EXPeRT

NUT CARCINOMA IN CHILDREN AND ADOLESCENTS

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STANDARD CLINICAL PRACTICE RECOMMENDATIONS

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1. Background and rationale

1.1.1 Summary

Pediatric very rare tumors (VRT) constitute an extremely heterogeneous group of neoplasms. Some of them are typical of pediatric age, while other more commonly arise during adulthood and only rarely develop in children. Using the definition "*any solid malignancy or borderline tumor characterized by an annual incidence < 2/million children < 18 years old"* the European Cooperative Study Group for Pediatric Rare Tumors (EXPeRT) has initially identified a number of pediatric VRT[1]. Due to the low number of patients, it is very difficult - or even impossible - to conduct clinical trials on them, and this makes it hard to reach evidence-based treatment guidelines. Consequently, the treatment of patients with VRT is often individualized.

Background:

NUT carcinoma (NC) is a rare and highly aggressive tumor occurring mainly in adolescents and young adults (AYA), defined by the presence of the *NUTM1* rearrangement. The incidence remains unknown, likely due to frequently undiagnosed or misdiagnosed cases. The first confirmed case was diagnosed in Japan in 1991, where the translocation t(15;19) was found in a thymic carcinoma[2]. However, only recently in 2004 the disease was defined by screening undifferentiated and poorly differentiated childhood and adolescent carcinomas for the *NUTM1* translocation[3]. NC mainly arises in midline structures (head, neck, and thorax), but other various organs may be affected, such as the lungs, bladder, pancreas, kidney, salivary glands, central nervous system, or bone. Patients usually present at an advanced stage of disease, with a rapidly growing mass that displays aggressive radiological feature such as destructive and infiltrative growth. Given the rarity of these tumors, no standardized recommendations for diagnosis and therapeutic management are available. The management strategy usually combines prolonged conventional combination chemotherapy, local surgery, and radiotherapy (RT). Despite intensive multimodal approaches, the overall prognosis is extremely poor (in particular for tumors with location within the thorax), with a median survival ranging from 6.5 to 9.5 months, due to the frequent resistance to chemotherapy and the early and rapid tumor progression following conventional therapy. In this context, new targeted therapies have been developed, including bromodomain (BRD) or HDAC (histone deacetylases) inhibitors, with some promising preliminary data, but preferably should be delivered in the context of prospective trials (if available).

Objective:

To establish internationally harmonized consensus recommendations for the diagnosis and treatment of children and adolescents with NC ("Standard of care recommendations for children with VRT").

1.1.2 Background

The *Nuclear protein of the testis* (NUT) carcinoma (NC) has been recently defined as a rare and aggressive tumor, associated with *NUTM1* rearrangement. Firstly described in 1991[2,4], NC was originally named as "midline carcinoma" because of propensity for origin in the midline structures including head, neck, and mediastinum. However, it can be found in various other organs, such as the lungs, bladder, pancreas, kidney, central nervous system, bone, or salivary glands, and the primary tumor is actually strictly lateralized in 2/3 of cases[5–9]. NC is an exceptionally rare cancer with unknown incidence, although probably underdiagnosed due to the absence of any specific clinical and histological features[10]. The histologic appearance of NC is nonspecific showing overlap with the histology of other poorly differentiated or undifferentiated tumors or small blue round cell tumors, such as Ewing sarcoma, olfactory neuroblastoma, and undifferentiated squamous cell carcinoma. Although first described in adolescents and young adults, NC has subsequently been reported in adults, and can actually occur at any age, even though it is extremely rare in young children[7,11,12]. Due to the rarity of the disease, no epidemiological studies of etiologic factors have been conducted. Nevertheless, no predisposition factors, either genetic, environmental or viral, have been reported to date[12,13]. NC classically shows an aggressive behavior with advanced locoregional invasion and distant metastases at diagnosis[5]. Despite initial and temporary response to chemotherapy, rapid tumor progression is often observed after a short period of time. Therefore, NC portends an extremely poor prognosis, despite an aggressive multimodality management, with an overall survival (OS) not exceeding 10 months in published series[5,7,10,14–17]. In a recent review of 141 patients from the international NC registry (www.NMCRegistry.org) published by Chau *et al.*, the 2-year OS and event-free survival (EFS) rates for all patients combined were only 22% [95%CI: 15-30%] and 15% [95%IC: 9-22%], respectively. The study reported 16 long-term survivors (i.e., 11%), defined as living at least 3 years. Adverse prognostic factors after univariate analysis were: age at diagnosis ≥ 18 years, thoracic primary tumor site, *BRD4-NUTM1* fusion transcript, lymph node involvement or metastases at diagnosis, incomplete surgery and absence of tumor response to chemotherapy[18]. Thus, Chau *et al.* described 3 different prognostic groups: A/ non-thoracic primary with BRD3- or NSD3-NUTM1 rearrangement (OS: 36.5 months [95%CI: 12.5 months-upper bound not reported]), whose localized nature and small size (< 6 cm) more often allow complete surgical resection; B/ non-thoracic primary with *BRD4-NUTM1* rearrangement (OS: 10 months [95%CI: 7-14.6 months]); and C/ thoracic primary regardless of the fusion transcript (OS: 4 months [95%CI: 3.5-5.6 months]), with no reported survivors[18].

