,104).

Subsequent studies revealed that BRD–NUTM1 does not act alone, but cooperates with the repression by EZH2 of TSGs that block the RB1–CDK2/CDK4/CDK6–cyclin D1 axis, which further promotes cell-cycle progression[6,103] (Fig. 1c). Indeed, co-inhibition of EZH2 and BRD–NUTM1, or of CDK4/CDK6 and BRD–NUTM1, is highly synergistic in inhibiting tumour growth, even causing tumour regression[103] and durable complete responses in mice[6]. In addition, p300 recruitment by NUT is essential for super-enhancer formation by BRD–NUTM1; co-inhibition of p300 and BRD–NUTM1 is also synergistic at inhibiting tumour growth, although less so than the aforementioned inhibitor combinations[105,106,126].

These preclinical studies provided the rationale for clinical investigation of small-molecule inhibitors in patients with NUT carcinoma. The first-in-human trials of BET inhibitor monotherapy were performed in patients with NUT carcinoma and demonstrated on-target activity of four different BET inhibitors, although with only modest clinical benefit (objective response rate of 21–33%, progression-free survival <3 months for most patients) owing, in part, to adverse effects such as thrombocytopenia, leading to dose interruptions and reductions[127–132].

On the basis of these results, as well as preclinical data indicating that BET inhibition alone does not fully address NUT carcinoma biology and/or has a therapeutic window that is too narrow, new trials using rational combinations with BET inhibitors have emerged, including BET inhibitors with the CDK4/6 inhibitor abemaciclib (NCT05372640) or with chemotherapy (etoposide and cisplatin; NCT05019716). Another promising strategy that is superior to BET inhibition alone in preclinical models is the use of a dual bromodomain inhibitor that targets the bromodomains of BET proteins and of p300 (NEO2734/EP316740; NCT05488548)[105]. If tolerated, these combinations might have improved activity over BET inhibitor monotherapy, paving the way for further clinical development.

It is important to understand that epigenetic mechanisms are fundamental not only in NUT carcinoma, but also in non-NUT carcinoma SCCs[62–64]. However, at least in part because NUT carcinomas are not classified as SCCs, SCCs have typically not been included in clinical trials using BET inhibitors, despite the presence of epigenetic alterations and preclinical evidence suggesting that BET inhibitors might provide a tractable treatment strategy[133–137]. Consequently, the relative oncogenic role and clinical therapeutic targeting of BET proteins remain under-investigated in SCC, thus re-enforcing its separate classification from NUT carcinoma.

Apart from novel small-molecule inhibitors and combination approaches, published case reports suggest that occasionally patients with NUT carcinoma, such as those with conventional SCC, benefit from ICI-based treatment (as discussed above). Another modality is the oncolytic virus talimogene laherparepvec (T-VEC), which can lyse tumour cells while stimulating an immune response to newly presented tumour-associated antigens[138]. NUT carcinoma cells are sensitive to T-VEC infection and lysis[138]. Combining T-VEC with chemotherapy and pembrolizumab resulted in a durable partial response in a patient with NUT carcinoma[139], and experimental evidence indicates that T-VEC can combine favourably with small-molecule inhibitors in killing NUT carcinoma cells[140].

Given the overlap between key oncogenic components of NUT carcinoma and SCC, progress in understanding their role in NUT carcinoma will advance the biological understanding of conventional SCCs. Furthermore, targeted vulnerabilities in NUT carcinoma will probably also be applicable to treatment of conventional SCCs. Reclassification of NUT carcinoma as an SCC will facilitate further investigation of these possibilities.

The case for and against reclassifying NUT carcinoma

Although differences exist between NUT carcinoma and conventional SCC, reclassifying NUT carcinoma as an SCC has clear advantages in light of emerging data regarding the biology and natural history of NUT carcinoma. Here, we summarize the arguments for and against reclassifying NUT carcinoma as an SCC (Table 1).

Biology

The many biological disparities between NUT carcinoma and conventional SCC include differences in aetiology, molecular drivers, pathogenetic mechanism and epigenetic profile; however, every biological difference arguing against reclassifying NUT carcinoma can be countered by compelling evidence in favour of classifying it as an SCC (Table 1). Although the pathogenesis of conventional SCC is driven by mutation-induced oncogenic drivers or virus-induced inactivation of tumour-suppressor proteins, these same oncogenic drivers and TSGs are activated and deactivated, respectively, in NUT carcinoma by epigenetic mechanisms — an example of convergent evolution. Even the aetiology of conventional SCCs is not consistently environmental (that is, tobacco exposure or viral infection); a subset of confirmed squamous NSCLCs and HNSCCs, similarly to NUT carcinoma, are driven by fusion oncogenes (for example, *FGFR3–TACC3* (refs. 141,142) and *DEK–AFF2* (ref. 143)). Incidentally, this fact argues in favour of an additional subcategory of SCCs characterized by oncogene drivers. Finally, three characteristics of NUT carcinoma provide powerful evidence that this entity is biologically an SCC (Table 1), namely origin from squamous epithelium in mouse models, transcriptomic co-classification with HNSCC and squamous NSCLC, and experimental induction of squamous differentiation.

Some features of NUT carcinoma, including young age at presentation and poorly differentiated tumour morphology and aggressive behaviour, recall features of embryonal-type, undifferentiated tumours, such as Ewing sarcoma, neuroblastoma or germ cell tumours. However, experimental findings from GEMMs, transcriptomics and epigenetic studies

demonstrate that BRD4–NUTM1 induces epigenetic blockade of differentiation of epithelial precursor cells that mimics the mutagen-driven molecular oncogenic pathways driving conventional SCC. In fact, the transcriptomics-based clustering of NUT carcinomas with SCCs of the lungs and head and neck, and not with blastomas of childhood, argues strongly against NUT carcinomas being embryonal tumours.

One or two features alone are not sufficient rationale for reclassification of NUT carcinoma as an SCC or for retaining its status as a standalone entity. An existing example is HPV-associated HNSCC, which is morphologically different and less aggressive clinically than tobacco-associated SCC, and is not mutationally driven like tobacco-associated SCC, yet remains a subtype of SCC. We believe that the sum of evidence, including anatomical, histological, immunohistochemical, molecular, transcriptomic, epigenetic and cell of origin, supports NUT carcinoma as being biologically an SCC.

