










BEDSIDE TO BENCH REPORT



Diagnosis of NUT carcinoma of lung origin by next-generation sequencing: case report and review of the literature

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ABSTRACT

NUT carcinoma (NC) is an aggressive squamous tumor characterized by *NUT* gene rearrangement, and the most common fusion form is *BRD4-NUT*. However, NC diagnosis is difficult for its rareness and often being confused with a variety of poorly differentiated tumors. A 21-year-old Chinese woman was referred to our hospital for cough and intermittent fever. Chest computed tomography (CT) imaging revealed a left lobe hilar mass. Fiberoptic bronchoscopy results showed that tumor cells were poorly differentiated. In combination with immunohistochemistry staining, she was misdiagnosed with Ewing's sarcoma/primitive neuroectodermal tumor. Next-generation sequencing (NGS) revealing *BRD4-NUT* fusion, and NUT immunohistochemistry confirmed the diagnosis of NC. Subsequently, left pneumonectomy and lymph node dissection were performed, and the patient received pemetrexed and lobaplatin treatment. NGS technology played an important role in NC diagnosis in this case, and it may have clinical use for rare cancer diagnosis and guidance of potential targeted therapies.

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KEYWORDS

NUT carcinoma; *BRD4-NUT*; next-generation sequencing

Background

NUT carcinoma (NC), an aggressive subtype of squamous cell carcinoma, is defined by an acquired rearrangement involving the *NUT* (NUT midline carcinoma family member 1) gene on chromosome 15. In most cases, *NUTM1* is fused to *BRD4* (bromodomain-containing protein 4) on chromosome 19.¹ NUT carcinoma can arise in different organs, including lung, mediastinum, bladder, pancreas, kidney and salivary gland,¹ which can affect any age or gender group.² Ewing's sarcoma (ES)/primitive neuroectodermal tumor (PNET) is a malignant prototypical primitive small round cell tumor that arises from lung, bone and other sites in children or adolescents,^{3,4} which can be indistinguishable from poorly differentiated NC.⁵

Here, we present a case that was previously misdiagnosed as ES/PNET but was later confirmed as NC through next-generation sequencing (NGS).

Case report

A 21-year-old Chinese woman presented with cough and intermittent fever. The patient had no hemoptysis, chest pain, shortness of breath, hoarseness, headache or bone pain. Chest computed tomography (CT) imaging revealed a left lobe hilar mass measuring 5.7 cm x 4.5 cm x 5.4 cm, enlarged hilar lymph nodes, mediastinal vessels invasion and inflammatory obliteration in the left lung lobe (Figure 1(a)). Bone scan and magnetic resonance imaging (MRI) for brain

showed no abnormality. Laboratory tests for blood showed elevated AFP (137.26 ng/mL; normal range 0.89–8.78 ng/mL) and β -HCG of 1.20 mIU/ml (negative). She underwent fiberoptic bronchoscopy, and pathologic examination showed poorly differentiated malignant tumor composing of cords and nests of small-sized cells with round conspicuous nucleus and translucent cytoplasm (Figure 2(a)) and demonstrated the presence of bronchus and blood vessel involvement, neutrophil infiltration and alveolar cleft-like defect (data not shown). Morphologically, squamous differentiation was absent by hematoxylin-eosin staining. A battery of immunohistochemistry markers was performed for tumor cells, but only CD99 was positive (Figure 2(b)). Despite the young age, malignant ES/PNET was considered. Differential diagnosis included adenocarcinoma (TTF-1, napsin A, CK7 and pancytokeratin negative), squamous carcinoma (P40 and CK5/6 negative), neuroendocrine carcinoma (NSE, chromogranin A and CD56 negative), embryonal rhabdomyosarcoma (desmin, MyoD1, myogenin and synaptophysin negative). There were surgery indications, so left pneumonectomy by video-assisted thoracic surgery (VATS) and lymph node dissection were performed (Figure 1(b)). Immunohistochemistry staining of the resection specimen demonstrated that tumor cells were positive for CD99, synaptophysin and FLI1, and negative for pancytokeratin, CK18, and P40 (data not shown). Fluorescence in situ hybridization (FISH) for resection specimen was negative for *EWSR1* (Ewing sarcoma breakpoint region 1) rearrangement. A few days after surgery, the level