Histologically, the tumor presents as poorly differentiated to un-differentiated carcinoma, with possible focal squamous differentiation[19–22]. Because there are no specific features that distinguish of NC from other neoplasms, misdiagnoses are frequent, and a definitive diagnosis requires the demonstration of NUT translocation by either immunohistochemical staining or molecular analyses[20,22].

Like other pediatric VRT, NC in children and adolescents presents diagnostic and therapeutic challenges for pathologists, ad surgeons, as well as radiation, pediatric and medical oncologists. Since specific standardized guidelines are lacking, management decisions are currently made at a case-by-case level, basically combining prolonged conventional chemotherapy, local surgery and RT. Beyond that,

understanding of the underlying molecular disorders in NC has suggested several potential therapeutic targets, such as histone deacetylase (HDAC) or bromodomain (BRD) inhibitors, also known as bromodomain and extra-terminal domain (BET) inhibitors[23–25], but exclusively based on preliminary data at present. These therapies preferably should be delivered in the context of prospective trials (if available).

Here, we present the internationally harmonized consensus recommendations for the diagnosis and treatment of children and adolescents with NC established by the European Cooperative Study Group for Pediatric Rare Tumors (EXPeRT).

2. Methodology

According to the Consensus Conference Standard Operating Procedure methodology, the levels of evidence can be classified from levels of evidence I to V and grades of recommendation A to E (*Table 1*)[26].

Levels of evidence	
I	Evidence from at least one large randomized, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomized trials without heterogeneity
II	Small, randomized trials or large randomized trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case-control studies
V	Studies without control group, case reports, expert opinions
Grades of recommendation	
A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, ...), optional
D	Moderate evidence against efficacy or for adverse outcome, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

Table 1. Levels of evidence and grades of recommendation (adapted from the Infectious Disease Society of America-United States Public Health Service Grading System)

EXPeRT members recognized that due to the rarity of this tumor, no evidence of Level I to II exists. Therefore, recommendations for VRTs are developed based on the evidence collected from some published prospective studies (Level III), but more frequently retrospective series (Level IV), case reports (Level V) and personal expertise (Level V). In addition, the "strength" of recommendations will be categorized by additional grading (Grade A to E).

To identify tumors that need shared recommendations, EXPeRT members designed the following procedure:

- Identification of the tumor of interest on the base of its relevance, and previous EXPeRT experience (i.e., data analysis and publication). Tumors should be classified as VRT (i.e. < 2/100000/inhabitants/

year), not already analyzed in previous Expo-r-Net project (pleuropneumoblastoma, pancreatoblastoma, thymic tumors, rare sarcomas), not included in specific international protocols and frequent enough to be of interest[1].

- Designation of two main coordinators for each VRT based on their experience (data analysis, publications, personal experience).

