ACCSCIENCE PUBLISHING

Tumor Discovery

EXPERT CONSENSUS

Expert consensus and current landscape of NUT carcinoma: A comprehensive strategy from diagnosis to treatment

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Abstract

Nuclear protein of the testis (NUT) carcinoma is a rare and highly aggressive malignancy characterized by the rearrangement of the NUT midline carcinoma family member 1 (*NUTM1*) gene. Nevertheless, standardized strategies for its diagnosis and treatment remain unavailable, underscoring the need for expert consensus. To address this gap, we conducted a systematic review to gather comprehensive information on NUT carcinoma from five databases: PubMed, Web of Science, Embase, Cochrane Library, and Ovid Medline. This expert consensus was collaboratively developed by a team of international multidisciplinary experts, in partnership with the NUT Carcinoma Diagnosis Working Group of the Chinese Anti-Cancer Association's Oncogene Diagnosis Professional Committee. This working group comprises medical oncologists, radiation oncologists, surgical oncologists, pathologists, nurses, molecular biologists, statisticians, and bioinformatics specialists. A systematic review, based on data from 526 patients across 199 articles, was conducted to comprehensively explore various characteristics, including demographic features (e.g., patient gender, distribution regions, and age), tumor-node-metastasis (TNM) classification stage, clinical symptoms, tumor size, metastatic patterns, immunohistochemical (IHC) findings, treatment modalities, prognosis-related information, and *NUTM1* fusion partners. We have developed an expert consensus on diagnosing and treating NUT carcinoma using a multidisciplinary approach. The guideline provides eight recommendations, addressing epidemiological characteristics, clinical and imaging manifestations, pathological findings, IHC features, molecular mechanisms and subtypes, prognosis, diagnosis, and treatment strategies for NUT carcinoma. Furthermore, an international platform has been established to disseminate NUT carcinoma knowledge and patient recruitment, providing patients and healthcare providers access to NUT carcinoma-related information and updates on clinical trial recruitment.

Keywords: Nuclear protein of the testis carcinoma; Expert consensus; Diagnosis; Treatment

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Citation: Ye Z, Li X, Xie F, *et al.* Expert consensus and current landscape of NUT carcinoma: A comprehensive strategy from diagnosis to treatment. *Tumor Discov.* 2024;3(4):4904. doi: 10.36922/td.4904

Received: September 22, 2024

Accepted: November 14, 2024

Published Online: December 24, 2024

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Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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1. Introduction

Nuclear protein of the testis (NUT) carcinoma, also known as midline carcinoma resulting from the rearrangement of the NUT gene, is a rare and highly aggressive malignant tumor. It is associated with rearranging the NUT midline carcinoma family member 1 (*NUTM1*) gene on chromosome 15q14. First described in 1991, subsequent cases of NUT carcinoma have primarily been reported in the midline structures of the human body, including the head, neck, and thoracic/mediastinal regions, which is why it was initially termed "midline carcinoma."[1-4] However, NUT carcinoma can also occur in various organs, including the lungs, bones, nasal cavity, salivary glands, central nervous system (CNS), and soft tissues. As a result, the World Health Organization renamed the condition "NUT carcinoma" to better reflect its broader clinical presentation.[5-15] The rearrangement of the *NUTM1* gene was initially considered a hallmark molecular alteration in NUT carcinoma. In recent years, *NUTM1* rearrangements have also been identified in tumor types, such as round-cell sarcoma, acute leukemia, hidradenoma, and hidradenocarcinoma, broadening the spectrum of diseases associated with this genetic change. Multiple partner genes that can fuse with *NUTM1* have now been identified, including members of the bromodomain protein family, the myelocytomatosis-

associated factor X (MAX) dimerization family, Capicua (CIC) transcription factor, and the transcriptional enhancer structural domain activation factor. This fusion results in the formation of the X: NUTM1 fusion protein.[2] The carboxy-terminal portion of the fusion protein retains nearly intact NUT protein, indicating the critical role of NUT in the epigenetic regulation of histone acetylation and its significance in the pathogenesis of these tumors. The pathological characteristics of NUT carcinoma exhibit low specificity,[16] which complicates clinical diagnosis. At present, the most commonly used diagnostic methods involve immunohistochemical (IHC) detection of NUT overexpression in the nucleus or identification of *NUTM1* gene (15q14) copy number variations or fusion events with common partner genes, often using fluorescence *in situ* hybridization (FISH) or next-generation sequencing (NGS).

The NUT carcinoma predominantly affects young individuals, although it can occur at any age.[17] The disease is highly invasive, often diagnosed at an advanced stage with distant metastasis, resulting in a poor prognosis.[18] Furthermore, the prognosis is closely linked to the specific fusion partners involved with *NUTM1*.[19] Radiation therapy and chemotherapy have limited efficacy, with rapid tumor progression typically occurring within months.[1,18,20] Recent studies indicate that bromodomain and extra-terminal domain (BET) inhibitors and histone deacetylase (HDAC) inhibitors may offer promising new treatment options for NUT carcinoma.[21,22]

The NUT Carcinoma Diagnosis Working Group of the Chinese Anti-Cancer Association's Oncogene Diagnosis Professional Committee was formally established in June 2023. This paper aims to provide more precise and standardized diagnostic and therapeutic guidelines for clinicians by summarizing and analyzing the general characteristics, clinical features, imaging, pathology, treatment, and prognosis of 526 cases of NUT carcinoma from 199 published articles. An expert consensus was developed through consultations with experts and scholars in relevant domestic and international research fields, and it has been periodically revised to incorporate the latest developments in the field to meet current clinical needs.

2. Methods

We followed the methodology recommended by the World Health Organization (WHO) handbook for guideline development[23] and utilized the Appraisal of Guidelines for Research and Evaluation (AGREE II) for assessment.[24] The draft of the final guideline was developed following the Reporting Items for Practice Guidelines in Healthcare (RIGHT).[25] An overview of the entire development process is provided in Figure S1.

2.1. Sponsor and supporters

This work was initiated and supported by the NUT Carcinoma Diagnosis Working Group of the Chinese Anti-Cancer Association's Oncogene Diagnosis Professional Committee, the Tumor Biomarker Professional Committee of the Chinese Anti-Cancer Association, and the China Food and Drug Corporation Quality and Safety Promotion Association.

2.2. Registration

This guideline has been registered on the International Practice Guideline Registry Platform under the registration number PREPARE-2023CN767 (http://www.guidelines-registry.cn).[26]

2.3. Scope

This guideline focuses on the diagnosis and treatment of NUT carcinoma. The primary target audience includes medical oncologists, radiation oncologists, surgical oncologists, pathologists, nurses, and the general public. Other healthcare professionals and decision-makers in cancer management may also benefit from this guideline.

2.4. Guideline working group

We established the following four groups to direct the development of the guidelines (Table S1):

(i) The steering committee comprised 10 chief oncologists and pathologists. They were responsible for guiding the overall design of the guidelines, making final decisions at each stage, and overseeing the entire process. They also wrote the guidelines.

(ii) The consensus panel consisted of 65 multidisciplinary experts, including 23 comprehensive oncology specialists, 12 pulmonary oncology specialists, six head and neck oncology specialists, five surgical oncology specialists, five pathology specialists, four gastrointestinal oncology specialists, two laboratory medicine specialists, two bone oncology specialists, two rare oncology specialists, along with experts in hematologic oncology, clinical pharmacology, pediatric oncology, and biochemistry and molecular biology. This group was responsible for clinical issues, voting on recommendations, and establishing consensus.

(iii) The evidence synthesis group, composed of four members, was responsible for gathering clinical issues, conducting literature searches, evaluating, synthesizing, and grading evidence, performing systematic reviews,

and creating summary tables of findings and decision tables for recommendations.

(iv) An external review group comprising 21 first-line healthcare professionals was responsible for providing external review and feedback on the guideline.

2.5. Ethical considerations

All steering committee and consensus panel members completed a conflict-of-interest disclosure form before participating in the guideline development process.[27] No relevant financial conflicts of interest were identified.

2.6. Systematic review and grading

To enhance an early and comprehensive understanding of NUT carcinoma and provide potentially effective treatment options for patients, we conducted a systematic review to collect detailed information on the key characteristics and treatment scenarios associated with this malignancy.

The protocol for this systematic review was registered with PROSPERO (CRD42021277083) on November 10, 2023. The review methodology followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. Given the highly heterogeneous patient populations and differences in study designs, a meta-analysis was deemed inappropriate.

We searched for relevant studies in the following databases: PubMed, Web of Science, Embase, Cochrane Library, and Ovid Medline, without language restrictions. The search strategy was based on the following terms: "NUT Carcinoma," "NUT midline carcinoma," "nuclear protein in testis carcinoma," "NUTM1," and "nuclear protein of the testis midline carcinoma." Further, details on the search terms are provided in Table S2. Each study was reviewed to confirm the presence of *NUTM1* gene rearrangement in the included patients, with diagnoses verified using pathological tissues and techniques such as IHC, FISH, or NGS. The final search was conducted on January 20, 2024. No restrictions were applied based on age, gender, country, or tumor stage. Some of the authors on our team were trained in the purpose and methodology of the review. The selection process was conducted independently by two reviewers (XL and ZMY), with disagreements resolved through discussion or by consulting a third reviewer (JZ). A PRISMA flow diagram illustrating the study selection procedure is provided in Figure S2.

Data extracted from each study included the following variables: author, year of publication, country, sample size, gender, region, age, smoking status, symptoms at first diagnosis, tumor location, metastatic lesions, tumor staging, maximum tumor diameter, type of treatment received, treatment sequence, IHC results, overall survival (OS), and *NUTM1* fusion partner (if known). The methodological quality of the studies was assessed using the Agency for Healthcare Research and Quality (Table S3). Two reviewers (LL and ZY) independently performed the quality rating, and a third reviewer (JZ) was consulted if disagreements occurred. Given that the most published studies on NUT carcinoma are observational, such as case reports, case series, and small retrospective cohort studies, they carry a higher risk of bias.

2.7. Data analysis and survival analysis

Based on the data extracted from the literature, we conducted a descriptive statistical analysis of NUT carcinoma's general characteristics, features, and current treatment strategies. In addition, we performed both univariate and multivariate analyses of OS. We ensured that each subgroup in the survival analysis cohort contained more than 10 individuals. Survival analyses were conducted for various factors, including patient gender, region of origin, age, site of metastasis, tumor-node-metastasis (TNM) classification stage, tumor diameter, antigen Kile 67 (Ki-67) index, *NUTM1* fusion partner, treatment type, and chemotherapy regimen. It is important to note that due to the rarity of NUT carcinoma and the lack of large-scale cohort data, the vast majority of data in this study comes from case reports, making the data non-continuous with significant heterogeneity. These data results are for clinical reference only, and the results of analyses and statistics based on these data should be interpreted and treated with caution.

2.8. Formulation of recommendations

After reviewing the literature and integrating clinical experience, we initially drafted a set of recommendations. The evidence levels and definitions of evidence-based medicine in this expert consensus are based on the Oxford Centre for Evidence-Based Medicine levels (Table S4). The initial recommendations underwent expert consultation, with three voting options: "agree," "uncertain," or "disagree." Experts were invited to provide suggestions for modification of each recommendation. After each round of consultation, the recommendations were revised or supplemented based on expert feedback. The recommendations were categorized as "strong recommendation," "recommendation," or "no consensus" based on expert votes. If the agreement rate (i.e., the proportion of experts selecting "agree") was ≥ 70%, the recommendation was considered to have reached a consensus. An agreement rate > 90% was classified as a "strong recommendation," 70 – 90% as a "recommendation," and anything less as "no consensus." The consensus draft was then submitted to the External Review Group for additional external review.