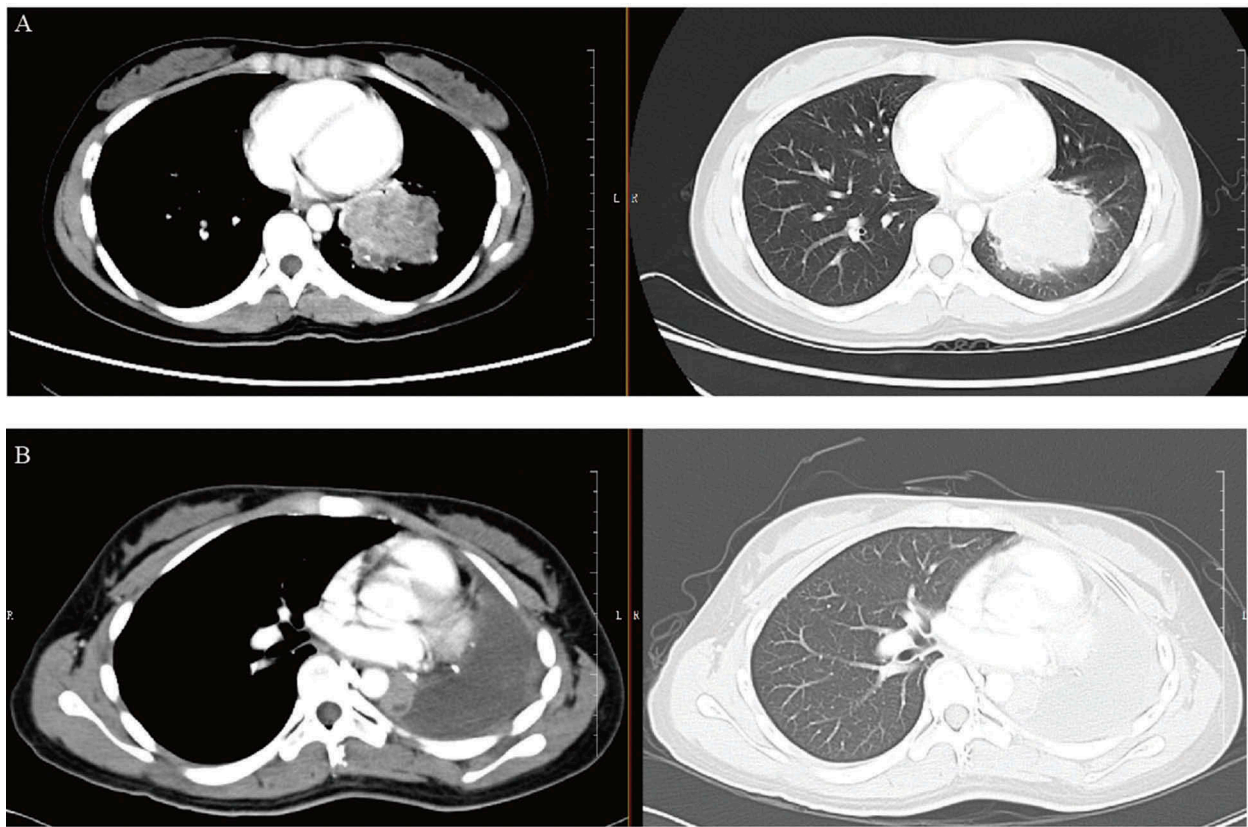


Figure 1. Disease status at diagnosis (a) and after left pneumonectomy and lymph node dissection (b) by CT scan (axial). CT, computed tomography.