Coordinators must:

- Analyze the medical literature and select the relevant papers.
- Propose a series of recommendations in a form of a first draft of recommendations.
- Identify the main diagnostic and therapeutic problems for the designated VRT. The first drafts will be shared and discussed, along with the relevant publications, into the selected EXPeRT group of PARTNER members and annotated.
- A mature version of recommendations will be produced, taking into account proposals from the group of selected EXPeRT members.
- The annotated draft will be then proposed to external experts identified by the coordinators based on a recognized experience on the tumor (pathologist, pediatric oncologist, medical oncologist, radiation oncologist, surgeon, …).
- The final version will be validated by the whole PARTNER group. In case of remaining disagreements, a vote will be done, during a physical consensus meeting, to agree on a final consensus.
- Validated version will be submitted for publication in an open-source peer review journal.

The final document including recommendations will be available on EXPeRT website.

NB: These guidelines may change over time according to new data available. Local clinicians remain responsible for the care of their patients. The EXPeRT members are not responsible for results or complications related to their use. If necessary, medical discussions are possible with EXPeRT members of these groups via the expert website: ***https://vrt.cineca.it***

3.3.1 Diagnostic Criteria

As mentioned above, NC may occur at any age, but mainly in adolescents and young adults, and it remains only exceptionally reported during infancy[6,11,12]. Thus, the median age at diagnosis may vary considerably depending on published series, ranging from 16 to 38 years (range 0 - 80 years)[5,7,10,14–17,20,27–29]. Recent retrospective largest series (including respectively 119 and 124 cases of NC) reported a median age of onset around 23 years[14,15]. Male and female are equally affected. Clinical manifestations are not specific and depend on the location and extent of the tumor, with mass-related symptoms. General and non-specific symptoms are also noticed, such as fever, weight loss, or asthenia[22]. NC classically shows aggressive clinical and radiological features, with early advanced locoregional invasion, and lymph node involvement or distant metastases at diagnosis[5,10,11,14,22,30]. When reported, the main sites of metastases include lymph nodes, bone, bone marrow, and pleura[5,31–33]. Despite the historical name of "midline carcinoma"[30], NC can arise in various location and organs, although it is mainly reported in the lung, mediastinum or head and neck. Thus, the recent World Health Organization (WHO) classification recently renamed it as "NUT carcinoma"[34].

Appropriate clinical and imaging studies at diagnosis are useful to assess disease stage and extent of locoregional and metastatic spread, and to discriminate from other differential diagnoses. Histology is mandatory for the diagnosis of NC, including immunolabelling by using anti-NUT antibodies and molecular testing for *NUTM1* rearrangement since histology by itself is insufficient for definitive histopathological diagnosis because of the great overlap with other poorly differentiated or undifferentiated carcinoma[22,35,36] [Level V; Grade A].

Discussion by a Multidisciplinary Team (MDT) is highly recommended early in the assessment process, and before any invasive procedure (including biopsy) [Level V; Grade A].

3.3.2 Imaging

Full clinical evaluation *is necessary in addition to imaging studies [Level V; Grade A*]. An endoscopic evaluation (like pan-endoscopy for H&N sites) depending on site of primary tumour and level of concern for invasion of neighboring structures may be appropriate *[Level IV; Grade C].*

3.3.2.1 Primary tumor and its loco-regional tumor extension:

- **Magnetic resonance imaging (MRI)** and/or **computed tomography (CT)**, depending on the tumor location, is essential to define the precise tumor site and extent with respect to adjacent structures and its operability [Level V; Grade A].
  Primary thoracic NC usually presents as a solid mass associated with enlarged hilar and mediastinal lymphadenopathy and pleural involvement (i.e., effusion thickening or pleural nodules)[37,38]. Tumors arising in the sinonasal tract (which represents the main site for head and

neck NC) present as a space-occupying mass with locoregional destruction and invasion, including cervical lymph node involvement[38].