3. Results

3.1. Patient characteristics

As compiled from the literature, the distribution and general characteristics of NUT carcinoma patients are summarized in Figure 1. Additional patient details are provided in Table S5.

3.1.1. Patient gender

This study evaluated 526 patients from 199 articles; no significant difference was observed in the gender ratio among patients with known gender. The overall male-to-female ratio was 1.18 (276 males and 232 females). In the thoracic group, the male-to-female ratio was 1.52 (144 males and 95 females), while in the head and neck group, the ratio was 0.97 (81 males and 83 females).

3.1.2. Patient distribution by region and age

Most patients were in the Americas (51.52%), followed by Asia (34.6%). The average age of all patients was 38 years. Thoracic NUT carcinoma patients had a slightly higher average age of 40.02 years (range: 6 – 86 years) than head-and-neck NUT carcinoma patients, whose average age was 33.82 years (range: 1 – 82 years).

3.1.3. Patient TNM classification of malignant tumors staging

Among NUT carcinoma patients with a known TNM stage, 50.9% (56/110) were at stage IV, and 33.6% (37/110) were at stage III. In thoracic NUT carcinoma patients, the proportion of those at stage III (36.36%, 24/66) and stage IV (53.03%, 35/66) was higher than in head-neck NUT carcinoma patients, whereas 26.32% (10/38) were at stage III and 47.37% (18/38) were at stage IV.

3.1.4. Clinical symptoms at initial diagnosis of NUT carcinoma in patients

A total of 24 clinical symptoms were identified by combining data from the literature. Patients with thoracic NUT carcinoma frequently present with non-specific symptoms. Common clinical symptoms at the initial diagnosis of thoracic NUT carcinoma include cough (56.45%, 70/124), pain (40.32%, 50/124), and dyspnea (30.65%, 38/124). In contrast, patients with head-and-neck NUT carcinoma primarily exhibited compression symptoms due to tumor enlargement (53.01%, 44/83), pain (37.35%, 31/83), nasal congestion (14.46%, 12/83),

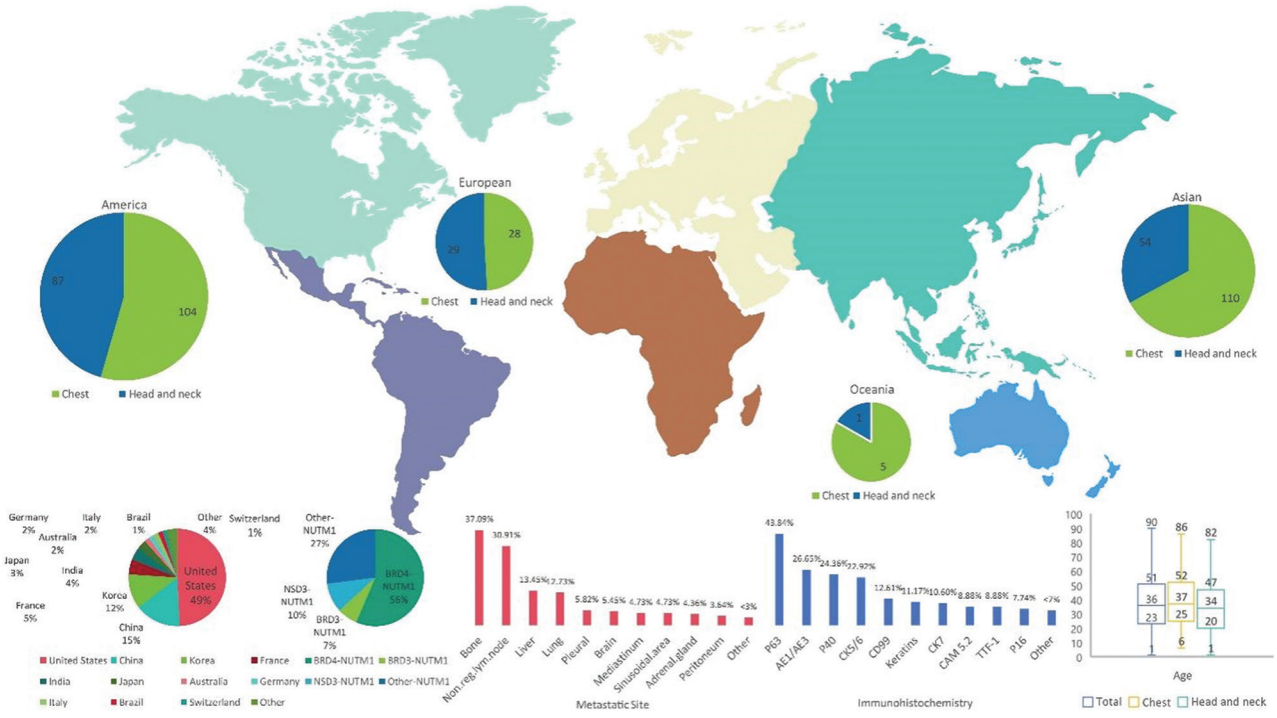


Figure 1. The distribution and general characteristics of nuclear protein of the testis carcinoma patients.
Note: The X-axis represents the specific indicators under each category, while the Y-axis indicates the percentage (%) of these indicators within the corresponding category.
Abbreviations: BRD3: Bromodomain-containing protein 3; BRD4: Bromodomain-containing protein 4; Non.reg.lym.node: Non-regional lymph node; NSD3: Nuclear receptor binding SET domain protein 3; NUTM1: Nuclear protein of the testis midline carcinoma family member 1.

and hemorrhage (13.25%, 11/83), resulting from local tumor compression and invasion of surrounding tissues.

3.2. Tumor characteristics

Tumor diameter: The average tumor diameter of NUT carcinoma patients was 6.04 cm. The average diameter in thoracic NUT carcinoma (6.86 cm) was larger than that of head-and-neck NUT carcinoma (4.08 cm).

3.2.1. NUT carcinoma fusion partners

Among patients with known specific *NUTM1* fusion partners, bromodomain-containing protein 4 (*BRD4*)::*NUTM1* was the most common (56.4%, 167/296), followed by nuclear receptor binding SET domain protein 3 (*NSD3*)::*NUTM1 (*9.80%, 29/296), *and* bromodomain-containing protein 3 (*BRD3*)::*NUTM1 (*6.76%, 20/296).

3.2.2. NUT carcinoma metastasis

A total of 29 metastatic sites were identified. About 52.28% (275/526) of all patients had distant metastasis. Among thoracic NUT carcinoma patients, 60.25% (144/239) had distant metastasis, while 40.85% (67/164) of head-and-neck NUT carcinoma patients had distant metastasis. These findings suggest that thoracic NUT carcinoma may be more prone to distant metastasis than head-and-neck NUT carcinoma. Based on the available data, the bones were the most common site of distant metastasis (37.09%, 102/275). The likelihood of bone metastasis in thoracic NUT carcinoma patients (48.61%, 70/144) was higher than in head-and-neck NUT carcinoma patients (35.82%, 24/67). In addition, NUT carcinoma, found in both thoracic and head/neck regions, was prone to metastasize to the liver (13.45%, 37/275).

3.2.3. NUT carcinoma histopathology and immunohistochemistry

Based on our comprehensive analysis of the available literature, the histopathology of NUT carcinoma predominantly demonstrates poor differentiation (81.90%) and undifferentiation (3.02%). Notably, 72 studies reported partial or focal squamous differentiation, while 32 studies indicated the absence of squamous differentiation. The remaining studies did not specifically address this aspect. Among the literature reporting tumor cell morphology of NUT carcinoma, tumor cells primarily present with three morphological patterns: round cell morphology (approximately 40%), spindle cell features (30%), and epithelioid characteristics (30%). The cells typically exhibit monomorphic growth patterns characterized by vesicular nuclei, prominent nucleoli, and varying amounts of cytoplasm. Microscopic examination frequently reveals sheet-like growth patterns accompanied by extensive necrosis. Regarding specific differentiation, 25% of cases demonstrated neuroendocrine differentiation, 15% exhibited myoepithelial features, and 60% showed no specific differentiation. Many cases presented with numerous mitotic figures and were often associated with geographic necrosis. The stromal component varies from minimal to fibrotic, with occasional infiltration of inflammatory cells. The most prevalent IHC findings for NUT carcinoma included tumor protein p63 (43.84%, 153/349), cytokeratin AE1/AE3 (26.65%, 93/349), ribonucleic acid-binding protein p40 (24.36%, 85/349), and cytokeratin 5/6 (CK5/6; 22.92%, 80/349). The average Ki-67 index expression was approximately 55.16% (15 – 95%).

3.3. Treatment strategy

We identified 17 primary treatment modalities for current NUT carcinoma patients based on the patient's previous treatment histories. NUT carcinoma is generally insensitive to chemotherapy and radiotherapy.[1,18,28] Evidence suggests that surgery, BET inhibitors, HDAC inhibitors, and immunotherapy may be potentially effective against NUT carcinoma.[1,18,28] However, the sample sizes for BET inhibitors, HDAC inhibitors, and immunotherapy are generally limited. When combined with chemotherapy and radiotherapy, the sample size for each group was insufficient (<10 patients). Therefore, we grouped patients who received surgery, BET inhibitors, HDAC inhibitors, and immunotherapy into a category labeled "include surgery/BET/immunotherapy." For example, patients treated with BET inhibitors in combination with chemotherapy or radiotherapy were collectively assigned to the "include BET" group.

3.3.1. Current treatment modalities

Approximately 59.32% (312/526) of patients have relatively comprehensive treatment data. Among the various therapeutic approaches, patients received chemotherapy (18.59%, 58/312), chemotherapy combined with radiotherapy (11.54%, 36/312), chemotherapy combined with surgery and radiotherapy (9.94%, 31/312), and surgery combined with immunotherapy (9.62%, 30/312). In thoracic NUT carcinoma patients, chemotherapy (28.28%, 41/145) and chemotherapy combined with radiotherapy (14.48%, 21/145) were the most common approaches, with 10.34% (30/312) of patients receiving chemotherapy combined with surgery. Conversely, head-and-neck NUT carcinoma patients more commonly received treatment plans based on surgery, including chemotherapy/radiotherapy/surgery (20.34%, 24/118), radiotherapy/surgery (19.49%, 23/118), and surgery combined with immunotherapy (16.95%, 20/118).

3.3.2. Treatment sequence

(a) Treatment sequence 1

We analyzed the treatment sequences of various therapeutic approaches based on the timing when patients initially received each treatment regimen. In the literature dataset, approximately 51.92% (81/156) of thoracic NUT carcinoma patients received chemotherapy first, while 12.18% (19/156) underwent surgery. In head-and-neck NUT carcinoma patients, the majority initially underwent surgery (41.27%, 52/126), with some receiving chemotherapy first (29.37%, 37/126).

(b) Treatment sequence 2

After initial treatment, many thoracic NUT carcinoma patients continued to receive further treatment or, due to the failure of previous treatments, underwent radiation therapy (14.1%, 22/156), chemotherapy (7.69%, 12/156), or BET inhibitors (8.97%,14/156). In contrast, head-and-neck NUT carcinoma patients had a higher proportion receiving subsequent radiation therapy (31.75%, 40/126), followed by chemotherapy (23.02%, 29/126), and surgery (9.52%, 12/126).