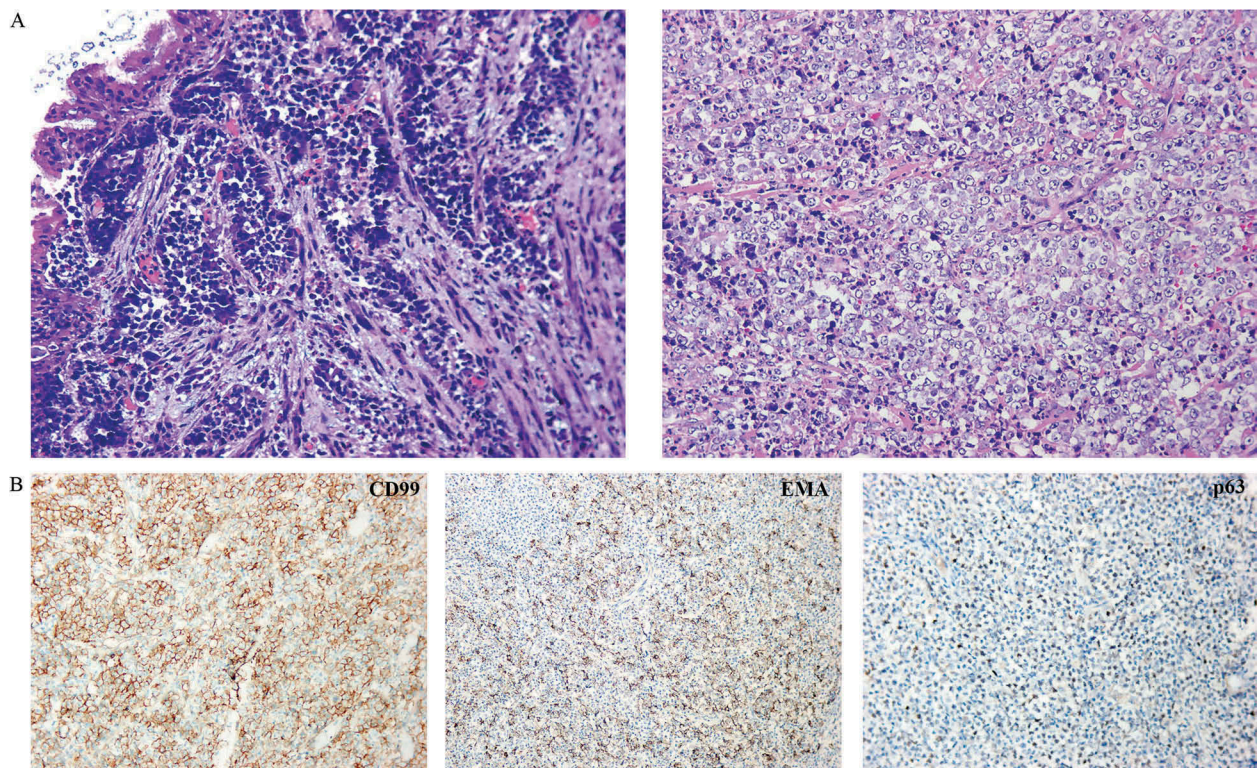


Figure 2. Clinicopathologic characteristics of this case. (a) Pathology image of fiberoptic bronchoscopy deposits by hematoxylin and eosin stain showing round undifferentiated tumor cells. (b) Immunohistochemistry staining of tumor cells from a resected specimen that are positive for CD99, EMA and p63.

of AFP dramatically decreased to 14.05 ng/ml (normal range 0.89–8.78 ng/ml).

The resected specimen was assayed using adaptor-ligation and hybrid capture NGS (Yuansu; Origimed Inc, Shanghai, China) for all coding exons from 450 cancer-related genes plus introns from 35 genes frequently rearranged in cancer. At least 50 ng of DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue from unstained slides, and peripheral blood was used as a control. The mean exon coverage depth of sequencing was >1000× and >200× for tissue and blood controls, respectively. Molecular profiling revealed a *BRD4* exon11-*NUT1* exon2 fusion (Figure 3(a,b)). The tumor mutational burden (TMB) was 0.8 muts/Mb.

Microsatellite status was stable in this specimen. Immunohistochemistry staining of previous fiberoptic bronchoscopy deposits demonstrated positive staining for p63 and EMA (Figure 2(b)). According to the NGS result, NUT antibody (C52B1 clone, Cell Signaling Technology, Danvers, MA) was used to confirm the diagnosis of NC (Figure 3(c)). It is worth mentioning that immunohistochemistry staining for NUT was performed by Guangdong General Hospital because this test is not available at our hospital.