Radiological findings (on both CT scan or MRI) are classically nonspecific and may mimic various pathological entities, such as lymphomas or other malignant solid tumors [39–42]. However, the common imaging characteristics frequently reported in the literature include a low-density mass with heterogeneous enhancement and central necrosis on CT scan, and T1 hypo-intensity and low-level T2 hyper-intensity with heterogeneous enhancement on MRI[30,39,41–43]. In addition, it classically shows aggressive and invasive features. Contrast-enhanced CT scan was considered the reference for the initial staging assessment. However, MRI provides useful additional information, particularly regarding vascular invasion, or - for head and neck tumors - bone marrow extension, perineural involvement, and skull base invasion[39]. For head and neck tumors, MRI represents the gold standard for diagnosis and correct staging[13]. For sinonasal/midface tumor assessment, combination of MRI (for soft tissue, orbital, dura and brain parenchyma) with CT scan (for maxillary bone, skull base and lymph nodes) is recommended [Level V; Grade B]. Ultrasound to guide lymph nodes cytoaspiration in case of doubt could help to confirm lymph nodes invasion [Level IV; Grade B].

3.3.2.2 Distant metastasis

The initial staging assessment must be systematically extensive, as metastatic disease is commonly present at diagnosis and may be widespread. It should include:

- ***Chest CT***
- ***^{18}F-fluorodeoxyglucose positron emission tomography/CT (FDG-PET/CT)*** has been proposed in various case reports as the imaging modality of choice for distant metastatic assessment[39–41,44–49]. Potential pitfalls include low FDG-avidity due to tumoral necrosis. However, FDG-avidity on PET/CT directly correlated with tumor burden on CT as well as the clinical disease status on published case reports[41,44]. Based on this literature, and despite the lack of cohort studies specifically assessing the additional value of FDG-PET/CT to standard imaging, we recommend performing it whenever possible, both for the initial staging as well as for monitoring treatment response [Level V; Grade B]. The value of whole-body PET/MRI is not defined yet [Level V; Grade C].
- ***Other examinations*** may be considered, guided by the clinical presentation and initial symptoms, particularly brain MRI in the presence of neurological signs [Level V; Grade A]. Bone X-rays or CT to understand bone stability to define “palliative radiotherapy” may be indicated if bone metastases are present [Level IV; Grade C]. Bone marrow trephine biopsies and cytoaspirations could be discussed in case of clinical concern or equivocal findings by PET [Level IV; Grade C].

3.3.3 Histopathology

When NC is suspected - based on clinical and radiological findings - **histology** must be *obtained [Level V; Grade A]*. Due to the lack of typical morphological characteristics, with a great overlap with other neoplasms, NC may be misdiagnosed on standard immunohistochemistry analyses. The most common description on hematoxylin and eosin staining is a poorly differentiated or undifferentiated carcinoma with focal squamous differentiation[12,19–22]. High grade features are classically present, including abundant mitoses, apoptotic bodies, and tumor necrosis. Similarly, the immunophenotypic profile of NC is non-specific and may include, in variable frequency and intensity, the expression of epithelial markers, such as cytokeratins AE1/AE3, or epithelial membrane antigen (EMA, MUC1); strong and diffuse p63 immunoreactivity is frequently reported, however neither always present nor specific to NC. In contrast, expression of neuroendocrine markers (synaptophysin, chromogranin, TTF1), desmin and myogenin, and lymphoid markers, is usually negative.