Pathology

Morphologically and immunohistochemically, NUT carcinoma falls within the poorly differentiated SCC spectrum, although with some differences, including monomorphism and focal, 'abrupt' keratinization[80,144] (Fig. 2). The pleomorphism that can occur in tobacco-related SCC is distinctly lacking in NUT carcinoma, which might be related to the absence of genomic instability that is characteristic of tobacco-related SCC and is linked to important aetiological and biological differences. However, these differences are also seen between HPV-related and tobacco-related SCCs. In fact, the characteristic basaloid morphology[145] and foci of abrupt keratinization[146] of HPV-associated SCC is quite similar to the morphology often observed in NUT carcinoma of the head and neck. In summary, although histopathological differences exist between NUT carcinoma and tobacco-associated SCC, when HPV-associated SCC is considered, NUT carcinoma is within the morphological spectrum of poorly differentiated SCC.

Classification as a site-specific SCC

The majority (92%) of NUT carcinomas occur in thoracic and head and neck sites[8]. Among thoracic primary tumours, the majority are probably of pulmonary origin and an unknown but probably small proportion arise in the thymus[147,148]. Although the uncommon occurrence of NUT carcinoma outside of the lungs and head and neck raises the question of whether classifying NUT carcinoma as an HNSCC and squamous NSCLC is too restrictive, it is important to recognize that this rare origin outside of the lungs and head and neck does not preclude classification as a NUT carcinoma arising in other sites. This classification is not unlike that for existing cancers that rarely occur at another site. For example, malignant pleural mesothelioma can also infrequently arise in the peritoneum, pericardium and testis. In the same way, NUT carcinoma should be classified as a subtype of HNSCC and squamous NSCLC when it occurs in these sites, but when it occurs elsewhere, should be categorized as a NUT carcinoma of the other site (for example, NUT carcinoma of the kidney or thyroid).

Treatment

The presentation of NUT carcinoma is similar in many important ways to squamous NSCLC and HNSCC, including clinical presentation, anatomical site of origin and organotropism, but differs in two important aspects — aggressive behaviour and occurrence in patients with a younger median age (23.6 years[8] versus 53–73 years[149]) (Table 3). A potential risk of classifying NUT carcinoma as a subtype of SCC is that it might not be diagnosed and treated with the needed urgency. However, the increased awareness of NUT carcinoma that would result from formal recognition by the lung and head and neck cancer community, a global multidisciplinary community familiar with fusion-driven cancers with distinct treatment paradigms (for example, *ALK*, *ROS1*, *RET* and *NTRK* fusions), will probably hasten rather than delay diagnosis and thus treatment. With improved rapid diagnosis, a greater number of patients with NUT carcinoma will be well enough to enrol in investigational trials of treatments targeting BRD4–NUTM1 or of other novel strategies. The knowledge that conventional treatment of adult NUT carcinoma using SCC-type chemotherapy is more appropriate and better tolerated than Ewing sarcoma-like regimens will increase the confidence of physicians managing NUT carcinoma and improve the diagnosis-to-treatment lag time.

A lingering concern relates to a lack of sufficient evidence that SCC-type treatment regimens used in adults with NUT carcinoma is appropriate for paediatric NUT carcinoma. At least four case reports describe long-term complete responses or cures with the ifosfamide-based, Ewing SSG IX regimen in paediatric patients with localized NUT carcinoma[43,115,116]. Such remarkable responses have not been described using SCC regimens in this patient population, suggesting that treatment of paediatric patients with non-metastatic NUT carcinoma needs further consideration. A potential concern is that classifying NUT carcinoma as a subtype of SCC in this group might lead oncologists to treat paediatric NUT carcinoma with squamous chemotherapy regimens. We argue that any treatment differences in this population are likely to reflect host differences and not differences in the underlying disease itself. Specifically, paediatric patients without other comorbidities might tolerate more intensive regimens such as the SSG IX regimen that an older patients might not tolerate so well. Thus, rather than considering paediatric NUT carcinoma as a standalone entity, paediatric NUT carcinoma should be recognized as an SCC.

Conclusions

NUT carcinoma is currently classified as a poorly differentiated carcinoma with *NUTM1* rearrangement. Despite arising predominantly in the lungs and head and neck, various factors have contributed to NUT carcinoma not being classified as a NSCLC or HNSCC. However, multiple lines of evidence support the idea that NUT carcinoma is an SCC.

The current classification of NUT carcinoma as a separate entity rather than a subset of SCC has led to varied and inconsistent treatment approaches, highlighting the need for reclassification to optimize and standardize therapeutic strategies. Furthermore, this classification contributes to a lack of awareness among oncologists about NUT carcinoma. This knowledge gap results in insufficient diagnostic testing, compromising care for patients who might miss the opportunity to participate in investigational trials from which they can potentially benefit.

Furthermore, the current classification represents a missed opportunity for advancing research and developing new therapeutic approaches. NUT carcinomas have been shown to respond to BET bromodomain targeted therapy in clinical trials and combining EZH2 inhibition or CDK4/6 inhibition with BET inhibitors leads to profound synergistic growth inhibition of NUT carcinoma in preclinical studies. Given that MYC, EZH2 and CDK4/6 are important drivers of squamous cancer, therapeutic progress in inhibiting these factors in NUT carcinoma can possibly have broader applicability to conventional SCCs. For this reason, it is crucial that NUT carcinoma is correctly diagnosed to facilitate enrolment of patients in the several ongoing clinical trials. Conversely, if NUT carcinoma is reclassified as an SCC, newly diagnosed patients with NUT carcinoma might benefit from a standard-of-care approach for conventional SCC.