Considering the poor prognosis of NC, postoperative adjuvant chemotherapy was performed. The patient received docetaxel plus carboplatin, but she was significantly allergic to docetaxel. Thus, chemotherapy was changed to pemetrexed

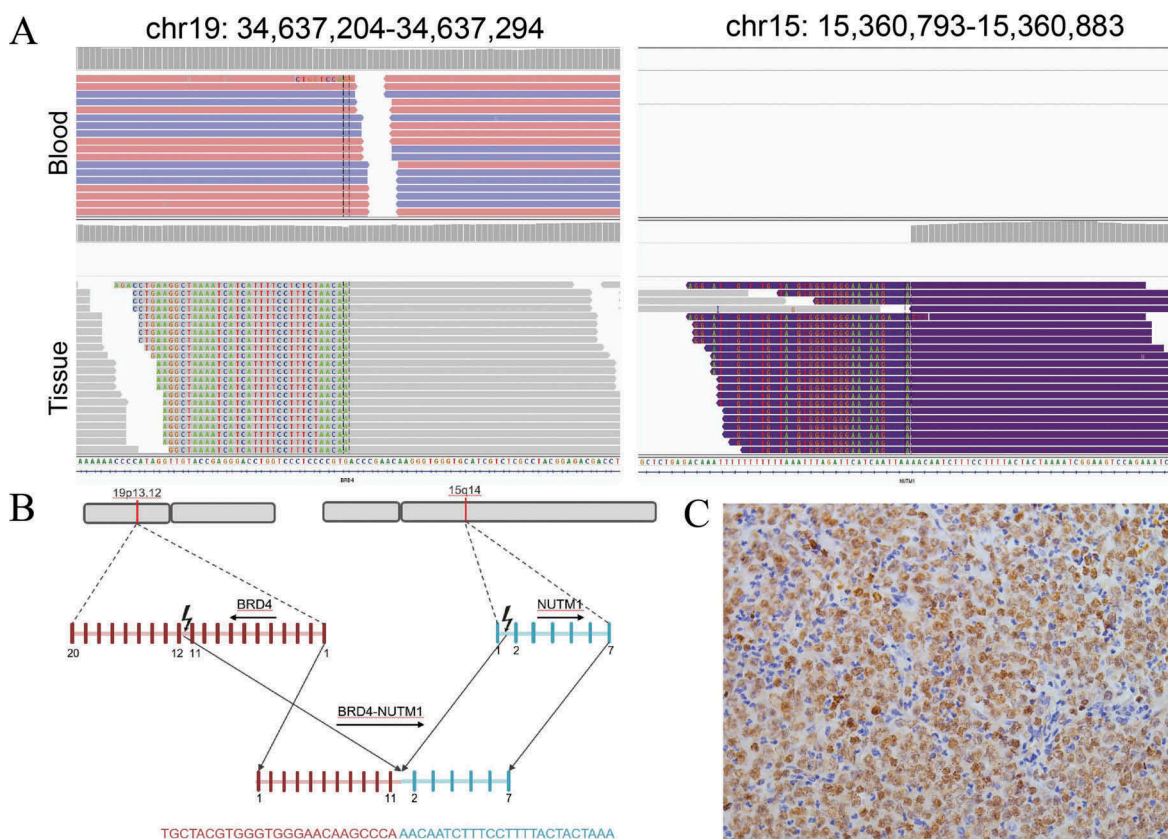


Figure 3. Detail presentation of *BRD4-NUT* fusion. (a) Screenshot of Integrative Genomics Viewer demonstrating uniquely mapped paired-end reads in the intronic regions of *BRD4* (Intron 11) on chromosome 19 and *NUT* (Intron 1) on chromosome 15. For no hybrid capture probes were designed for *NUT* gene, tissue sample showed aberrant arrangement reads. (b) Schematic representation of the translocation responsible for the generation of the *BRD4-NUT* fusion gene. (c) Immunohistochemistry staining of tumor cells from resected specimen were positive for anti-NUT antibody.

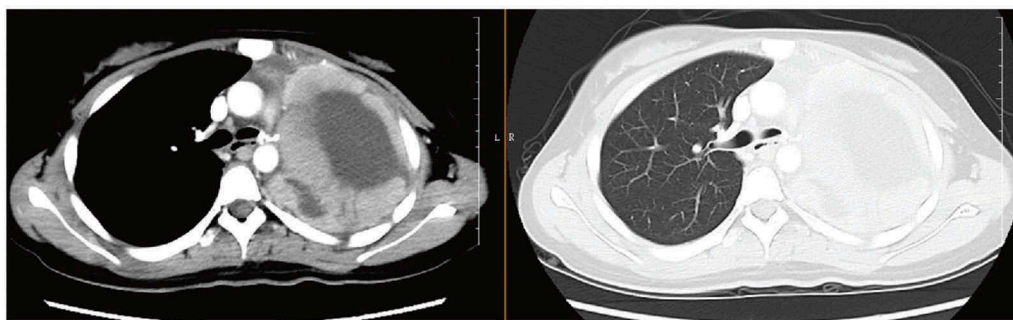


Figure 4. Disease status after three weeks of chemotherapy by CT scan (axial). CT, computed tomography.