Due to the difficulties of the histopathological diagnosis, the definitive diagnosis is confirmed either on the demonstration of *NUTM1* rearrangement by either immunohistochemical staining or molecular analyses[20] [Level IV; Grade A]. Immunolabelling using anti-NUT antibodies provides a highly accurate easy to use and rapid test to guide the diagnosis. According to Haack's study, the presence of diffuse and strong nuclear reactivity, with a cut-off value of 50% or more (according to the WHO), can confirm the diagnosis with a sensitivity of 87% and a specificity of 100%[34,50,51]. Weak cytoplasmic staining may be present in benign and malignant epithelial cells, representing probable non-specific background staining, but does not interfere with diagnostic work-up in the absence of nuclear expression. Moreover, caution should be made on interpretation - particularly in the case of a mediastinal tumor - considering that NUT immunostaining may be focally seen in some germ cell tumors (mainly dysgerminomas), probably due to expression of normal NUTM1. Nevertheless, in germ cell tumors, the nuclear reactivity is consistently weak and focal, in contrast with NC. Furthermore, other tumors with a translocation involving the *NUTM1* gene and a positive nuclear staining for NUT-like a subset of sarcomas have to be considered as differential diagnosis. In addition, morphological characteristics that may help to differentiate these two entities as NC may include (but not always) focal squamous differentiation. Finally, if any doubt remains, additional immunostaining including germ cell markers (OCT3/4, SALL4) - which are consistently negative in NC - should be performed[20]. In the presence of poorly differentiated or undifferentiated carcinoma, anti-NUT staining should be extensively used, regardless of the patient's age or history, as histological and immunophenotypic profile (apart from anti-NUT antibody) are not pathognomonic[22,35,36].

Due to the non-specificity of NC histology, many differential diagnoses may be suggested, depending on the location, such as Ewing sarcoma, rhabdomyosarcoma, synovial sarcoma, undifferentiated carcinomas (including undifferentiated carcinoma nasopharyngeal type (UCNT) or salivary gland), desmoplastic small round cell tumors and all other tumors with a small cell morphology. Molecular biology, searching for specific transcripts, constitutes a valuable tool to exclude some of these diagnoses[52]. Expert pathology for second opinion or central pathology review are essential in this rare tumor.

3.3.4 Molecular pathology

Contrary to most other carcinomas, NC is characterized by a simple karyotype, often with a single cytogenetic aberration, i.e. translocation involving *NUTM1* (the main t(15;19)(q14;p13.1) resulting in a *BRD4/NUT* fusion transcript)[7]. The presence of a fusion transcript and the NUTM1 partner is confirmed by molecular analyses (fluorescence in situ hybridization [FISH], reverse-transcriptase polymerase chain reaction [RT-PCR], or next-generation sequencing [NGS]). In about 2/3 of cases, *NUTM1* (chromosome 15) is fused to *BRD4* (chromosome 19). Description of the molecular mechanism leading to oncogenesis of *NUTM1* and *BRD4* is represented in *Appendix 1*. Fusion transcript involving *BRD3*, which is a homologue of *BRD4*, has also been reported. More rarely, other variants have been observed, such as *NSD3, ZNF532, ZNF592*[40,53–56]. At present, translocations involving *BRD3/4* and *NUTM1* are considered specific for NC[11]. However, other tumors involving the *NUTM1* gene have also been reported[56,57].

In addition, due to the difficulty of diagnosing NC and the great overlap with other tumor types, negative results for transcripts of other cancers (such as *EWSR1* transcripts for Ewing sarcoma, or *SYT-SSX* transcripts for synovial sarcoma) are often a valuable tool to exclude various differential diagnoses[52].

3.3.5 Additional assessments

- Before chemotherapy, a classic **laboratory work-up** (full blood count, liver and renal function tests), tumor markers alpha1-fetoprotein and ß human chorionic gonadotropin to exclude secreting germ cell tumors and **specific evaluations** depending on chemotherapeutic agents (e.g., audiometry, echocardiography) are required, in order to limit side effects *[Level V; Grade A]*.
- Before RT in the head and neck area, **detailed dental assessment** including clinical evaluation, dental panoramic radiography ± dental scan is necessary[58,59]. Depending on the tumor site, **endocrine testing, vision, hearing** analyses could be necessary [Level V; Grade C].
- For thoracic tumors, **pulmonary function tests or cardiac specific evaluations** may be required depending on the organs included in the irradiation fields such as echocardiography *[Level V; Grade A]*.
- **Fertility preservation options** are not necessary before surgery and/or locoregional RT *[Level V; Grade A]* but could be considered before chemotherapy taking into account the poor prognosis of NC.

4. Treatment details

4.1.1 General considerations

- **MDT** consultation is mandatory at diagnosis and during therapy *[Level IV; Grade A]*.