On the basis of the findings discussed herein, we propose consideration of the following changes to the WHO classification of lung and head and neck tumours. We propose to make the following changes to the sixth edition of *Thoracic Tumours* based on the current fifth edition[10]. Within the chapter entitled, "Tumours of the Lung", we suggest that the "NUT carcinoma of the lung"[18] subsection of "Other Epithelial Tumours" be removed. Instead, we propose the inclusion of another, new subsection entitled "NUT carcinoma", added to the "Squamous cell carcinomas" section, alongside the subsections "Squamous cell carcinoma" and "Lymphoepithelial carcinoma". The NUT carcinoma subsection under "Thymic carcinoma"[10] can remain or be subsumed as a subcategory of "Squamous cell carcinoma" within "Thymic carcinoma". Furthermore, in the next edition of the *Classification of Head and Neck Tumours* (sixth edition) the following changes could be made compared with the fifth edition[38]: within the chapter entitled, "Nasal, paranasal and skull-based tumours", we propose adding "NUT carcinoma" as a subsection to "Non-keratinizing squamous cell carcinoma" within the "Carcinomas" section. Importantly, around 10% of NUT carcinomas arise in sites outside of the thorax and head and neck, so we propose that NUT carcinomas arising in these sites (for example, kidney, skin, or thyroid), remain within a standalone subcategory of "NUT carcinoma" in their respective sections in the WHO classification of tumours. The current fifth edition of *WHO Paediatric Tumours* only lists NUT carcinoma, next to "nasopharyngeal carcinoma", within the "Head and Neck Tumours" chapter in a section entitled, "Malignant tumours"[73]. We suggest that a new subsection called "Squamous cell carcinoma" be created within "Malignant tumours", under which "nasopharyngeal carcinoma" and "NUT carcinoma" can be listed. NUT carcinoma is not listed as a subtype in the "Thoracic tumours" chapter. We propose that it be added as a subsection of the "Lung tumours" section. Given that squamous cell carcinoma is not currently listed as a lung tumour subtype of paediatric tumours, it may be premature to create this as a new subsection if NUT carcinoma is the only type of squamous cancer listed. Instead, NUT carcinoma should be described as an SCC in the text of this new subsection.

Published online: 03 February 2025

References

1. Yuan, M., Huang, L. L., Chen, J. H., Wu, J. & Xu, Q. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. *Signal. Transduct. Target. Ther.* **4**, 61 (2019).
2. Makarem, M. & Janne, P. A. Top advances of the year: targeted therapy for lung cancer. *Cancer* **130**, 3239–3250 (2024).
3. Dotto, G. P. & Rustgi, A. K. Squamous cell cancers: a unified perspective on biology and genetics. *Cancer Cell* **29**, 622–637 (2016).
4. Durall, R. T. et al. The BRD4–NUT fusion alone drives malignant transformation of NUT carcinoma. *Cancer Res.* **83**, 3846–3860 (2023).
5. Grayson, A. R. et al. MYC, a downstream target of BRD–NUT, is necessary and sufficient for the blockade of differentiation in NUT midline carcinoma. *Oncogene* **33**, 1736–1742 (2014).
6. Huang, Y. et al. EZH2 cooperates with BRD4–NUT to drive NUT carcinoma growth by silencing key tumor suppressor genes. *Cancer Res.* **83**, 3956–3973 (2023).
7. Wang, R. et al. Activation of SOX2 expression by BRD4–NUT oncogenic fusion drives neoplastic transformation in NUT midline carcinoma. *Cancer Res.* **74**, 3332–3343 (2014).
8. Chau, N. G. et al. An anatomical site and genetic based prognostic model for patients with NUT midline carcinoma: analysis of 124 patients. *JNCI Cancer Spectr.* **4**, pkz094 (2019).
9. Luo, J. et al. Initial chemotherapy for locally advanced and metastatic NUT carcinoma. *J. Thorac. Oncol.* **19**, 829–838 (2024).
10. French, C. A., Badve, S., den Bakker, M. A. & Jain, D. in *WHO Classification of Tumours: Thoracic Tumours* 5th edn (eds Chan J. K. C. et al.) 364–367 (International Agency for Research on Cancer, 2021).
11. French, C. A. et al. BRD4–NUT fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer Res.* **63**, 304–307 (2003).
12. French, C. A. et al. BRD–NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. *Oncogene* **27**, 2237–2242 (2008).
13. Wu, S. J. et al. Novel BRD2::NUTM1 fusion in NUT carcinoma with exceptional response to chemotherapy: a case report. *JTO Clin. Res. Rep.* **5**, 100625 (2024).
14. French, C. A. et al. NSD3–NUT fusion oncoprotein in NUT midline carcinoma: implications for a novel oncogenic mechanism. *Cancer Discov.* **4**, 928–941 (2014).
15. Alekseyenko, A. A. et al. Ectopic protein interactions within BRD4–chromatin complexes drive oncogenic megadomain formation in NUT midline carcinoma. *Proc. Natl Acad. Sci. USA* **114**, E4184–E92 (2017).
16. Shiota, H. et al. "Z4" complex member fusions in NUT carcinoma: implications for a novel oncogenic mechanism. *Mol. Cancer Res.* **16**, 1826–1833 (2018).
17. Agaimy, A. et al. Misleading germ cell phenotype in pulmonary NUT carcinoma harboring the ZNF532–NUTM1 fusion. *Am. J. Surg. Pathol.* **46**, 281–288 (2022).
18. French, C. A., Badve, S., den Bakker, M. A. & Jain, D. in *WHO Classification of Tumours: Thoracic Tumours* 5th edn (eds Borczuk, A. C. et al.) (International Agency for Research on Cancer, 2021).
19. Dickson, B. C. et al. NUTM1 gene fusions characterize a subset of undifferentiated soft tissue and visceral tumors. *Am. J. Surg. Pathol.* **42**, 636–645 (2018).
20. Stevens, T. M. et al. NUTM1-rearranged neoplasia: a multi-institution experience yields novel fusion partners and expands the histologic spectrum. *Mod. Pathol.* **32**, 764–773 (2019).
21. Van Treeck, B. J. et al. NUTM1-rearranged colorectal sarcoma: a clinicopathologically and genetically distinctive malignant neoplasm with a poor prognosis. *Mod Pathol.* **34**, 1547–1557 (2021).
22. Sekine, S. et al. Recurrent YAP1–MAML2 and YAP1–NUTM1 fusions in poroma and porocarcinoma. *J. Clin. Invest.* **129**, 3827–3832 (2019).
23. Xu, B. et al. NUTM1-fusion positive malignant neoplasms of the genitourinary tract: a report of six cases highlighting involvement of unusual anatomic locations and histologic heterogeneity. *Genes. Chromosomes Cancer* **61**, 542–550 (2022).
24. Luo, W. et al. NUTM1-rearranged neoplasms-A heterogeneous group of primitive tumors with expanding spectrum of histology and molecular alterations—an updated review. *Curr. Oncol.* **28**, 4485–4503 (2021).
25. Zheng, D. et al. Brd4::Nutm1 fusion gene initiates NUT carcinoma in vivo. *Life Sci. Alliance* **7**, e202402602 (2024).
26. Haack, H. et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. *Am. J. Surg. Pathol.* **33**, 984–991 (2009).
27. Kroening G. et al. Multiomic characterization and molecular profiling of nuclear protein in testis carcinoma. *JCO Precis. Oncol.* **8**, e2400334 (2024).
28. Okamura, R. et al. Analysis of NTRK alterations in pan-cancer adult and pediatric malignancies: implications for NTRK-targeted therapeutics. *JCO Precis. Oncol.* https://doi.org/10.1200/PO.18.00183 (2018).
29. Takeuchi, K. et al. RET, ROS1 and ALK fusions in lung cancer. *Nat. Med.* **18**, 378–381 (2012).
30. Lassche, G. et al. Identification of fusion genes and targets for genetically matched therapies in a large cohort of salivary gland cancer patients. *Cancers* **14**, 4156 (2022).
31. French, C. A. et al. Midline carcinoma of children and young adults with NUT rearrangement. *J. Clin. Oncol.* **22**, 4135–4139 (2004).
32. Lee, A. C. et al. Disseminated mediastinal carcinoma with chromosomal translocation (15;19). A distinctive clinicopathologic syndrome. *Cancer* **72**, 2273–2276 (1993).
33. Kubonishi, I. et al. Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. *Cancer Res.* **51**, 3327–3328 (1991).
34. Kees, U. R., Mulcahy, M. T. & Willoughby, M. L. Intrathoracic carcinoma in an 11-year-old girl showing a translocation t(15;19). *Am. J. Pediatr. Hematol. Oncol.* **13**, 459–464 (1991).
35. Travis, W. D. in *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart* 185–186 (International Agency for Research on Cancer, 2004).
36. French, C. A. & den Bakker, M. A. in *WHO Classification of Head and Neck Tumours* 4th edn (eds Slootweg, P. J. et al.) 229–231 (International Agency for Research on Cancer, 2015).
37. Marx, A. et al. The 2021 WHO classification of tumors of the thymus and mediastinum: what is new in thymic epithelial, germ cell, and mesenchymal tumors? *J. Thorac. Oncol.* **17**, 200–213 (2022).
38. French, C. A., Stelow, E. B. & Hiroshi, M. in *WHO Classification of Tumours: Head and Neck Tumours Part A* 5th edn (eds Bishop, J. A. et al.) 65–67 (International Agency for Research on Cancer, 2024).
39. Lee, J. K. et al. Complex chromosomal rearrangements by single catastrophic pathogenesis in NUT midline carcinoma. *Ann. Oncol.* **28**, 890–897 (2017).
40. Bauer, D. E. et al. Clinicopathologic features and long-term outcomes of NUT midline carcinoma. *Clin. Cancer Res.* **18**, 5773–5779 (2012).
41. Viswanathan, K. et al. The histological spectrum and immunoprofile of head and neck NUT carcinoma: a multicentre series of 30 cases. *Histopathology* **85**, 317–326 (2024).
42. Farooq, A., Kerper, A. L., Boland, J. M. & Lo, Y. C. Nuclear protein in testis (NUT) carcinoma: a comprehensive immunohistochemical analysis of 57 cases with consideration of interpretation and pitfall recognition. *Arch. Pathol. Lab. Med.* **148**, 898–904 (2023).
43. Mertens, F., Wiebe, T., Adlercreutz, C., Mandahl, N. & French, C. A. Successful treatment of a child with t(15;19)-positive tumor. *Pediatr. Blood Cancer* **49**, 1015–1017 (2007).
44. Li, W. & Chastain, K. NUT midline carcinoma with leukemic presentation mimicking CD34-positive acute leukemia. *Blood* **132**, 456 (2018).
45. Numakura, S. et al. P63-negative pulmonary NUT carcinoma arising in the elderly: a case report. *Diagn. Pathol.* **15**, 134 (2020).
46. Luo, J. et al. Presenting features and diagnostic delays of NUT carcinoma: a report from the NUT carcinoma registry. *J. Thorac. Oncol.* **19**, S71–S72 (2024).