Table 1. Clinicopathologic characteristics of NUT carcinomas of lung origin.

Case	Age	Sex	Initial diagnosis	Squamous differentiated	AFP	Immunohistochemistry staining		RT-PCR	NC confirmation	Survival time (months)	Reference
						Positive	Negative				
1	16	M	Squamous cell carcinoma	positive	NA	pancytokeratin	PLAP, CD34	NA	BRD4-NUT (FISH)	35	French et al., 2004 ⁶
2	14	M	Unspecified sarcoma or undifferentiated carcinoma	NA	NA	CK1/5/10/14, CK5, CK8, vimentin, TTF-1, p63, CD99	CK7, CK19, CK20, EMA, CD5, CD34, neurofilament, synaptophysin, chromogranin A, desmin, muscle actin, SMA, WT1	EWSR1-FLI1, EWSR1-ERG, EWSR1-WT1	anti-NUT antibody (IHC) and BRD4-NUT (RT-PCR)	12	Tanaka et al., 2012 ⁷
3	7	F	squamous cell carcinoma	positive	NA	CK1/5/10/14, CK5, CK7, CK19, EMA, p63	CK20, vimentin, TTF-1, CD5, CD34, CD99, neurofilament, synaptophysin, chromogranin A, desmin, muscle actin, SMA, WT1	NA	anti-NUT antibody (IHC) and BRD4-NUT (RT-PCR)	4	Tanaka et al., 2012 ⁷
4	36	M	nonseminomatous primary mediastinal germ cell tumor	NA	NA	NA	pancytokeratin, CK7, OSCAR, CAM 5.2, EMA, CD45, S100, CD117, TTF-1, PLAP, Oct-4, CD31, CD34, FLI1	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	2	Parikh et al., 2013 ⁸
5	12	F	NC	positive	NA	pancytokeratin, CAM5.2, p63, CD56	chromogranin A, synaptophysin, neurofilament, S-100	NA	BRD4-NUT (FISH)	21	Ueki et al., 2014 ⁹
6	68	F	Lymphoma	NA	NA	p63, MNF-116	TTF-1, synaptophysin, chromogranin, CD56	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	1	Sholl et al., 2015 ¹⁰
7	21	F	NC	NA	NA	p63, pancytokeratin, Cam5.2	NA	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	4	Sholl et al., 2015 ¹⁰
8	23	M	Thymic carcinoma	NA	NA	p63, pancytokeratin	TTF-1	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	2	Sholl et al., 2015 ¹⁰
9	63	F	Poorly differentiated NSCLC	NA	NA	p63, TTF-1, pancytokeratin, CK5/6, CK7, CK20	synaptophysin, chromogranin	NA	variant NUT rearrangement (FISH) and anti-NUT antibody (IHC)	2	Sholl et al., 2015 ¹⁰
10	37	M	Germ cell tumor	NA	NA	p63, TTF-1, Cam5.2, pancytokeratin, CD56	synaptophysin, chromogranin	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	1	Sholl et al., 2015 ¹⁰
11	63	M	NC	NA	NA	p63, CK7, CK20, CK5/6	TTF-1, synaptophysin, chromogranin, CD56	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	1	Sholl et al., 2015 ¹⁰
12	26	M	Adenosquamous carcinoma	NA	NA	p63, Cam5.2, pancytokeratin, CK5/6	TTF-1, synaptophysin	NA	BRD4-NUT (FISH) and anti-NUT antibody (IHC)	2	Sholl et al., 2015 ¹⁰
13	30	M	Poorly differentiated carcinoma	NA	NA	p63, Cam5.2, pancytokeratin, synaptophysin	TTF-1, chromogranin	NA	BRD4-NUT (FISH) and anti-NUT antibody (IHC)	5	Sholl et al., 2015 ¹⁰
14	29	F	NSCLC	NA	NA	p63, TTF-1	NA	NA	BRD4-NUT (FISH) and anti-NUT antibody (IHC)	3	Sholl et al., 2015 ¹⁰
15	34	M	NC	negative	NA	p63, Ki-67	CAM5.2, CK7, CK20, CK5/6, lymphoid, myeloid, chromogranin, synaptophysin, CD56, S-100	NA	anti-NUT antibody (IHC)	1	Polcarpio-Nicolas et al., 2015 ¹¹
16	36	F	NC	positive	NA	EMA, p63, pancytokeratin, CAM5.2, CD138, vimentin	NA	NA	anti-NUT antibody (IHC) and BRD4-NUT (FISH)	10	Kuroda et al., 2015 ¹²