(c) Treatment sequence 3 – 4

The number of patients receiving third-line or later treatment was relatively small. Maintenance treatment was primarily based on radiotherapy (7.96%, 27/339) and immunotherapy (2.95%, 10/339) among these patients. Due to the generally poor prognosis of NUT carcinoma patients, the number of patients who received third-line and subsequent treatments remained low. In our data, the proportion of patients receiving immunotherapy (1.47%) was slightly higher than those receiving other treatments.

3.4. Univariate and multivariate analyses

Both multivariate and univariate analyses revealed that the risk ratio for *BRD3::NUTM1* and *BRD4::NUTM1* fusion partners was higher than that for other *NUTM1* fusion partners, such as MAX gene-associated protein (MGA). The risk ratio for NUT carcinoma originating in the thoracic region was higher than that for carcinoma originating in the head and neck. Patients with bone metastasis had a worse prognosis. Conversely, having undergone radiotherapy, immunotherapy, or surgery was identified as a protective factor for patient survival. In addition, receiving radiation therapy as part of salvage treatment prolonged the survival of NUT carcinoma patients. Specific results are available in Figure S3.

3.5. Survival analysis

Further, details on survival data are provided in Table S6.

3.5.1. Patient characteristics

There were no statistically significant differences in prognosis based on patient gender. Asian patients with thoracic NUT carcinoma had better prognoses compared to those from America (overall median survival [mOS]: 5 months) and Europe (mOS: 5 months), with an mOS of 11.4 months. No significant association between age and prognosis in NUT carcinoma was observed. TNM stage, however, was statistically associated with prognosis: patients in stages I–II had the best prognosis (mOS: 54 months), followed by stage III (mOS: 14.9 months), and stage IV had the poorest prognosis (mOS: 6 months). Among thoracic NUT carcinoma patients, a statistically significant difference ($P < 0.05$) in prognosis was observed between stage III (mOS: 12.9 months) and stage IV (mOS: 3 months).

3.5.2. Tumor characteristics

As with other malignancies, tumor size can reflect prognosis to some extent. NUT carcinoma tumors larger than 7 cm had the worst prognosis (mOS: 5 months), followed by tumors between 5 cm and 7 cm (mOS: 8 months). The best prognosis was observed in patients with tumors smaller than 5 cm (mOS: 12 months). However, in thoracic NUT carcinoma, different tumor sizes showed statistically significant differences in prognosis, with a dividing point at a diameter of 5 cm (mOS: 11.6 months). The prognosis of patients with thoracic NUT carcinoma and tumors between 5 – 7 cm (mOS: 4 months) was not significantly different from those with tumors larger than 7 cm (mOS: 4 months). The prognosis for patients with *BRD4::NUTM1* (mOS: 7 months) was significantly worse than that for patients with *NSD3::NUTM1* (mOS: 21 months), with similar findings observed in head and neck carcinoma patients. Prognosis also varied with different metastatic sites. The prognosis for patients with bone metastasis (mOS: 6 months) and lung metastasis (mOS: 6 months) was similar. There were no statistically significant differences in survival analysis across different subgroups of the Ki-67 index (p=0.084). Minimal difference in prognosis was observed between the Ki-67 40–70% and >70% groups (mOS: 8 months [3.6, not available {NA}] versus 8 months [5.7, NA]).

3.5.3. Treatment strategy

We conducted survival analyses separately for patients who received surgery and immunotherapy, comparing them to those who did not. The results demonstrated that patients who underwent surgical treatment achieved a median survival time of 18.7 months (12.2-29), while patients without surgical intervention had a median survival time

of 6 months (5-7.93). Regarding immunotherapy, patients who received immunotherapy treatment reached a median survival time of 19.5 months (12-NA), while those without immunotherapy treatment had a median survival time of 7.93 months (6-10.6).

Although surgery and immunotherapy demonstrated significant survival benefits ($p<0.05$) in the thoracic NUT carcinoma group, immunotherapy had the most pronounced effect. In thoracic NUT carcinoma patients, those who received immunotherapy in combination with other therapeutic modalities achieved a median overall survival (mOS) of 19.5 months. For patients who did not receive immunotherapy, the median overall survival was 6 months. Regarding surgical intervention, patients who underwent surgery demonstrated a median overall survival of 18.7 months (12.2 – 24.6), while those who did not receive surgical treatment had a median overall survival of 10 months (8.4 – 26). In head-and-neck NUT carcinoma patients, those who underwent surgical treatment achieved a median overall survival of 16.5 months, while patients who did not receive surgery had a median overall survival of 7.46 months. Regarding immunotherapy, patients who received combined immunotherapy demonstrated a median overall survival of 31.4 months, whereas those who did not receive immunotherapy had a median overall survival of 11 months.

Patients who received combined immunotherapy during treatment (mOS: 19.5 months [: 12, NA]) showed better survival outcomes compared to those who did not receive immunotherapy (mOS: 7.93 months [6,10.6]), particularly among patients undergoing combined surgery and immunotherapy (mOS: 31.43 [10, NA]). Unfortunately, our study recorded a low incidence of PD-L1/programmed cell death protein 1 (PD-1) positivity, making it challenging to assess the efficacy of immunotherapy in NUT carcinoma patients with negative PD-L1/PD-1 expression. Thus, validation through large-scale cohort studies is necessary.

A survival analysis was conducted on 339 patients, with survival data categorized by treatment type. The treatments were ranked by median survival time as follows (Figure 2): include surgery + immunotherapy (mOS: 31.43 months), radiotherapy + surgery (mOS: 24 months), chemotherapy + radiotherapy + surgery (mOS: 14 months), chemotherapy + surgery (mOS: 12 months), include immunotherapy (mOS: 12 months), chemotherapy + radiotherapy (mOS: 7.93 months), surgery (mOS: 7 months), include BET (mOS: 6 months), radiotherapy (mOS: 5.95 months), include HDAC (mOS: 5.8 months), and chemotherapy (mOS: 4 months). For thoracic NUT carcinoma patients, treatment types were ranked by median survival time as follows: chemotherapy + surgery (mOS: 19 months), including immunotherapy (mOS: 12 months), chemotherapy + radiotherapy (mOS: 6 months), and chemotherapy (mOS: 4 months).

Due to the limited analyzable data, we only evaluated the first and second treatment regimens. The benefit of surgery was most pronounced regardless of whether it was the first or second treatment (sequence 1 mOS: 15 months, sequence 2 mOS: 16.5 months).

Among patients unable to undergo surgery as the first-line treatment, the median survival times were ranked as follows: chemotherapy (mOS: 8 months), radiotherapy (mOS: 7.46 months), and BET (mOS: 6 months). In thoracic NUT carcinoma patients, those receiving radiotherapy (mOS: 6.95 months) had a slightly higher median survival time compared to those receiving chemotherapy (mOS: 6 months). However, this trend was reversed in head-and-neck NUT carcinoma patients, where chemotherapy [mOS: 10 months (8.4,28)] showed a better outcome than radiotherapy [mOS: 7.46 months (5.5,NA)]. No statistically significant differences were observed between the second-line treatment or after surgery.

We also analyzed treatment regimens and specific chemotherapy drugs for NUT carcinoma patients who received chemotherapy exclusively. No significant statistical difference in survival was found between ifosfamide (IFO; mOS: 8 months) alone and platinum-containing chemotherapy regimens without IFO (mOS: 8 months). However, chemotherapy regimens containing IFO had higher OS times compared to other drugs, including Abraxane, docetaxel, paclitaxel, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and etoposide. However, the difference between IFO and platinum-based drugs was not statistically significant.

In thoracic NUT carcinoma patients, chemotherapy regimens containing IFO still provided slightly better survival outcomes than other drugs. Conversely, in head-and-neck NUT carcinoma patients, regimens including platinum-based drugs showed better outcomes than other drugs (Table S6).

Finally, we conducted a separate comparison of regimens containing multiple chemotherapy drugs. The IFO+etoposide+platinum (IEP) regimen provided the best survival benefits (mOS: 10 months) compared to regimens containing only platinum (Taxanes +platinum [TP]: 8.4 months [6,16], etoposide+platinum [EP]: 7 months [4, NA]), or only IFO (IFO+etoposide: 8 months [4.5, NA]). However, no statistically significant difference was found

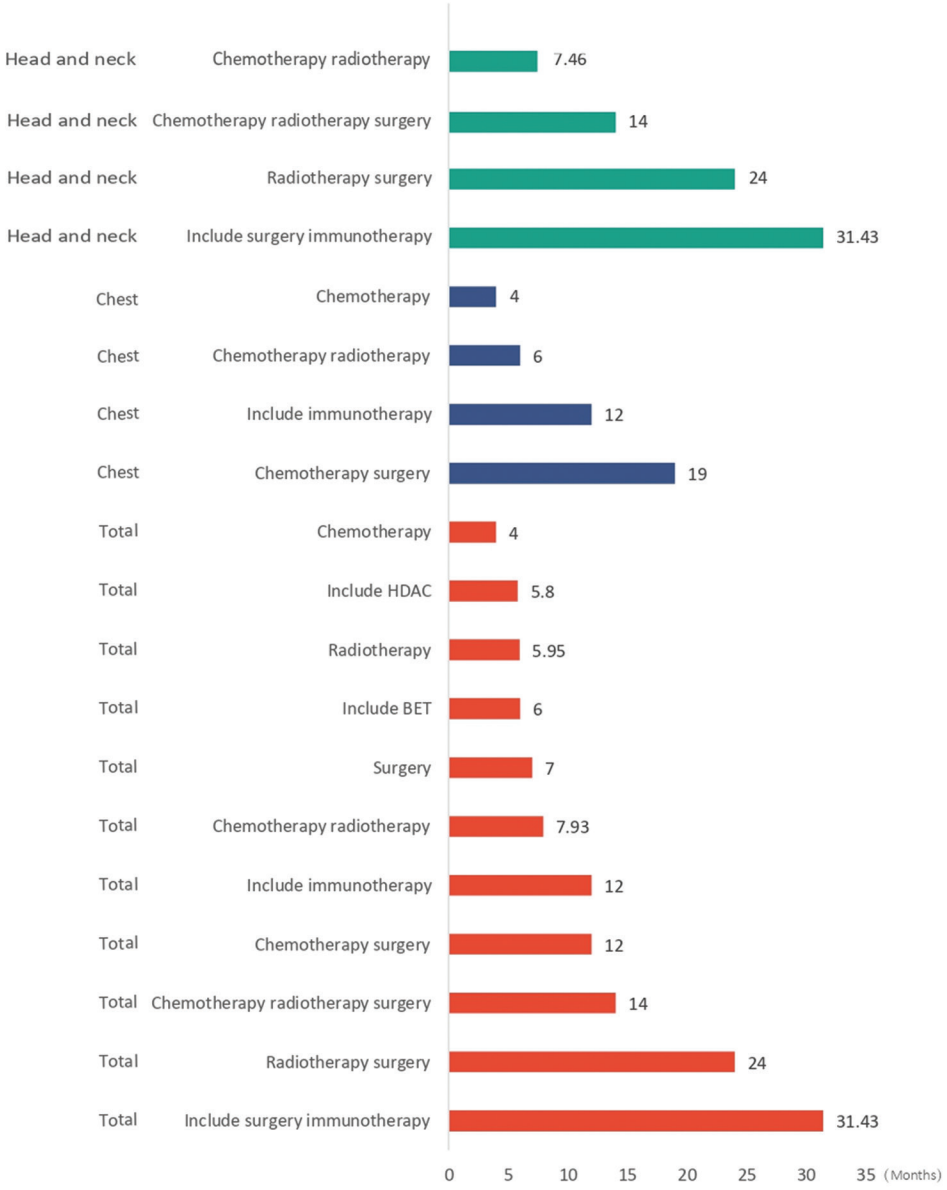


Figure 2. Treatment types ranked by median survival time
Abbreviations: BET: Bromodomain and extra-terminal domain; HDAC: Histone deacetylase.

between these regimens.