47. Hormann, F. M. et al. 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**, e455–e459 (2019).
48. McEvoy, C. R., Fox, S. B. & Prall, O. W. J. Emerging entities in NUTM1-rearranged neoplasms. *Genes Chromosomes Cancer* **59**, 375–385 (2020).
49. Li, J. et al. Emerging molecular subtypes and therapeutic targets in B-cell precursor acute lymphoblastic leukemia. *Front. Med.* **15**, 347–371 (2021).
50. French, C. A. et al. BRD4 bromodomain gene rearrangement in aggressive carcinoma with translocation t(15;19). *Am. J. Pathol.* **159**, 1987–1992 (2001).
51. Rahman, S. et al. The Brd4 extraterminal domain confers transcription activation independent of pTEFb by recruiting multiple proteins, including NSD3. *Mol. Cell Biol.* **31**, 2641–2652 (2011).
52. Gilan, O. et al. Functional interdependence of BRD4 and DOT1L in MLL leukemia. *Nat. Struct. Mol. Biol.* **23**, 673–681 (2016).
53. Yokoyama, A. Molecular mechanisms of MLL-associated leukemia. *Int. J. Hematol.* **101**, 352–361 (2015).
54. Alekseyenko, A. A. et al. The oncogenic BRD4–NUT chromatin regulator drives aberrant transcription within large topological domains. *Genes. Dev.* **29**, 1507–1523 (2015).
55. Hammerman, P. S., Hayes, D. N. & Grandis, J. R. Therapeutic insights from genomic studies of head and neck squamous cell carcinomas. *Cancer Discov.* **5**, 239–244 (2015).
56. Polo, V. et al. Squamous cell carcinomas of the lung and of the head and neck: new insights on molecular characterization. *Oncotarget* **7**, 25050–25063 (2016).
57. Sands, J. M. et al. Next-generation sequencing informs diagnosis and identifies unexpected therapeutic targets in lung squamous cell carcinomas. *Lung Cancer* **140**, 35–41 (2020).
58. Hai, J. Next generation mouse models of squamous cell lung cancer for translational immuno-oncology. *Oncotarget* **11**, 4463–4464 (2020).
59. Kloker, L. D. et al. Clinical management of NUT carcinoma (NC) in Germany: analysis of survival, therapy response, tumor markers and tumor genome sequencing in 35 adult patients. *Lung Cancer* **189**, 107496 (2024).
60. Koh, C. M. et al. Myc enforces overexpression of EZH2 in early prostatic neoplasia via transcriptional and post-transcriptional mechanisms. *Oncotarget* **2**, 669–683 (2011).
61. Wu, X. et al. BRD4 regulates EZH2 transcription through upregulation of C-MYC and represents a novel therapeutic target in bladder cancer. *Mol. Cancer Ther.* **15**, 1029–1042 (2016).
62. Balinth, S. et al. EZH2 regulates a SETDB1/DeltaNp63alpha axis via RUNX3 to drive a cancer stem cell phenotype in squamous cell carcinoma. *Oncogene* **41**, 4130–4144 (2022).
63. Xu, M., Hou, Y., Li, N., Yu, W. & Chen, L. Targeting histone deacetylases in head and neck squamous cell carcinoma: molecular mechanisms and therapeutic targets. *J. Transl. Med.* **22**, 418 (2024).
64. Yuan, G. et al. Elevated NSD3 histone methylation activity drives squamous cell lung cancer. *Nature* **590**, 504–508 (2021).
65. Tamura, R. et al. Novel MXD4–NUTM1 fusion transcript identified in primary ovarian undifferentiated small round cell sarcoma. *Genes Chromosomes Cancer* **57**, 557–563 (2018).
66. Diolaiti, D. et al. A recurrent novel MGA–NUTM1 fusion identifies a new subtype of high-grade spindle cell sarcoma. *Cold Spring Harb. Mol. Case Stud.* **4**, a003194 (2018).
67. Le Loarer, F. et al. Clinicopathologic features of CIC–NUTM1 sarcomas, a new molecular variant of the family of CIC-fused sarcomas. *Am. J. Surg. Pathol.* **43**, 268–276 (2019).
68. Barletta, J. A. et al. NUTM1-rearranged carcinoma of the thyroid: a distinct subset of NUT carcinoma characterized by frequent NSD3–NUTM1 fusions. *Am. J. Surg. Pathol.* **46**, 1706–1715 (2022).
69. Allison, D. B. et al. Thyroid carcinoma with NSD3::NUTM1 fusion: a case with thyrocyte differentiation and colloid production. *Endocr. Pathol.* **33**, 315–326 (2022).
70. den Bakker, M. A. et al. NUT midline carcinoma of the parotid gland with mesenchymal differentiation. *Am. J. Surg. Pathol.* **33**, 1253–1258 (2009).
71. Agaimy, A. et al. 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**, 877–884 (2018).
72. Shehata, B. M. et al. NUT midline carcinoma in a newborn with multiorgan disseminated tumor and a 2-year-old with a pancreatic/hepatic primary. *Pediatr. Dev. Pathol.* **13**, 481–485 (2010).
73. Bishop, J. A., Stelow, E. & French, C. A. in *WHO Classification of Tumours: Paediatric Tumours* 5th edn (ed. Thompson, L. D. R.) 908–909 (International Agency for Research on Cancer, 2022).
74. Iqbal, A. et al. Prognostic factors and survival outcomes in squamous cell carcinoma of the thyroid: a surveillance, epidemiology, and end results (SEER) database analysis. *Cureus* **16**, e63326 (2024).
75. Gluck, G. et al. Comparative study of conventional urothelial carcinoma, squamous differentiation carcinoma and pure squamous carcinoma in patients with invasive bladder tumors. *J. Med. Life* **7**, 211–214 (2014).
76. Ford, J. A., Bhatt, A., Kim, R. C., Larkins, M. & Burke, A. M. Primary squamous cell carcinoma of the pancreas: an update on a rare neoplasm from the SEER database. *Front. Oncol.* **13**, 1272740 (2023).
77. Liang, K., Yuan, Y., Lv, B. & Ke, Z. Primary squamous cell carcinoma of renal parenchyma: a case report and literature review. *Front. Oncol.* **13**, 1037156 (2023).