(Continued)

Table 1. (Continued).

Case	Age	Sex	Initial diagnosis	Squamous differentiated	AFP	Immunohistochemistry staining		RT-PCR	NC confirmation	Survival time (months)	Reference
						Positive	Negative				
17	23	F	non-Hodgkin's lymphoma	negative	NA	synaptophysin, polypytic keratin, OSCAR keratin, CD99, EMA	LCA, CD20, BCL-2, CAM5.2, desmin, CD3, CD30, BCL-6, CK7, myogenin, CD5, CD34, MUM-1, p16, FLI1, CD10, CD79a, Ber-Ep-4, p63, OCT3/4, CD15, BCL-1, pancytokeratin, TTF-1, S100	NA	anti-NUT antibody (IHC) and <i>BRD4-NUT</i> (FISH)	2	Shatavi et al., 2016 ¹³
18	15	F	ES/PNET	NA	NA	pancytokeratin, CD99	NA	NA	<i>BRD4-NUT</i> (FISH)	17	Lemelle et al., 2017 ¹⁴
19	16	F	Undifferentiated carcinoma	NA	NA	KL1 cytokeratins	CD99	NA	t(15;19)(q13;p13) (cytogenetics)	3	Lemelle et al., 2017 ¹⁴
20	39	M	non-small cell lung cancer	positive	NA	p63	TTF-1, napsin A, synaptophysin	NA	NGS and anti-NUT antibody (IHC)	4	Baras et al., 2018 ¹⁵
21	21	F	ES/PNET	negative	elevated	CD99, EMA, p63, FLI1, synaptophysin	TTF-1, napsin A, CK7, p40, CK5/6, NSE, CgA, CD56, pancytokeratin, desmin, MyoD1, myogenin	NA	NGS and anti-NUT antibody (IHC)	5	present study

IHC: Immunohistochemistry staining; NC: NUT carcinoma

700 mg and lobaplatin 40 mg on day 1. After three weeks, the patient came back to the hospital for dull ache in the chest and upper back and intermittent abdominal pain. Laboratory tests for blood showed that CEA, CA125, CA15-3, CA19-9, SCCAg and AFP were negative, while TK1 was elevated (4.91 pM/L; normal range 0–2.0 pM/L). Brain MRI showed no obvious abnormalities. Chest CT revealed decreased absorption of pleural fluid, left mediastinal shift, pleural thickening and soft tissue density on the left lung region (Figure 4). We suggested ultrasound-guided fine needle aspiration of pleural fluid, but the patient and her family refused all testing and treatment and left the hospital. The patient died within five months after the onset of symptoms.

Here, we have summarized the clinicopathologic characteristics of NC cases of lung origin with *BRD4-NUT* fusion in previous studies along with our present study (Table 1).

Discussion

NC is a rare aggressive squamous malignancy and is often difficult to diagnosis due to its rarity and non-specific histology¹. NC is histologically described as poorly differentiated, which is indistinguishable from other poorly differentiated cell carcinomas such as ES/PNET, especially in the absence of squamous differentiation. ES/PNET is positive for CD99, a cell surface glycoprotein, and has been reported to be positive in all 52 cases.¹⁶ *EWSR1* was reportedly fused with *FLI1* in only 85% of cases³; thus, ES/PNET was initially diagnosed, although *EWSR1* rearrangement was negative in our case. Additionally, D'Ambrosio et al. presented an NC case in which elevated AFP was observed.¹⁷ However, not all previous NC cases presented with AFP.¹⁸