4. Expert consensus

Eight recommendations were formulated covering the entire process of diagnosing and treating NUT carcinoma (Table S7). These recommendations address key aspects such as epidemiological characteristics, clinical manifestations, imaging findings, pathological histology and IHC characteristics, molecular mechanisms and subtypes, prognosis, diagnosis, and treatment.

4.1. Epidemiological characteristics

The NUT carcinoma can occur at any age, with varying median ages of onset reported across different studies. More than 80% of NUT carcinoma patients belong to the adolescent and young adult age groups, with onset ranging from newborns to 81 years of age.[15,18,29]

4.2. Clinical manifestations

The clinical symptoms and laboratory test results of NUT carcinoma lack specificity,[28] and the disease typically

presents as rapidly growing masses, often associated with pain and low-grade fever. Between 60% and 77% of cases exhibit distant metastasis at the initial diagnosis,[17,18,30-32] with approximately 50.9% of patients in our dataset classified as stage IV. The most common sites of distant metastasis include the bones, lungs, pleura, liver, brain, adrenal glands, kidneys, and skin. In addition, 68% of cases show involvement of regional lymph nodes.[31] NUT carcinoma typically originates along the midline, with approximately 51% of cases occurring in the thoracic/mediastinal region.[33] In our data, 45.4% of cases were of thoracic origin, and 31.2% were of head-and-neck origin. Clinical manifestations of thoracic NUT carcinoma are often dominated by a persistent cough, with other common symptoms including wheezing, chest tightness, dyspnea, thoracic pain, shoulder pain, back pain, hemoptysis or bloody sputum, and fever.[34,35] Beyond the thoracic region, NUT carcinoma frequently involves the head and neck, particularly the sinonasal area.[31] Most head-and-neck NUT carcinoma cases originate in the nasal cavity, with 30.2% involving the paranasal sinuses and 14.3% affecting the salivary glands. Clinical manifestations of head-and-neck NUT carcinoma include pain at the lesion site, firm swelling of the skin, difficulty opening the mouth, nasal congestion, and other symptoms. At the time of diagnosis, 56.7% of head-and-neck NUT carcinoma patients show invasion of surrounding tissues, with 26.7% exhibiting regional lymph node involvement.[12] Patients with head-and-neck NUT carcinoma may also exhibit pain, swelling, decreased vision, diplopia, facial numbness, choking on liquids, epistaxis, and other symptoms due to tumor invasion of adjacent tissues.

4.3. Imaging manifestations

Computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, positron emission tomography (PET)-CT, and other imaging modalities are crucial for the diagnosis, clinical staging, treatment response assessment, and follow-up monitoring of NUT carcinoma. Endoscopic examinations may also be performed when appropriate, depending on the anatomical sites involved. Contrast-enhanced CT or MRI is recommended for anatomical regions affected by NUT carcinoma. Due to the high malignancy of NUT carcinoma, distant metastasis can occur early, with metastases commonly observed in multiple organs. The skeleton is the most frequent metastatic site, although metastases can occur in other locations, including the brain, liver, adrenal glands, and kidneys.[31] Approximately 75% of bone metastases in NUT carcinoma are osteolytic lesions.[31] Patients with NUT carcinoma often present with single or widespread bone metastases at initial diagnosis (Figure 3). Some patients may exhibit early, moderate osteolytic metastases without obvious clinical symptoms. Metastases may not be present initially but can develop during treatment. It is recommended that a comprehensive, whole-body contrast-enhanced CT scan of the chest, abdomen, pelvis, and brain MRI is performed at the initial visit. If feasible, PET-CT or a whole-body bone scan should be performed to establish baseline data for subsequent treatment planning and efficacy assessment.

Imaging findings in NUT carcinoma are typically nonspecific, resembling those of other common malignant solid tumors in the same anatomical location. These findings are characterized by non-uniform enhancement of low-density masses with significant invasiveness.[36] Primary lung NUT carcinoma typically presents as centrally located masses with invasive, infiltrative growth patterns, and irregular shapes. About 36.8% of cases exhibit pleural effusion, and 57.9% are associated with obstructive atelectasis or obstructive pneumonia.[37] Head-and-neck NUT carcinoma typically manifests as large, poorly defined, and space-occupying masses with internal necrosis and hemorrhage. These tumors often

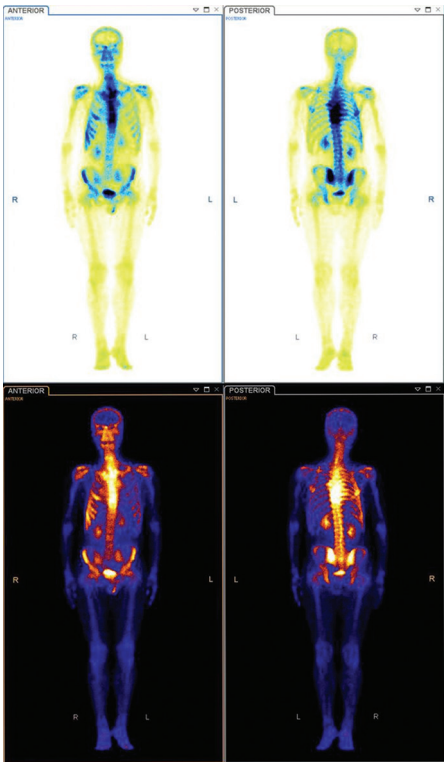


Figure 3. Whole-body bone scan of a 30-year-old male patient with thoracic nuclear protein of the testis carcinoma. The patient, with no history of smoking or alcohol consumption, presented with the right-sided chest pain for 1 month. Bone scan images reveal abnormal radionuclide shadows in multiple right-sided ribs, vertebral bodies in the thoracic spine and the left iliac bone, suggesting bone metastasis.

invade adjacent tissues, such as the sinus walls, muscles, and nerves, and may involve cervical lymph nodes.[12] On CT imaging, head-and-neck NUT carcinoma typically appears as low-density masses with internal necrosis and hemorrhage.

In contrast, MRI findings of NUT carcinoma typically show low-signal intensity on T1-weighted images and high-signal intensity on T2-weighted images, along with heterogeneous enhancement.[36] Both primary and metastatic lesions in thoracic and head-and-neck NUT carcinoma exhibit high fluorodeoxyglucose uptake on PET-CT imaging.[31] Lung NUT carcinoma tumors typically have a maximum standardized uptake value (SUV) exceeding 10, with an average SUV of 12, ranging from 5 to 40. Extra-pulmonary NUT carcinoma tumors have an average SUV of 13.8, ranging from 4.5 to 64.1.[38,39] Imaging features of NUT carcinoma in other locations typically include primary masses accompanied by metastatic lymph node enlargement and widespread distant metastases. These features resemble those of advanced tumors in the respective sites, posing challenges in distinguishing from other malignancies.[38,39] While bone metastases can be evaluated using bone scintigraphy or PET-CT, current data suggest that NUT carcinoma bone metastases are predominantly osteolytic. Since bone scintigraphy relies on enhanced sodium phosphate metabolism driven by osteoblastic activity,[40] PET-CT is recommended for a more accurate assessment.

4.3.1. Recommendation 1

NUT carcinoma frequently presents as large masses, often accompanied by regional lymph node metastases and distant metastases. Due to its lack of specificity, its imaging features closely resemble those of advanced tumors in the corresponding anatomical regions. Bone metastases are commonly observed in NUT carcinoma, and clinicians should be particularly vigilant when patients present with extensive bone metastases at the time of initial diagnosis. Enhanced CT/MRI examinations of the relevant anatomical sites and bone scans or PET-CT (more recommended) are beneficial for staging and treatment evaluation (level of evidence: Grade 4; recommendation level: Strong recommendation).

4.4. Pathological histology and IHC features

4.4.1. Sampling of pathological specimens

Suitable pathological samples for analysis include surgical specimens, biopsy tissues, sputum, fiberoptic bronchoscope brushings, and shed cells from pleural or peritoneal fluid. These samples can be used for various tests, such as hematoxylin and eosin staining, IHC, FISH, NGS, and liquid biopsy for molecular testing. However, samples for molecular testing must undergo enrichment and quality control to ensure reliability. It is essential to note that tissue samples obtained through endoscopy, fine-needle aspiration, or core-needle biopsy may not fully represent the morphology and structure of the tumor. This limitation could hinder the identification of typical histological features, such as abrupt keratinization in NUT carcinoma, thereby increasing the diagnostic challenge.[41] Since NUT carcinoma is frequently diagnosed in advanced stages when tumor samples cannot be obtained through surgery, liquid biopsy samples such as circulating-tumor DNA (ctDNA) and high-throughput sequencing can be considered supplementary diagnostic tools. In addition, endoscopy, fine-needle aspiration, or core-needle biopsy may still be helpful in these cases.

4.4.2. Pathological histological features

Due to its rarity and nonspecific manifestations, NUT carcinoma is often misdiagnosed during the initial pathological examination. In most cases, NUT carcinoma lacks specific histological features, with cancer cells typically showing poorly differentiated morphology.[42] Approximately 33% to 40% of NUT carcinomas may exhibit focal squamous differentiation with abrupt keratinization (Figure 4). In such cases, squamous cells can abruptly form small, round cell clusters with abundant keratinized cytoplasm and nuclear shrinkage, lacking stratification, and gradual differentiation processes.[43] The stroma may show

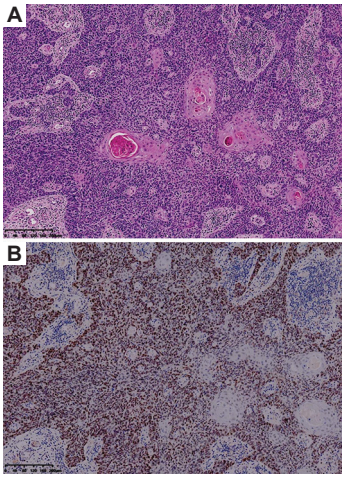


Figure 4. Immunohistochemical (IHC) characteristics of nuclear protein of the testis carcinoma tumor tissue. (A) Hematoxylin and eosin stain (magnification = 10×; scale bar = 200 μm) of a head-and-neck nuclear protein of the testis carcinoma biopsy, revealing areas of sudden keratinization surrounded by primitive, undifferentiated, or poorly differentiated small round cells, with minimal lymphocytic infiltration in the stroma. (B) Nuclear protein of the testis IHC staining (magnification = 10×; scale bar = 200 μm) of the same biopsy, showing diffuse nuclear staining.

edema, mucinous appearance, and fibrous changes, often accompanied by varying connective tissue proliferation. Neutrophil infiltration may also be observed, occasionally with intraepithelial and stromal lymphocyte infiltration.[42]

4.4.3. IHC markers

Immunohistochemistry in NUT carcinoma can reveal a range of epithelial markers to varying extents, including AE1/AE3, epithelial membrane antigen p63, p40, as well as sex-determining region Y-box 2 (SOX2), thyroid transcription factor-1, paired box 8, and MYC. In addition, other relevant markers such as neuroendocrine markers (e.g., synaptophysin, chromogranin, cluster of differentiation [CD] 56), lymphocyte markers (e.g., CD56, CD30, CD34, CD43, CD138, and CD45), germ cell tumor markers (e.g., CD30, placental alkaline phosphatase, and split-like protein 4), vascular markers (e.g., friend leukemia integration 1 transcription factor [FLI1] and CD34), and Ewing's sarcoma markers (e.g., FLI1 and CD99) can also be expressed in NUT carcinoma.[2] In our analysis, the top positive IHC markers were p63 (43.84%), AE1/AE3 (26.65%), p40 (24.36%), CK5/6 (22.92%), and CD99 (12.61%). With few exceptions, the NUT protein was expressed in nearly all cases of NUT carcinomas;[44] however, it was also widely expressed in other solid tumors. The characteristic diagnostic feature of NUT carcinoma is the diffuse nuclear dot-like pattern of NUT staining positivity.[45]

(a) Recommendation 2

Apart from surgical specimens, NUT carcinoma tissue samples can be obtained through endoscopy, aspiration, and core-needle biopsy. The histology of NUT carcinoma is often nonspecific, with some cases showing focal squamous differentiation accompanied by sudden keratinization and neutrophil infiltration. IHC staining for NUT typically reveals diffuse nuclear dot-like patterns. While NUT positivity is a key feature, other IHC markers in NUT carcinoma do not exhibit specificity (level of evidence: grade 4; recommendation level: strong recommendation). If the diagnosis remains inconclusive through these methods, supplementary diagnostic approaches, such such as high-throughput sequencing via blood ctDNA, can be considered (level of evidence: grade 5; recommendation level: strong recommendation).