78. Sholl, L. M. et al. Primary pulmonary NUT midline carcinoma: clinical, radiographic, and pathologic characterizations. *J. Thorac. Oncol.* **10**, 951–959 (2015).
79. Ferone, G. et al. SOX2 is the determining oncogenic switch in promoting lung squamous cell carcinoma from different cells of origin. *Cancer Cell* **30**, 519–532 (2016).
80. French, C. A. & den Bakker, M. A. in *WHO Classification of Head and Neck Tumours* (eds El-Naggar et al.) 20–21 (International Agency for Research on Cancer, 2017).
81. Matsuda, K., Kashima, J. & Yatabe, Y. The isoform matters in NUT carcinoma: a diagnostic pitfall of p40 immunohistochemistry. *J. Thorac. Oncol.* **15**, e176–e178 (2020).
82. Tilson, M. P. & Bishop, J. A. Utility of p40 in the differential diagnosis of small round blue cell tumors of the sinonasal tract. *Head. Neck Pathol.* **8**, 141–145 (2014).
83. Zhuang, X. P. et al. Primary pulmonary NUT carcinoma: a clinicopathological analysis of seven cases. *Zhonghua Bing. Li Xue Za Zhi* **52**, 1244–1248 (2023).
84. Comitani, F. et al. Diagnostic classification of childhood cancer using multiscale transcriptomics. *Nat. Med.* **29**, 656–666 (2023).
85. Ruiz, E. J. et al. USP28 deletion and small-molecule inhibition destabilizes c-MYC and elicits regression of squamous cell lung carcinoma. *eLife* **10**, e71596 (2021).
86. Boumahdi, S. et al. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* **511**, 246–250 (2014).
87. Rocco, J. W., Leong, C. O., Kuperwasser, N., DeYoung, M. P. & Ellisen, L. W. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. *Cancer Cell* **9**, 45–56 (2006).
88. Martin-Padron, J. et al. Plakophilin 1 enhances MYC translation, promoting squamous cell lung cancer. *Oncogene* **39**, 5479–5493 (2020).
89. Justilien, V. et al. The PRKCI and SOX2 oncogenes are coamplified and cooperate to activate Hedgehog signaling in lung squamous cell carcinoma. *Cancer Cell* **25**, 139–151 (2014).
90. Akervall, J. et al. The gene ratios c-MYC:cyclin-dependent kinase (CDK)N2A and CCND1:CDKN2A correlate with poor prognosis in squamous cell carcinoma of the head and neck. *Clin. Cancer Res.* **9**, 1750–1755 (2003).
91. Pickering, C. R. et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov.* **3**, 770–781 (2013).
92. Saladi, S. V. et al. ACTL6A is co-amplified with p63 in squamous cell carcinoma to drive YAP activation, regenerative proliferation, and poor prognosis. *Cancer Cell* **31**, 35–49 (2017).
93. Bass, A. J. et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat. Genet.* **41**, 1238–1242 (2009).
94. Brennan, J. A. et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* **332**, 712–717 (1995).
95. Werness, B. A., Levine, A. J. & Howley, P. M. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* **248**, 76–79 (1990).
96. Reynoird, N. et al. Oncogenesis by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chromatin domains. *EMBO J.* **29**, 2943–2952 (2010).
97. Ezhkova, E. et al. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell* **136**, 1122–1135 (2009).
98. Behrens, C. et al. EZH2 protein expression associates with the early pathogenesis, tumor progression, and prognosis of non-small cell lung carcinoma. *Clin. Cancer Res.* **19**, 6556–6565 (2013).
99. Xie, Q. et al. Increased expression of enhancer of Zeste Homolog 2 (EZH2) differentiates squamous cell carcinoma from normal skin and actinic keratosis. *Eur. J. Dermatol.* **24**, 41–45 (2014).
100. Temam, S. et al. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J. Clin. Oncol.* **25**, 2164–2170 (2007).
101. Redon, R. et al. A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. *Cancer Res.* **61**, 4122–4129 (2001).
102. Weiss, J. et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci. Transl. Med.* **2**, 62ra93 (2010).
103. Liao, S., Maertens, O., Cichowski, K. & Elledge, S. J. Genetic modifiers of the BRD4-NUT dependency of NUT midline carcinoma uncovers a synergism between BETis and CDK4/6is. *Genes. Dev.* **32**, 1188–1200 (2018).
104. Filippakopoulos, P. et al. Selective inhibition of BET bromodomains. *Nature* **468**, 1067–1073 (2010).
105. Morrison-Smith, C. D. et al. Combined targeting of the BRD4–NUT–p300 axis in NUT midline carcinoma by dual selective bromodomain inhibitor, NEO2734. *Mol. Cancer Ther.* **19**, 1406–1414 (2020).
106. Zhang, X. et al. Therapeutic targeting of p300/CBP HAT domain for the treatment of NUT midline carcinoma. *Oncogene* **39**, 4770–4779 (2020).
107. Kim, M. et al. Regulation of mouse embryonic stem cell neural differentiation by retinoic acid. *Dev. Biol.* **328**, 456–471 (2009).
108. Guo, X., Stice, S. L., Boyd, N. L. & Chen, S. Y. A novel in vitro model system for smooth muscle differentiation from human embryonic stem cell-derived mesenchymal cells. *Am. J. Physiol. Cell Physiol.* **304**, C289–C298 (2013).
109. Niederkohr, R. D., Cameron, M. J. & French, C. A. FDG PET/CT imaging of NUT midline carcinoma. *Clin. Nucl. Med.* **36**, e124–e126 (2011).
110. Bishop, J. A. & Westra, W. H. NUT midline carcinomas of the sinonasal tract. *Am. J. Surg. Pathol.* **36**, 1216–1221 (2012).
111. Thompson, L. D. Small round blue cell tumors of the sinonasal tract: a differential diagnosis approach. *Mod. Pathol.* **30**, S1–S26 (2017).