NC is characterized by a *NUT* rearrangement, which can be detected by immunohistochemistry, karyotype, FISH, RT-PCR or NGS. Immunohistochemistry staining for *NUT* is effective for *NUT* diagnosis. However, in this case, we did not determine NC in the initial differential diagnosis. Because, an immunohistochemistry antibody for *NUT* is not available in our hospital or other hospitals in our province. FISH has been adopted to detect *NUT* gene rearrangement in NC using dual-color *NUT* split-apart probes, but is not widely available or commercialized.^{8,19,20} *BRD4-NUT* fusion can also be detected using RT-PCR.¹⁴ *BRD4-NUT* fusion, the most common rearrangement in NC cases, has attracted interest for the development of small molecules that target *BRD4*,²¹ a member of the BET (bromodomain and extra terminal domain) family that contains two bromodomains.¹ A novel oral BET inhibitor, OTX015/MK-8628, is currently in clinical development for targeting *BRD2/3/4/T*, and two NC patients have responded rapidly to this inhibitor.²² NGS can be applied for detecting the *NUT* rearrangement,^{23–26} especially for the *BRD4-NUT* fusion, which is crucial for guiding targeted therapy. Thus, NGS can be used not only to detect multiple *NUT* rearrangements for NC diagnosis but also to guide targeted therapy of NC.

For now, there is no effective treatment for NC. The rarity of NC renders multiple treatment paradigms used in the clinic, such as those for sarcoma and non-small lung cancer.²⁷ Bauer et al. reported improved survival for gross total resection in NC

patients. However, the NC patients still had poor prognosis, and the median overall survival for patients was 6.7 months.²⁸ In addition to the BET inhibitor mentioned above, the histone deacetylase inhibitor (HDACi) CUDC-907 showed effectiveness in NC xenograft tumors.²⁹ A child with NC showed an objective response to the FDA-approved HDAC inhibitor vorinostat after five weeks of therapy.³⁰

In summary, NC is an extremely aggressive carcinoma that can be diagnosed by NGS. It is necessary to consider NC when poor differentiation is observed by pathological examination. NGS technology can be an effective tool for rare disease diagnosis and further targeted therapy. Poor prognosis is the main characteristic of NC. Surgery and radiotherapy are still the main treatment options for NC. However, some novel targeted therapies such as BETi and HDACi have been developed.

Abbreviations

NC:	NUT carcinoma
NUT:	NUT carcinoma, family member 1, former known as NUT
CT:	computed tomography
ES/PNET:	Ewing's sarcoma/primitive neuroectodermal tumor
BRD4:	bromodomain-containing protein 4
TTF-1:	thyroid transcription factor 1
FLI1:	a member of the ETS transcription factor family
CK7:	cytokeratin 7
CK5/6:	cytokeratin 5/6
MyoD1:	myogenic differentiation 1
CK18:	cytokeratin 18
AFP:	alpha-fetoprotein
NGS:	next-generation sequencing
FISH:	Fluorescence in situ hybridization
RT-PCR:	reverse transcription polymerase chain reaction.
EWSR1:	Ewing sarcoma breakpoint region 1
FFPE:	formalin-fixed, paraffin-embedded
TMB:	tumor mutational burden
BET:	bromodomain and extra terminal domain
CEA:	carcinoembryonic antigen
CA125:	cancer antigen 125
CA15-3:	cancer antigen 15-3
CA19-9:	cancer antigen 19-9
SCCAg:	human squamous cell carcinoma related antigen
TK1:	thymidine kinase 1
MRI:	magnetic resonance imaging.

Ethics approval and consent to participate

The patient provided written informed consent and gave permission for the use of biopsies and publication of case details.

This study was approved by the Medical Ethics Committee of the Affiliated Tumor Hospital of Guangxi Medical University.

Consent for publication

Written informed consent was obtained from the patient for the publication of this case report.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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




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Authors' contributions

Naiquan Mao, Zhiling Liao, Junwei Wu, Kai Liang, Shoufeng Wang, Shaomian Qin, Hanqing Lin and Xiaowei Dong were involved in diagnostic flow and patient follow-up. Hanqing Lin and Ying Dou contributed to the interpretation of published data and were involved in drafting of the manuscript. Zhiling Liao and Xiaowei Dong are licensed pathologists. All the authors read and gave their final approval of the version to be published.

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