4.5. Molecular mechanisms and molecular subtypes of NUT carcinoma

The *NUTM1* gene on chromosome 15 encodes the NUT protein, which is usually expressed in testicular and ovarian germ cells. Chromosomal translocations or rearrangements lead to fusion with various partner genes.[42] In over 70% of NUT carcinoma patients, *NUMT1* fuses with *BRD4*, a gene commonly expressed in the cell nucleus. Comparative genomic analyses suggest that the classification, clinical behavior, and treatment strategies for NUT carcinoma are best defined by the fusion partner of *NUTM1* rather than by tumor morphology or IHC profile.[46] Evidence indicates that NUT carcinoma is driven by the NUT fusion oncoprotein, with BRD4 being the most common fusion partner. Less common fusion partners include *BRD3::NUTM1* t(15;19)(q14;p34.2), *NSD3::NUTM1*, t(8;15)(p12;q15), and zinc finger protein (*ZNF*) *532,* among others.

The survival rate of NUT carcinoma patients is closely related to the tumor site and the specific fusion partners of NUTM1, with BRD4 fusion generally associated with the poorest prognosis.[18] BRD4 is a member of the BET protein family, which includes two bromodomains that bind to acetylated histones and an additional C-terminal protein interaction domain.[47] Bromodomain-containing proteins 2 (BRD2), BRD3, and BRD4 share sequence homology and participate in the transcriptional regulation of numerous genes involved in cell growth, proliferation, survival, and inflammation. Among these, BRD4 acts as a coactivator in the transcription of genes involved in the G1 phase of the cell cycle (such as MYC, B-cell lymphoma 2 [BCL2], and cyclin-dependent kinase 9 [CDK9]).[48] Fusion of NUTM1 with BRD2, BRD3, or BRD4 generates chimeric genes that encode BRDX-NUTM1 fusion proteins; fusions with other partner genes (such as *NSD3*) are collectively referred to as NUT variants.[49] *BRD4::NUTM1* typically inhibits cellular differentiation and supports the growth of NUT carcinoma cells by binding to acetylated chromatin through its dual bromodomains. It recruits histone acetyltransferase p300 to enhance wild-type BRD4 activity, markedly increasing the transcription of target genes, thereby activating oncogenes such as MYC and SOX2.[50] MYC and SOX2 maintain cells in a relatively undifferentiated, stem-like state, similar to cells in NUT carcinomas. Overexpression of oncogenes such as MYC activates cyclin D1 and cyclin D2, driving the cell cycle[51] while simultaneously inhibiting p53 activation, which blocks apoptosis[52] and facilitates tumor progression. A genetically engineered mouse model of NUT carcinoma based on the *BRD4::NUTM1* fusion gene closely mirrors human NUT carcinoma. This model provides strong evidence that BRD4-NUT alone drives squamous progenitor cells into a cancerous stage, with BET inhibitors inducing differentiation and extending survival.[53]

In addition, it is important to note that NUT carcinoma is generally considered a rare, undifferentiated squamous cell carcinoma, typically characterized by fusion partners such as BRD4, BRD3, NSD3, ZNF532, or ZNF592, predominantly affecting the mediastinum or head and

neck regions. As next-generation sequencing technologies have expanded, more NUTM1 fusion partners have been identified. Consequently, NUT carcinoma is now recognized as a subtype of NUTM1-rearranged neoplasms (NRNs), which also include skin tumors, sarcomas, CNS tumors, and hematologic malignancies.[29]

Skin tumors associated with NUTM1 rearrangements include classic poroma, hidradenoma simplex, dermal duct tumor, poroid hidradenoma, porocarcinoma, and malignant poroid hidradenoma/poroid hidradenocarcinoma. The fusion transcripts in these tumors primarily include yes1 associated transcriptional regulator (*YAP1*)::*NUTM1*, WW domain containing transcription regulator 1 (*WWTR1*):*NUTM1*, and endoplasmic reticulum membrane protein complex subunit 7 (*EMC7*)::*NUTM1*. Sarcomas with NUTM1 rearrangements can originate in soft tissues, dura mater, thoracic structures, ovaries, or the colon. The relevant fusion transcripts include MAX dimerization protein 1 (*MXD1*)::*NUTM1*, MAX interactor 1 (*MXI1*)::*NUTM1*, MAX dimerization protein MGA (*MGA*)::*NUTM1*, MAX dimerization protein 4 (*MXD4*)::*NUTM1*, B-cell lymphoma 6 corepressor like 1 (*BCORL1*)::*NUTM1*, and *CIC*::*NUTM1*, among others. These sarcomas can present with fibrosarcoma, epithelioid, and rhabdomyoblastic morphology. CNS tumors linked to NUTM1 rearrangements include fusion transcripts such as *CIC*::*NUTM1*, ataxin 1 (*ATXN1*)::*NUTM1*, par-3 family cell polarity regulator beta (*PARD3B*)::*NUTM1*, and spectrin beta, non-erythrocytic 4 (*SPTBN4*)::*NUTM1*. Hematologic malignancies associated with NUTM1 rearrangements can occur in B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), and acute myeloid leukemia (AML). The fusion transcripts in B-ALL typically include apoptotic chromatin condensation inducer 1 (*ACIN1*)::*NUTM1*, cut-like homeobox 1 (*CUX1*)::*NUTM1*, *ZNF618*::*NUTM1*, solute carrier family 12 member 6 (*SLC12A6*)::*NUTM1*, chromodomain helicase DNA binding protein 4 (*CHD4*)::*NUTM1*, bromodomain containing 9 (*BRD9*)::*NUTM1*, AF4/FMR2 family member 1 (*AFF1*)::*NUTM1*, ATPase family AAA domain containing 5 (*ATAD5*)::*NUTM1*, Runt-related transcription factor 1 (*RUNX1*)::*NUTM1*, Ikaros family zinc finger 1 (*IKZF1*)::*NUTM1*, nucleosome assembly protein 1 like 4 (*NAP1L4*)::*NUTM1*, and apoptosis and caspase activation inhibitor (*AVEN*)::*NUTM1*. Meanwhile, T-ALL shows fusion transcripts such as bromodomain PHD finger transcription factor (*BPTF*)::*NUTM1*, and AML demonstrates fusion transcripts such as zinc finger and BTB domain containing 7A (*ZBTB7A*)::*NUTM1*, adenylate cyclase 7 (*ADCY7*)::*NUTM1*, and apoptosis and caspase activation inhibitor (*AVEN*)::*NUTM1*. Myeloid neoplasms associated with NUTM1 mainly involve the nucleosome assembly protein 1 like 4 (*NAP1L4*)::*NUTM1* fusion transcript.[2,29]

The distinction between NUT carcinoma and other non-NUT carcinoma is not entirely clear and is mainly based on fusion partners, tumor origin, and pathology. The oncogenic mechanisms of NUT carcinoma and other non-NUT carcinoma may differ.[2,29,42,54] Moreover, non-NUT carcinoma tends to respond more favorably to standard therapies, exhibit lower malignancy and invasiveness, and show better overall prognoses than NUT carcinoma.[29,55]

4.6. Prognosis

NUT carcinoma is associated with a poor prognosis and rapid progression, with a median survival period of 6.7 – 9.5 months. Many patients lose the opportunity for surgery due to local progression and distant metastasis.[2,15,17,30,56,57] For NUT carcinoma patients aged 7 – 16 years, the median survival is 8 months (ranging from 4.5 months to 2.4 years), with 60% of cases originating in the thoracic region (median survival of 4.5 – 15 months) and 40% in the head, neck, or adjacent to the spine (median survival of 10 months – 2.4 years).[58] In a study by Chau *et al.*[18] involving 124 patients, it was reported that the prognosis for primary NUT carcinoma originating in the thorax is worse compared to other locations, with a higher likelihood of distant metastasis, larger tumors (>6 cm) that are difficult to remove completely, and a mOS of only 4.4 months, resulting in a 2-year survival rate of 5%. Patients with thoracic NUT carcinoma carrying the *BRD4*::*NUTM1* fusion gene have the worst prognosis.[18,59] In contrast, those without this fusion (such as *BRD3*::*NUTM1* and *NSD3*::*NUTM1*) exhibit the best prognosis among non-thoracic NUT carcinoma patients, with a 2-year survival rate of 64% and a mOS of 36.5 months.[18] The mOS for non-thoracic NUT carcinoma tumors with the *BRD4*::*NUTM1* fusion is 10 months.[29] Patients with head and neck NUT carcinoma tend to have a better prognosis compared to those with tumors in the lungs and other locations (OS: 16 months vs. 6 months).[31]

4.7. Diagnosis

Diagnosing NUT carcinoma is challenging due to its nonspecific clinical manifestations and histopathological features, which require differentiation from other small round cell tumors such as Ewing sarcoma, primitive neuroectodermal tumor (PNET), rhabdomyosarcoma, synovial sarcoma, undifferentiated carcinoma (including nasopharyngeal or salivary gland undifferentiated carcinoma), proliferative fasciitis-like low-grade fibromyxoid sarcoma, and other poorly differentiated or undifferentiated tumors.[1] Similar to hematologic malignancies, NUT carcinoma can also present with

diffuse bone infiltration and express CD34, which can lead to misdiagnosis as acute leukemia or other hematologic diseases.[60] NUT carcinoma lacks specific diagnostic morphological features; it is a poorly differentiated or undifferentiated tumor, with some cases exhibiting sudden keratinized foci, leukocyte infiltration, and extensive necrosis. At present, NUTM1 rearrangement can be confirmed using IHC staining for NUT positivity or FISH (with over 20% of tumor cells showing the t(15;19)(q14;p13.1) separation probe-positive), along with other molecular techniques such as cell cytogenetics, reverse transcription polymerase chain reaction, and second-generation sequencing, all of which assist in diagnosing NUT carcinoma.[18] In the 2021 edition of the WHO classification of lung cancer, NUT carcinoma is categorized under "other epithelial tumors of the lung."