112. Stelow, E. B. et al. NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. *Am. J. Surg. Pathol.* **32**, 828–834 (2008).
113. Pan, M. & Chang, J. S. Durable complete remission of PD-L1 positive NUT carcinoma treated with concurrent chemotherapy and radiation. *Perm. J.* **25**, 1–3 (2020).
114. Ueki, H. et al. A case of NUT midline carcinoma with complete response to gemcitabine following cisplatin and docetaxel. *J. Pediatr. Hematol. Oncol.* **36**, e476–e480 (2014).
115. Leeman, R. et al. NUT carcinoma without upfront surgical resection: a case report. *J. Pediatr. Hematol. Oncol.* **43**, e707–e710 (2020).
116. Storck, S. et al. Pediatric NUT–midline carcinoma: therapeutic success employing a sarcoma based multimodal approach. *Pediatr. Hematol. Oncol.* **34**, 231–237 (2017).
117. Murano, C. et al. Vimentin-positive and alpha-fetoprotein-elevated nuclear protein of the testis midline carcinoma: a case report and review of the literature. *Intern. Med.* **60**, 3645–3649 (2021).
118. Parikh, S. A. et al. NUT midline carcinoma: an aggressive intrathoracic neoplasm. *J. Thorac. Oncol.* **8**, 1335–1338 (2013).
119. Gupta, R. et al. NUT midline lung cancer: a rare case report with literature review. *AME Case Rep.* **6**, 2 (2022).
120. Fossella, F. V. et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. *J. Clin. Oncol.* **18**, 2354–2362 (2000).
121. Klingberg, D. et al. Association of chemotherapy dose intensity and age with outcomes in patients with Ewing's family sarcoma. *Asia Pac. J. Clin. Oncol.* **21**, 87–94 (2023).
122. Davis, A., Mahar, A., Wong, K., Barnet, M. & Kao, S. Prolonged disease control on nivolumab for primary pulmonary NUT carcinoma. *Clin. Lung Cancer* **22**, e665–e667 (2021).
123. Riess, J. W. et al. Genomic profiling of solid tumors harboring BRD4–NUT and response to immune checkpoint inhibitors. *Transl. Oncol.* **14**, 101184 (2021).
124. Jung, M. et al. Clinicopathological and preclinical findings of NUT carcinoma: a multicenter study. *Oncologist* **24**, e740–e748 (2019).
125. Xie, X. H. et al. Clinical features, treatment, and survival outcome of primary pulmonary NUT midline carcinoma. *Orphanet J. Rare Dis.* **15**, 183 (2020).
126. Tontsch-Grunt, U. et al. Therapeutic impact of BET inhibitor BI 894999 treatment: backtranslation from the clinic. *Br. J. Cancer* **127**, 577–586 (2022).
127. Piha-Paul, S. A. et al. Phase 1 study of molibresib (GSK525762), a bromodomain and extra-terminal domain protein inhibitor, in NUT carcinoma and other solid tumors. *JNCI Cancer Spectr.* **4**, pkz093 (2020).
128. Lewin, J. et al. 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**, 3007–3014 (2018).
129. Shapiro, G. I. et al. A phase 1 study of RO6870810, a novel bromodomain and extra-terminal protein inhibitor, in patients with NUT carcinoma, other solid tumours, or diffuse large B-cell lymphoma. *Br. J. Cancer* **124**, 744–753 (2020).
130. Hilton, J. et al. Initial results from a phase I/IIa trial evaluating BMS-986158, an inhibitor of the bromodomain and extra-terminal (BET) proteins, in patients (pts) with advanced cancer. *Ann. Oncol.* **29** (2018).
131. Stathis, A. et al. Clinical response of carcinomas harboring the BRD4–NUT oncoprotein to the targeted bromodomain inhibitor OTX015/MK-8628. *Cancer Discov.* **6**, 492–500 (2016).
132. French, C. A. et al. Report of the first international symposium on NUT carcinoma. *Clin. Cancer Res.* **28**, 2493–2505 (2022).
133. Yamamoto, T. et al. BRD4 promotes metastatic potential in oral squamous cell carcinoma through the epigenetic regulation of the MMP2 gene. *Br. J. Cancer* **123**, 580–590 (2020).
134. Wu, Y. et al. Therapeutic targeting of BRD4 in head neck squamous cell carcinoma. *Theranostics* **9**, 1777–1793 (2019).
135. Zhang, W. et al. Combinational therapeutic targeting of BRD4 and CDK7 synergistically induces anticancer effects in head and neck squamous cell carcinoma. *Cancer Lett.* **469**, 510–523 (2020).
136. Wu, Q. et al. BRD4 drives esophageal squamous cell carcinoma growth by promoting RCC2 expression. *Oncogene* **41**, 347–360 (2022).
137. Fisher, M. L. et al. BRD4 regulates transcription factor deltaNp63alpha to drive a cancer stem cell phenotype in squamous cell carcinomas. *Cancer Res.* **81**, 6246–6258 (2021).
138. Ohnesorge, P. V. et al. Efficacy of oncolytic herpes simplex virus T-VEC combined with BET inhibitors as an innovative therapy approach for NUT carcinoma. *Cancers* **14**, 2761 (2022).
139. Kloker, L. D. et al. Case report: immunovirotherapy as a novel add-on treatment in a patient with thoracic NUT carcinoma. *Front. Oncol.* **12**, 995744 (2022).
140. Sotiriadis, S. et al. Multimodal therapy approaches for NUT carcinoma by dual combination of oncolytic virus talimogene laherparepvec with small molecule inhibitors. *Viruses* **16**, 775 (2024).
141. Pham, C., Lang, D. & Iams, W. T. Successful treatment and retreatment with erdafitinib for a patient with FGFR3-TACC3 fusion squamous NSCLC: a case report. *JTO Clin. Res. Rep.* **4**, 100511 (2023).