The 2024 National Comprehensive Cancer Network clinical practice guidelines for non-small-cell lung cancer recommend testing for NUT rearrangement in all poorly differentiated lung cancers that lack glandular differentiation or specific causes, especially in nonsmokers or young patients. However, this recommendation is not widely followed due to the rarity of NUT carcinoma. Moreover, when NUT carcinoma masses are detected in non-midline anatomical structures (e.g., head/neck/thorax) or in elderly patients with limited tumor samples that lack typical NUT carcinoma morphology, NUT IHC staining may not be performed, potentially leading to misdiagnosis.[61] According to the 2023 Chinese Society of Clinical Oncology guidelines for head-and-neck tumors, NUT IHC testing is recommended as a level II diagnostic tool. It is advised for patients with poorly differentiated carcinoma, regardless of age or medical history. According to the WHO recommendations, NUT antibody IHC staining with a cutoff value of ≥50% positivity for the C52B1 clone has 100% specificity and 87% sensitivity for diagnosing NUT carcinoma.[45] Although transcriptome sequencing has higher sensitivity in NGS than DNA-based NGS, false negatives are still possible. Therefore, NGS results should be validated using other molecular detection methods to ensure diagnostic accuracy.

Fluorescence *in situ* hybridization with a NUTM1 breakpoint 15q14 probe is considered one of the essential methods for diagnosing NUT carcinoma.[2] However, FISH has low resolution and may miss potential breakpoints. Therefore, when cases are IHC NUT-positive, full-length NUTM1 probe FISH or second-generation sequencing (preferably transcriptome sequencing) is recommended to confirm the diagnosis.[2]

At present, NUT carcinoma can be diagnosed using NUTM1 IHC or FISH. While NUT carcinoma shows high IHC specificity, molecular testing can identify various fusion partners of NUTM1, which helps predict patient prognosis. BET inhibitors may have suboptimal efficacy for patients with gene fusions not dependent on BRD4. Therefore, identifying NUTM1 fusion partners and available drug targets through second-generation sequencing can validate the pathological diagnosis, predict disease prognosis, and inform treatment options, including later-stage drug therapies and combination treatments.[41,62]

4.7.1. Recommendation 3

The clinical presentation, histopathology, and conventional IHC features of NUT carcinoma lack specificity, highlighting the importance of using specific NUT antibodies for IHC staining in its diagnosis. For patients with an initial diagnosis accompanied by rapid progression or distant metastasis, especially those with poorly differentiated carcinomas or small round cell tumors, it is strongly recommended to consider NUT IHC examination (evidence level: grade 4; recommendation level: strong recommendation). In addition, FISH or high-throughput sequencing should be considered to identify the type of NUTM1 fusion partner (such as BRD4, BRD3, and NSD3) (evidence level: grade 5; recommendation level: strong recommendation).

4.8. Treatment

Given the highly invasive nature and poor prognosis of NUT carcinoma, it is essential to emphasize the importance of multidisciplinary team (MDT) consultations, including the involvement of a Molecular Tumor Board (MTB). In the MTB, experts from various disciplines collaboratively analyzed the patients' clinical manifestations, imaging, pathology, and molecular biology/genetic data to comprehensively assess the patient's general condition, disease diagnosis, extent of invasion, and prognosis. Based on existing treatment standards and evidence-based medicine, combined with available treatment methods, treatment plans should be adjusted in real time according to changes in the patient's condition to develop the most appropriate treatment strategy.

NUT carcinoma can occur in various organs, and currently, there is no specific staging system exists for this cancer. Therefore, the staging system from the American Joint Committee on Cancer for tumors in corresponding locations may be applied to NUT carcinoma.

A study of 124 patients with NUT carcinoma showed an objective remission rate of 49% for treatments involving surgery, radiotherapy, and chemotherapy.[18] New treatment possibilities may emerge from targeting

fusion partners, including inhibiting DNA binding with BET inhibitors or modifying downstream histones with HDAC inhibitors to disrupt the function of fusion partners. NUT carcinoma is a newly classified tumor by the WHO, and awareness of it remains limited globally. At present, most treatment evidence for NUT carcinoma comes from retrospective analyses and case reports, with relatively less evidence from treatment-related studies in NUT carcinoma patients. Moreover, given that NUT carcinoma can arise in various organs, treatment guidelines for other tumors in affected locations may be adopted for NUT carcinoma. Based on relevant literature, clinical experience with NUT carcinoma, and expert opinions, this consensus refers to guidelines for other tumors in the specific locations affected by NUT carcinoma. It summarizes the principles for treating NUT carcinoma (Figure 5). Patients with NUT carcinoma who are candidates for surgery should undergo the procedure.

For patients unsuitable for surgery or require adjuvant therapy post-surgery, other treatment modalities, such as radiotherapy and systemic therapies (e.g., chemotherapy and targeted therapy), can be considered based on the patient's condition. Considering the rapid progression and high malignancy of NUT carcinoma, particularly in cases with poor prognosis, such as those carrying the *BRD4::NUTM1* fusion gene, participation in clinical trials is strongly recommended. Maintenance therapy based on prior treatments can continue if lesions stabilize following initial treatment. In cases of disease progression, potential drugs should be selected based on high-throughput sequencing results, with treatment plans tailored to the patient's previous treatment history. If necessary, MDT consultations can guide further diagnosis and treatment. We encourage patients to participate in prospective clinical trials and explore potential new treatment options.

4.8.1. Surgery

Surgery plays a pivotal role in the comprehensive management of NUT carcinoma. Surgical strategies should be developed based on the patient's overall health, the extent of tumor invasion, tumor staging, and the feasibility of resection, which can be assessed through enhanced CT or MRI evaluation. Surgery is the primary curative method for NUT carcinoma, with surgical techniques often adapted from protocols used for similar cancers in specific anatomical sites. Before surgery, a specialized medical team should evaluate or discuss in an MDT meeting whether NUT carcinoma patients should undergo neoadjuvant therapy. Patients eligible for direct surgical excision should be assessed based on pre-operative and post-operative TNM staging, pathological findings, surgical resection margins (R0, R1, and R2), and post-operative lymph node involvement to determine the need for post-operative adjuvant therapy. Surgical treatment remains a viable option for patients with localized tumor recurrence, which provided that they are still deemed suitable for surgery. For patients unsuitable for surgery, alternative treatment modalities such as radiotherapy and systemic treatment (e.g., chemotherapy and targeted therapy) may be considered.

Relying solely on chemotherapy is unlikely to result in a favorable prognosis. In cases where NUT carcinoma lesions are localized without distant metastasis, comprehensive surgical resection should be actively considered, as it can lead to a better prognosis when followed by radiotherapy and chemotherapy. Some studies on head and neck NUT carcinoma suggest that prophylactic cervical lymph node dissection should be considered even in the absence of lymph node metastasis (N0).[1] Patients with head-and-neck NUT carcinoma who undergo surgical resection followed by radiotherapy tend to experience longer OS and progression-free survival (PFS). In head-and-neck NUT carcinoma, the extent of surgical resection, the achievement of negative margins, and the response to initial treatment are all significantly associated with better PFS and OS. For instance, patients who underwent surgery had a 2-year OS rate of 50%, compared to just 7% for those who did not (p=0.003). The degree of surgical resection also correlated with PFS and OS in a graded manner: patients with negative margins had a 2-year OS of 80%, those with gross total resection but positive margins had a 2-year OS of 44%, and those who underwent debulking had a 2-year OS of 37%.[30,56] However, these findings require further validation through prospective research.[56] Palliative surgical debulking may also be an option to improve quality of life.[63]

In addition, collaboration among multidisciplinary surgical teams may be crucial due to the aggressive nature of NUT carcinoma, mainly when it affects multiple anatomical regions. Following surgery, proactive planning should include radiotherapy for the primary tumor site and affected lymph nodes, along with timely systemic treatment.[12,56]

(a) Recommendation 4

Patients with resectable NUT carcinoma should undergo curative surgery as the primary treatment as early as possible. Post-operative adjuvant therapy should be determined based on pre-operative and post-operative TNM staging, pathological conditions, surgical margins (R0, R1, and R2), and post-operative lymph node status (level of evidence: level 3; recommendation grade: strongly

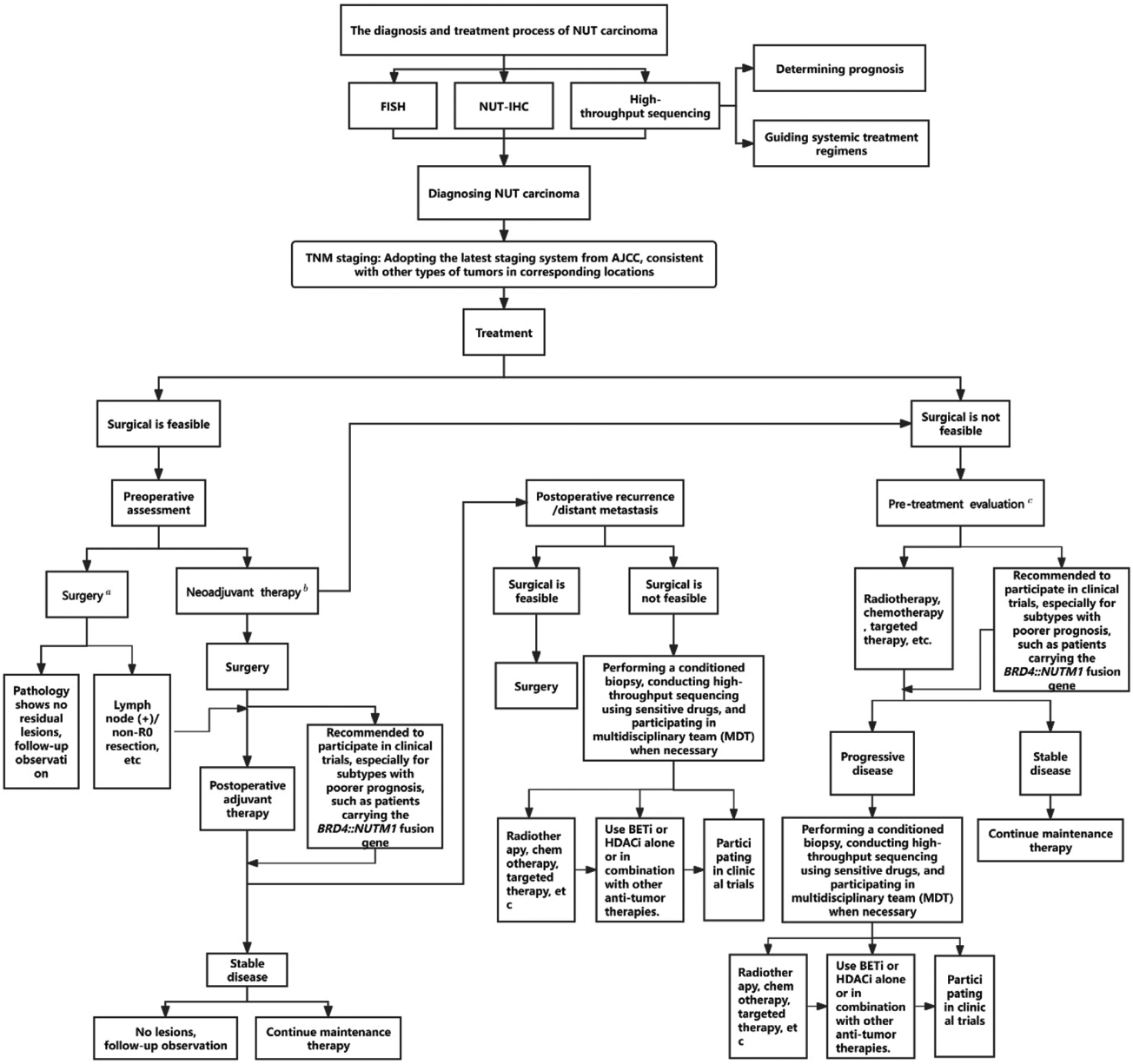


Figure 5. Flowchart of diagnosis and treatment for the nuclear protein of the testis carcinoma

Notes: [a]Prior to surgery, assessment by specialists or a multidisciplinary team can determine whether direct surgery is feasible. Postoperatively, decisions on whether to undergo adjuvant therapy are based on factors such as the patient's pre-operative TNM staging, pathological findings, surgical margin status (R0, R1, R2), and lymph node positivity. [b]Prior to surgery, evaluation by specialists or a multidisciplinary team can assess the feasibility of neoadjuvant therapy for potentially resectable nuclear protein of the testis carcinoma. Specific neoadjuvant treatment modalities are recommended based on guidelines for other tumor types at the specific site of NUT carcinoma. [c]Non-surgical patients with nuclear protein of the testis carcinoma should undergo evaluation by specialists or a multidisciplinary team before treatment. Treatment decisions should be based on guidelines for other tumor types at the specific site of nuclear protein of the testis carcinoma, as well as the patient's overall condition.

Abbreviations: AJCC: American Joint Committee on Cancer; BRD4: Bromodomain-containing protein 4; FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemistry; NUT: Nuclear protein of the testis; NUTM1: Nuclear protein of the testis midline carcinoma family member 1; TNM: TNM classification of malignant tumors.

recommended). Pre-operative assessment by specialized physicians or an MDT can help determine the feasibility and approach of neoadjuvant therapy (level of evidence: level 4; recommendation grade: strongly recommended).

4.8.2. Radiotherapy

Considering the high malignancy and aggressive invasiveness of NUT carcinoma, along with its propensity for recurrence, it is recommended that post-operative

NUT carcinoma patients who have undergone non-R0 resections, exhibit lymph node positivity, and receive post-operative adjuvant treatments. Many NUT carcinoma patients are not candidates for surgery at the time of diagnosis; for such patients, concurrent chemoradiotherapy becomes a viable treatment option for inoperable tumors. Standardization of radiation doses for NUT carcinoma remains elusive. Radiation plans should incorporate at least three-dimensional conformal techniques, with a preference for intensity-modulated radiotherapy. The radiation dose and target area should be tailored to the patient's overall health, tumor site involvement, and tumor staging. These parameters should be informed by radiation doses and target areas used for advanced malignancies in similar tumor types.

Radiation therapy generally employs curative doses, although palliative doses may be used if the tumor is located near critical organs or if the patient has limited tolerance for treatment. Radiation doses typically range from 50 Gy to 70 Gy, with studies showing that NUT carcinoma patients who received doses exceeding 50 Gy have a higher survival rate.[56,64] Before radiation therapy, a comprehensive evaluation of the patient's overall health, dietary habits, speech, and any organs that may be irradiated within the target area is required. It is recommended that the primary tumor area is irradiated with 65 – 70 Gy and the affected regional lymph nodes with 50 – 54 Gy" to: "In cases where radiotherapy is used exclusively, it is recommended to deliver a dose of 65 – 70 Gy to the primary tumor and lymph node lesions, and 50 – 54 Gy to elective lymph node regions.[1] The radiation dose should be adjusted based on the lesions and the extent of local disease involvement, including tumor margins and neurovascular invasion. The target area should encompass as many microlesions as possible, with recommendations to irradiate the expanded primary tumor site and any involved lymph node regions.

Chemotherapy drugs used during concurrent chemoradiotherapy for NUT carcinoma patients are typically based on those used for other malignancies at the same anatomical sites, with platinum-based agents being the most common. In one case of head and neck NUT carcinoma with primary lesions in the ethmoid and sphenoid sinuses, the lesions involved both optic nerves and the skull base 1.5 months postoperatively. After 11 sessions of concurrent chemoradiotherapy and hyperthermia (total radiation dose: 69.96 Gy, 2.12 Gy × 33F; cisplatin, temozolomide 75 mg/m^2), the lesions shrank significantly. However, due to side effects from radiotherapy, the patient was unable to complete the treatment, which led to an increase in the dose of temozolomide (200 mg/m^2), but the tumor continued to progress. Eventually, adding lapatinib led to further shrinkage of the lesions, but this effect lasted only 2 months, resulting in an OS of 17 months.[65] In another case, a 22-year-old patient with supraglottic NUT carcinoma (stage cT3N2bM0) achieved a complete response after concurrent chemoradiotherapy (total radiation dose: 70 Gy, 2 Gy × 35F; cisplatin 80 mg/m^2 every 3 weeks), although ovarian metastasis occurred 3 weeks after completing radiotherapy.[66] In the case of pulmonary NUT carcinoma, combined radiotherapy and anlotinib resulted in a 78% reduction in tumor size within 2 months.[67] A patient with locally advanced head-and-neck NUT carcinoma achieved complete remission after concurrent chemoradiotherapy and alternating regimens of vincristine, doxorubicin, and cyclophosphamide, with IFO and etoposide.[68] Adolescent patients tend to have a poor prognosis, with a median survival of 8 months (range: 4.5 – 28.8 months) for five NUT carcinoma patients aged 7 – 16 years who received radiotherapy and chemotherapy.[58]

Before radiotherapy, it is necessary to assess the patient's general condition, diet, speech, and any organs affected by irradiation. For NUT carcinoma patients with high-risk factors such as positive lymph nodes or non-R0 resection and those who are unsuitable for surgery, an MDT evaluation should be conducted before treatment to determine the most appropriate comprehensive treatment, which should primarily involve concurrent or sequential chemoradiotherapy. The radiation target area and dose can be based on those used for other malignancies at corresponding sites.

(a) Recommendation 5

Patients with high-risk factors, such as lymph node positivity or non-R0 resection, especially those with inoperable NUT carcinoma, particularly in the head-and-neck region, should undergo evaluation by specialized physicians or an MDT before treatment, aiming to select comprehensive therapy, primarily based on concurrent chemoradiotherapy. The radiotherapy protocol and dosage can be guided by delineating target areas and radiation doses for other corresponding malignant tumors (level of evidence: grade 3; recommendation level: strongly recommended).

4.8.3. Chemotherapy

The response rate of NUT carcinoma to chemotherapy is approximately 40%, but it tends to develop resistance regardless of the type of chemotherapy regimen used.[20,64] Anthracyclines, cisplatin, alkylating agents, etoposide, gemcitabine, irinotecan, and taxanes have been reported as part of chemotherapy combinations for NUT carcinoma. However, almost all chemotherapy regimens demonstrate only temporary efficacy or no therapeutic response.[1,20,56,64,69]

Some studies suggest an association between chemotherapy and improved survival rates,[56] but consensus has not been reached on the optimal chemotherapy regimen and timing. Relying solely on chemotherapy is often ineffective in controlling the progression of NUT carcinoma.

At present, the effective chemotherapy regimens used for achieving some degree of relief in multimodal treatments – combined with surgery, radiotherapy, and targeted therapy – are primarily based on anthracyclines, alkylating agents, and platinum compounds.[69,70] In a cohort study of 118 NUT carcinoma patients, the objective response rate with chemotherapy, including IFO, was 75%, compared to only 31% with platinum compounds; however, both regimens showed no difference in OS and PFS.[71] Some reported cases suggest that patients achieved favorable outcomes using high-dose etoposide+doxorubicin+IFO (VAI) and cisplatin+doxorubicin+IFO (PAI) as part of the Scandinavian Sarcoma Group IX (SSG IX) protocol for sarcoma.[72,73] For example, a patient with *BRD3::NUT* head-and-neck NUT carcinoma (stage T4bN0M0) achieved partial response (PR) after alternating treatment with VAI and PAI, followed by radiotherapy and a total of 4 cycles of chemotherapy. On resection, no residual cancer was found, indicating a pathological complete response.[72] Another patient with a vast mediastinal mass showed tumor shrinkage after one cycle of EP and the SSG IX regimen, but the tumor progressed again after 1 month.

Despite subsequent treatments with radiotherapy, anlotinib, bevacizumab, paclitaxel chemotherapy, and pembrolizumab immunotherapy, the patient passed away 4.7 months after diagnosis. Among these treatments, only radiotherapy, the EP regimen, and the SSG IX chemotherapy regimen alleviated the patient's pain and temporarily controlled tumor progression.[74] Two pediatric patients with head-and-neck NUT carcinoma maintained tumor remission for at least 6 years and 8 months, respectively, after receiving three cycles of SSG IX chemotherapy regimen, along with surgical resection and radiotherapy.[70] At present, the most effective chemotherapy regimens reported for NUT carcinoma are those related to sarcoma treatment protocols. However, there are relatively few documented cases of effective chemotherapy specifically for NUT carcinoma, warranting further exploration. It is recommended that chemotherapy drugs be selected based on anthracyclines, alkylating agents, and platinum compounds in combination with chemotherapy regimens for other types of malignancies in corresponding anatomical sites of NUT carcinoma. In addition, patients should actively consider high-throughput sequencing to help identify potentially sensitive personalized drug therapy regimens.

At present, no prospective randomized controlled trials demonstrate induction chemotherapy's effectiveness in improving survival outcomes for NUT carcinoma. For operable NUT carcinoma patients undergoing induction chemotherapy, close monitoring of tumor size is necessary to prevent rapid progression and loss of surgical opportunities. For inoperable NUT carcinoma, a comprehensive treatment model combining induction chemotherapy with radiotherapy may be considered. However, no prospective randomized controlled trials have demonstrated that adjuvant chemotherapy improves survival in NUT carcinoma after radiotherapy. Given the high recurrence rate of NUT carcinoma, traditional treatment (surgery, radiotherapy, and chemotherapy) generally only temporarily controls disease progression. It is, therefore, recommended to consider appropriate systemic treatments after concurrent chemoradiotherapy or radiotherapy, depending on factors such as the patient's overall condition. Considering chemotherapy's toxicity, a pre-chemotherapy assessment and consideration of the cumulative chemotherapy dose is necessary to reduce both acute and long-term side effects. Effective chemotherapy regimens for NUT carcinoma remain limited, and clinical trials for rare disease chemotherapy regimens should be prioritized to identify potentially effective first-line treatments.

(a) Recommendation 6

Clinicians may consider selecting chemotherapy drugs primarily based on IFO or platinum-based agents or combining them with chemotherapy regimens used for other types of malignancies in corresponding anatomical sites of NUT carcinoma (level of evidence: level 4; recommendation grade: strongly recommended). High-throughput sequencing can be considered to identify potentially sensitive personalized drug regimens (level of evidence: level 5; recommendation grade: recommended). For inoperable NUT carcinoma, a comprehensive treatment model combining induction chemotherapy with radiotherapy should be considered (level of evidence: level 4; recommendation grade: strongly recommended). Specific chemotherapy regimens should be evaluated based on the patient's condition after concurrent chemoradiotherapy (level of evidence: level 4; recommendation grade: strongly recommended).