142. Wang, C. G., Peiris, M. N., Meyer, A. N., Nelson, K. N. & Donoghue, D. J. Oncogenic driver FGFR3–TACC3 requires five coiled-coil heptads for activation and disulfide bond formation for stability. *Oncotarget* **14**, 133–145 (2023).
143. Amin, S. E. et al. DEK::AFF2 fusion-associated squamous cell carcinoma: a case series with literature review on an emerging and challenging entity. *Head Neck Pathol.* **18**, 86 (2024).
144. McLean-Holden, A. C. et al. NUT carcinoma in a patient with unusually long survival and false negative FISH results. *Head Neck Pathol.* **15**, 698–703 (2021).
145. Begum, S. & Westra, W. H. Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. *Am. J. Surg. Pathol.* **32**, 1044–1050 (2008).
146. Fujimaki, M. et al. Histological subtypes and characteristic structures of HPV-associated oropharyngeal carcinoma: study with Japanese cases. *Diagn. Pathol.* **8**, 211 (2013).
147. Petrini, P. et al. NUT rearrangement is uncommon in human thymic epithelial tumors. *J. Thorac. Oncol.* **7**, 744–750 (2012).
148. Gokmen-Polar, Y., Cano, O. D., Kesler, K. A., Loehrer, P. J. & Badve, S. NUT midline carcinomas in the thymic region. *Mod. Pathol.* **27**, 1649–1656 (2014).
149. Chen, S. et al. A prognostic model for elderly patients with squamous non-small cell lung cancer: a population-based study. *J. Transl. Med.* **18**, 436 (2020).
150. Schaefer, I. M. et al. CIC–NUTM1 fusion: a case which expands the spectrum of NUT-rearranged epithelioid malignancies. *Genes Chromosomes Cancer* **57**, 446–451 (2018).
151. Sturm, D. et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. *Cell* **164**, 1060–1072 (2016).
152. Andersson, A. K. et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. *Nat. Genet.* **47**, 330–337 (2015).
153. Gu, Z. et al. Genomic analyses identify recurrent MEF2D fusions in acute lymphoblastic leukaemia. *Nat. Commun.* **7**, 13331 (2016).
154. Liu, Y. F. et al. Genomic profiling of adult and pediatric B-cell acute lymphoblastic leukemia. *eBioMedicine* **8**, 173–183 (2016).
155. Lilljebjorn, H. et al. Identification of ETV6–RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukaemia. *Nat. Commun.* **7**, 11790 (2016).
156. Liu, Y. et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. *Nat. Genet.* **49**, 1211–1218 (2017).
157. Chaturvedi, A. K. et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J. Clin. Oncol.* **29**, 4294–4301 (2011).
158. Chau, N. G. et al. Intensive treatment and survival outcomes in NUT midline carcinoma of the head and neck. *Cancer* **122**, 3632–3640 (2016).
159. Sung, H. et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **71**, 209–49 (2021).
160. Windon, M. J. et al. Increasing prevalence of human papillomavirus-positive oropharyngeal cancers among older adults. *Cancer* **124**, 2993–9 (2018).
161. SEER*Explorer: an interactive website for SEER cancer statistics. *National Cancer Institute* https://seer.cancer.gov/statistics-network/explorer/ (2024).
162. Crook, T. et al. Status of c-myc, p53 and retinoblastoma genes in human papillomavirus positive and negative squamous cell carcinomas of the anus. *Oncogene* **6**, 1251–1257 (1991).
163. Sarbia, M. et al. Expression of Bcl-2 and amplification of c-myc are frequent in basaloid squamous cell carcinomas of the esophagus. *Am. J. Pathol.* **155**, 1027–1032 (1999).
164. Tonon, G. et al. High-resolution genomic profiles of human lung cancer. *Proc. Natl Acad. Sci. USA* **102**, 9625–9630 (2005).
165. Stransky, N. et al. The mutational landscape of head and neck squamous cell carcinoma. *Science* **333**, 1157–1160 (2011).
166. Hussenet, T. et al. SOX2 is an oncogene activated by recurrent 3q26.3 amplifications in human lung squamous cell carcinomas. *PLoS ONE* **5**, e8960 (2010).
167. Hussenet, T. & du Manoir, S. SOX2 in squamous cell carcinoma: amplifying a pleiotropic oncogene along carcinogenesis. *Cell Cycle* **9**, 1480–1486 (2010).
168. Sheu, J. J. et al. Functional genomic analysis identified epidermal growth factor receptor activation as the most common genetic event in oral squamous cell carcinoma. *Cancer Res.* **69**, 2568–2576 (2009).
169. Koole, K. et al. FGFR1 is a potential prognostic biomarker and therapeutic target in head and neck squamous cell carcinoma. *Clin. Cancer Res.* **22**, 3884–3893 (2016).
170. Murugan, A. K., Hong, N. T., Fukui, Y., Munirajan, A. K. & Tsuchida, N. Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. *Int. J. Oncol.* **32**, 101–111 (2008).
171. Schwartz, B. E. et al. Differentiation of NUT midline carcinoma by epigenomic reprogramming. *Cancer Res.* **71**, 2686–2696 (2011).
172. Shiota, H. et al. Chemical screen identifies diverse and novel histone deacetylase inhibitors as repressors of NUT function: implications for NUT carcinoma pathogenesis and treatment. *Mol. Cancer Res.* **19**, 1818–1830 (2021).