4.8.4. Immunotherapy

The NUT carcinoma is driven by the fusion of the NUT oncogenic protein, which is typically associated with a low tumor mutation burden, and the tumor is often insensitive to immunotherapy.[75] Only a subset of NUT carcinoma patients expresses programmed cell death ligand 1 (PD-L1). Aside from a few reported cases, there

are currently no prospective randomized controlled trials that demonstrate the effectiveness of immunotherapy in improving the survival of NUT carcinoma patients. A case report of primary thyroid NUT carcinoma revealed a PD-L1 combined positive score of ≥30, with the patient surviving for 10 months following a combination of chemotherapy and immunotherapy.[57] Some studies have reported cases of thoracic NUT carcinoma patients with high PD-L1 expression who were treated with immunotherapy, leading to significantly prolonged survival. These patients, who also received combined surgery, chemotherapy, and other treatments, survived for over 7.5 years.[76] In contrast, other studies suggest that PD-L1-negative NUT carcinoma patients can experience tumor shrinkage after receiving immune checkpoint inhibitors. While a few patients have shown partial or complete remission, these responses have generally been short-lived, with lesions rapidly relapsing or metastasizing.[77] Moreover, there are cases in which a combination of immunotherapy and oncolytic viruses has relieved both primary and metastatic lesions in patients.[78]

(a) Recommendation 7

For NUT carcinoma patients who are PD-L1/PD-1 positive, it is strongly recommended to consider combined immunotherapy as part of standard anti-tumor treatment. For patients who are PD-L1/PD-1 negative, the decision to use combined therapy should be made based on individual patient preference and circumstances (level of evidence: level 4; recommendation grade: strongly recommended).

4.8.5. Targeted therapy

Early research suggests that BET inhibitors or HDAC inhibitors can induce epithelial differentiation of NUT carcinoma cells and inhibit cell proliferation.[78] This event, along with a deeper understanding of NUT carcinoma's pathogenesis, has spurred the development of targeted therapies, particularly given the limited long-term efficacy of conventional treatments.

Histone deacetylase inhibitors, such as vorinostat, can induce histone acetylation, squamous differentiation, and growth arrest. For example, an 11-year-old patient with *BRD4::NUTM1* fusion salivary gland NUT carcinoma experienced partial remission of both primary and metastatic lesions after undergoing four cycles of chemotherapy (IFO+etoposide) combined with the HDAC inhibitor vorinostat as part of multimodal therapy.[79] While some cases with favorable prognoses have been reported, the evidence on using HDAC inhibitors for NUT carcinoma treatment remains insufficient and requires further investigation.[80,81]

Bromodomain and extra-terminal domain inhibitors work by competing with acetylated histones at bromodomain-chromatin interaction sites and histone tails, disrupting the binding of BRD4 protein to chromatin. This inhibition affects gene transcription during the M and G1 phases, impairs mitosis, induces squamous differentiation, and exerts antiproliferative effects. Non-*BRD4::NUTM1* fusion NUT carcinomas, such as those with *BRD3::NUTM1*, *NSD3::NUTM1*, or *ZNF532::NUTM1* fusions, also rely on BRD4 for oncogenicity, as the NUT fusion partners interact with BRD2, BRD3, and BRD4.[50] This dependency makes BET inhibitors a promising therapeutic option. A statistical analysis of clinical trials involving BET inhibitors revealed tumor control rates ranging from 75% to 87.5%, with the highest control rate observed at 87.5%, notably surpassing the efficacy of chemotherapy.[82-84] In a Phase I study of birabresib (OTX015/MK-8628), the recommended dose of BET inhibitors was 80 mg once daily orally. The response durations ranged from 1.4 to 8.4 months. Among nine NUT carcinoma patients, three showed PRs, three had stable disease (SD), two experienced disease progression (PD), and one was not evaluated (NE). Dose-limiting toxicities included thrombocytopenia and hyperbilirubinemia.[84] In a Phase I clinical trial of RO6870810, administered subcutaneously, eight NUT carcinoma patients showed two PRs, five SDs, and one PD, yielding an objective response rate of 25%. Treatment-related adverse events included fatigue (42%), decreased appetite (35%), and injection site erythema (35%).[82] The Phase I trial of molibresib, which enrolled the largest cohort of NUT carcinoma patients (*N*=19), showed primary adverse events, including thrombocytopenia (51%), gastrointestinal discomfort (e.g., diarrhea, vomiting, nausea, anorexia, and taste disturbances; 22 – 42%), anemia (22%), and fatigue (20%). The recommended dose was 80 mg, and the efficacy assessment revealed 4 PRs (21.1%), 8 SDs (42.1%), 4 PDs (21.1%), and 3 NEs (15.8%).[83] Several clinical trials related to BET inhibitors (e.g., NCT02516553 and NCT03936465) are ongoing, though formal data specific to NUT carcinoma have not been reported. NUT carcinoma has been observed in children and an ongoing Phase I clinical trial is recruiting pediatric patients with solid tumors, brain tumors, and lymphomas to assess the therapeutic effects of BET inhibitors (BMS-986158 and BMS-986378) on pediatric NUT carcinoma.[84] Given the poor prognosis associated with conventional treatments, BET inhibitors are poised to become a crucial option for first-line therapy. However, it is important to note that the blood-brain barrier may impede the entry of BET inhibitors into the brain, which could affect their efficacy in treating brain metastases.[85,86]

Given the poor prognosis of NUT carcinoma and its propensity for recurrence and metastasis, targeted therapies like BET inhibitors demonstrate superior control

rates compared to conventional treatments, offering a new promising therapeutic avenue. Considering the poor prognosis of NUT carcinoma, where most patients have short survival times and are unsuitable for repeat surgery, post-operative recurrent or metastatic patients should prioritize refining high-throughput sequencing to identify sensitive drugs. Given that there are currently no approved targeted therapeutic agents specifically for NUT carcinoma, it is recommended that patients either directly participate in clinical trials involving BET inhibitors, HDAC inhibitors, and other novel therapies, or on disease progression following conventional anti-tumor treatments, patients are encouraged to actively participate in clinical trials involving BET inhibitors, HDAC inhibitors, and other novel therapies.

(a) Recommendation 8

For patients with post-operative recurrence or distant metastasis who are unsuitable for further surgery, apart from optimizing high-throughput sequencing to select sensitive drugs. It is recommended that patients either directly participate in clinical trials involving BET inhibitors, HDAC inhibitors, and other novel therapies, or on disease progression following conventional anti-tumor treatments, patients are encouraged to actively participate in clinical trials involving BET inhibitors, HDAC inhibitors, and other novel therapies (evidence level: grade 3; recommendation level: strongly recommended). In cases of treatment progression, high-throughput sequencing should be refined, suitable drugs should be selected based on the results, and participation in relevant clinical trials should be actively considered (evidence level: grade 5; recommendation level: strongly recommended).

5. Limitations

This study has several limitations. First, high-quality data were not available for some of the topics discussed. Observational studies inherently carry a high risk of incomplete outcome data and selective outcome reporting. Publication bias may also influence researchers to prioritize reporting positive results over negative ones. In case series, selective reporting may lead to an emphasis on successful surgical outcomes. Other potential sources of bias include baseline imbalances, such as population selection in observational studies.

In addition, some relevant articles may have been overlooked. Second, due to the extreme rarity and poor prognosis of NUT carcinoma, physicians often lack sufficient awareness of the disease. Consequently, we aimed to include all available data on NUT carcinoma, but large-scale studies remain scarce. Much of the data analyzed in this review derived from case reports, leading to gaps and non-continuous data. The 199 reviewed papers did not uniformly or comprehensively study and report all relevant indicators, resulting in potential discrepancies in IHC and fusion gene results. These findings must be interpreted within the clinical context. Future large-scale, multi-center studies are required for a more precise investigation.

6. Conclusion

Based on the existing literature and insights from multidisciplinary experts, we have developed an expert consensus on the diagnosis and treatment of NUT carcinoma. This consensus provides comprehensive and practical recommendations for healthcare providers managing NUT carcinoma throughout patient care.

Given the poor prognosis and high invasiveness of NUT carcinoma as a rare tumor, its non-specific features complicate diagnosis, and the overall effectiveness of existing conventional treatment methods remains insufficient. Although the prospects for targeted agents such as BET and HDAC inhibitors are promising, overcoming drug resistance to these agents and selecting the most appropriate treatment combinations still require further exploration. Therefore, focused research on NUT carcinoma patients is essential to enhance our understanding of the disease and improve diagnostic and treatment strategies in the future. Specialized committees have recently been established in various countries.[87-89] For example, the International NUT Carcinoma Registry was established in the United States to record the clinical, genetic, and pathological characteristics and outcomes of NUT carcinoma patients diagnosed since 1993.[18] However, the patients included are predominantly from the American and European populations.

According to the most recent data on population and vital statistics from the United Nations (April 2024),[90] the United States and Europe account for 4.16% (331,449,281/7,975,105,000) and 9.32% (743,556,000/7,975,105,000) of the global population, respectively. In contrast, China and the other Asian countries account for 17.8% (1,419,873,864/7,975,105,000) and 59.2% (4,722,635,000/7,975,105,000) of the total population, respectively. These demographic data indicate a significant potential number of NUT carcinoma cases in China and Asia. In response to these statistics, we established the first specialized center for NUT carcinoma in China in January 2023. In June 2023, the NUT Carcinoma Gene Diagnosis Working Group was established under the Tumor Gene Diagnosis Special Committee of the Chinese Anti-Cancer Association. We initiated a real-world cohort study (NCT06073938) investigating the efficacy of the BET inhibitor (NHWD 870 HCl) for solid

tumors with NUT rearrangement, which began in 2022. From June 2023 to April 2024, we diagnosed and treated 80 cases of NUT carcinoma. However, due to insufficient awareness among most physicians and the high rate of misdiagnoses, the actual number of NUT carcinoma cases could be much higher. To address these issues, we collaborated with multidisciplinary experts from various countries to establish an international platform to increase public awareness and improve the understanding of NUT carcinoma among medical professionals. This platform (http://resource.yin-lab.com/NUT/) provides scientific information about NUT carcinoma and continuously updates all published cases and available cohort data, facilitating better research on this rare disease. Furthermore, it will support the publication of relevant clinical trials on NUT carcinoma in different countries, making it easier for patients to access appropriate clinical trials. With growing awareness and a broader understanding of NUT carcinoma among clinicians, pathologists, radiologists, and cancer researchers, we believe that the diagnosis and treatment of NUT carcinoma can improve, ultimately benefiting more patients in the future.

Acknowledgments

We extend our sincere gratitude to all the experts who contributed to the development of the Expert Consensus on the Diagnosis and Treatment of NUT Cancer and the members who participated in the retrieval, screening, and summarization of clinical evidence. We thank all the guideline development group members for their valuable contributions, particularly the external reviewers who provided insightful comments on the draft guidelines.

Funding

This work was supported in part by the Chongqing Wanzhou Municipal Science and Health Joint Medical Research Project Key Program (grant no. wzstc-kw2023001), the Chongqing Wanzhou PhD "Through Train" Research Project (grant no. Wzstc20230402), and the Chongqing Natural Science Foundation (grant no. CSTB2023NSCQ-MSX0658).

Conflict of interest

Mingzhu Yin and Helmut H. Popper are the Editors-in-Chief and Xin Li, Paolo Boffano, Steven Brower, Xinpei Deng, Nejat Duzgunes, Pierfrancesco Franco, Weilin Jin, Kalevi Kairemo, Mohamed Kamal, Evan T. Keller, Mikael S. Lindström, Yanqing Liu, Jose Manuel Lopes, Wenping Ma, Amancio Carnero Moya, Athanasios G. Papavassiliou, Axel Schonthal, Vishal Shelat, Alexander Shtil, Hifzur R Siddique, Shaoquan Zheng, and Qin Yan are Editorial Board Member of this journal. However, none of these individuals were involved in this paper's editorial or peer-review process, either directly or indirectly. In addition, the other authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data supporting the findings of this study are available from the corresponding author on reasonable request. Some data may not be made available due to privacy or ethical restrictions.

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