Acknowledgements

The work of J.L., G.I.S. and C.A.F. is funded by K12TR004381 (Harvard Catalyst, and the Harvard Clinical and Translational Science Center; J.L.), Lowe Center of Thoracic Oncology (J.L.), Dana–Farber Department of Medical Oncology (J.L.), R01 CA124633 (C.A.F.), U01 CA294062 (C.A.F.), R01 CA285308 (G.I.S. and C.A.F.) and R21 CA277316 (J.L., G.I.S. and C.A.F.). The Dana–Farber/Brigham and Women's Hospital NUT Carcinoma Program receives philanthropic support from the Friends of Jay Dion Memorial Classic, the Ryan Richards Foundation, the McDevitt Strong Foundation, the Max Vincze Foundation, the Victor Family Foundation, the Alexandra Hallock Memorial Fund, and the Fortisure Foundation Fund for NUT Carcinoma.

Author contributions

All authors contributed significantly to the manuscript concepts and content. J.L. and C.A.F. researched data for the article, wrote the manuscript and drafted the initial figures. J.B., S.G.D., G.J.H., L.M.S., E.B.S., L.D.R.T. and G.I.S. reviewed and edited the manuscript.

Competing interests

J.L. reports honoraria from Cancer GRACE, Community Cancer Education Inc., Physicians' Education Resource, Targeted Oncology and VJ Oncology; advisory board participation for Amgen, Astellas and AstraZeneca; institutional research support from Erasca, Genentech, Kronos Bio, Novartis and Revolution Medicines; and personal fees from Blueprint Medicines, Daiichi Sankyo and Erasca. A patent filed by Memorial Sloan Kettering Cancer Center related to multimodal features to predict response to immunotherapy (PCT/US2023/115872) is pending. G.J.H. reports grants or contracts from ACCRF, Actuate Therapeutics, ASCO CCF, Bicara, Bristol Myers Squibb, Elevar Therapeutics, Exicure, Gateway for Cancer Research, Genentech, GSK, ImmunityBio, Kartos, Kite (a Gilead company), KSQ Therapeutics, Kura Oncology, Regeneron, Repertoire, Sanofi Genzyme, Secura Bio and V Foundation; and advisory roles for and/or honoraria from Bicara, Bio-Rad, Boxer Capital, Bristol Myers Squibb, Coherus, Elevar, Exicure, General Catalyst, Guardian Bio, KSQ Therapeutics, Kura Oncology, Massachusetts Medical Society, Merck, Naveris, Nextech, Prelude, Rain, Regeneron, Remix, Replimune, Sanofi Genzyme, SIRPant and Surface Oncology. S.G.D. reports honoraria from and/or advisory board participation for Amgen, Bayer, InhibRx and Jazz Pharmaceuticals; and travel expenses from LOXO Oncology, Roche and Salarius. G.I.S. reports personal fees from Artios, Bayer, Bicycle Therapeutics, Blueprint Medicines, Boehringer Ingelheim, Concarlo Holdings, Cybrexa Therapeutics, CytomX Therapeutics, ImmunoMet, Janssen, Kymera Therapeutics, Merck KGaA/EMD-Serono, Syros, Xinthera and Zentalis; grants from Bristol Myers Squibb, Eli Lilly, Merck KGaA/EMD-Serono, Pfizer and Tango; has a patent for "Dosage regimen for sapacitabine and seliciclib", issued to Cyclacel Pharmaceuticals and G.I.S., and a patent for "Compositions and methods for predicting response and resistance to CDK4/6 inhibition", issued to Liam Cornell and G.I.S. C.A.F. reports research funding and consultancy fees from Boehringer Ingelheim.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41571-025-00986-3.

Peer review information *Nature Reviews Clinical Oncology* thanks the anonymous reviewers for their contribution to the peer review of this work.

[1]Department of Medical Oncology, Dana–Farber Cancer Institute, Boston, MA, USA. [2]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [3]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA. [4]Dana–Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA, USA. [5]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [6]Department of Pathology, University of Virginia Medical Center, Charlottesville, VA, USA. [7]Head and Neck Pathology Consultations, Woodland Hills, CA, USA.