- Patients/families should be invited to participate in a **prospective clinical trial** when available, with **data collection** in national, NMC registry or international databases to improve the knowledge of this disease *[Level IV; Grade B]*.
- Treatment of NC usually combines, through a **multimodal and aggressive approach**, prolonged conventional combination chemotherapy, local surgery, and RT, considering that NC is usually refractory to conventional chemotherapy and thus with the risk of early tumor progression on-treatment or following prolonged interruption of systemic therapy.

The treatment flowchart proposed by the EXPeRT group is detailed in *Appendix 2*.

4.1.2 Chemotherapy

NC is usually poorly sensitive to chemotherapy. Thus, while response at the onset of systemic therapy is common, NC becomes rapidly refractory and tumor progression occurs early on-treatment in most cases[60]. According to the literature, chemotherapy response rates do not exceed 40% and are only transient, regardless of the chemotherapeutic regimen used[5,10,13]. No consensus has been reached about the optimal chemotherapy regimens. Anthracyclines, cisplatin, alkylating agents, vincristine, gemcitabine, vinorelbine, and taxanes-based combinations have been reported, but with only transient response in almost all cases[13,31,60–63]. Storck *et al.* suggested that applying "Ewing sarcoma- like protocols" with multimodal chemotherapy combining alkylating agents, anthracycline ± cisplatin in association with surgery and RT could be a valuable option for patients with NC[63]. Other case reports describe favorable outcome for patients treated with sarcoma-like approach, with either dose-intensified VDC/IE (vincristine-doxorubicin-cyclophosphamide/ifosfamide-etoposide) or VAI/PAI (vincristine-adriamycin-ifosfamide/cisplatin-adriamycin-ifsofamide)[8,63,64]. In all cases, caution should be made and pre-chemotherapeutic evaluation is required, as well as total cumulative dose monitoring, in order to reduce acute and long-term side effects. Chemotherapy may be considered as first-line treatment (neo-adjuvant chemotherapy) for unresectable or metastatic tumor, or in the case of resectable but locally advanced tumor to avoid mutilating surgery, and rapidly followed by local treatment (i.e. 3 courses) *[Level IV; Grade B]*. Though the precise duration is poorly defined, adjuvant post-irradiation chemotherapy is recommended, for a total of 9 to 12 courses *[Level IV; Grade B]*, but limiting the cumulative dosages of doxorubicin to a maximum of 400-500 mg/m² if myocardial irradiation is necessary *[Level IV; Grade C]*. For first-line resected tumors, concomitant chemotherapy to RT may be discussed, if possible *[Level IV; Grade B]*. Finally, maintenance chemotherapy could be considered for metastatic disease *[Level V; Grade C]*. As far as possible, these treatments should therefore be delivered in the context of prospective clinical trials (if available) *[Level V; Grade B]*.

4.1.3 Local treatment (surgery, radiotherapy)

Local treatment appears to play an important role in disease control according to several studies, as the extent of surgical resection and initial RT have been reported as independently predictive of OS and EFS[10,14,16]. Nevertheless, the study based on retrospective data of 119 patients among 64 publications, showed that chemotherapy and RT were associated with improved survival, but not surgery[15]. Considering the poor chemosensitivity of NC with a high-risk of early progression on-treatment or after

systemic therapy interruption, local therapy remains a cornerstone of the treatment strategy. Indeed, in localized NC, a complete microscopic surgical resection should be attempted whenever and as soon as possible, followed by irradiation of the primary tumor and involved lymph nodes area[16] *[Level IV; Grade B]*. For head and neck tumors, a systematic neck dissection might be considered, even if N0 *[Level V; Grade C]*.

Given the rarity of the disease, and the complexity of the surgery due to the frequent high locoregional invasion, and the possible need of several surgical teams (head and neck surgeon, neurosurgeon, visceral surgeon, …), referral to a specialized surgical oncology center is highly recommended *[Level V; Grade A]*. However, given the poor prognosis and the high metastatic risk of this aggressive malignancy, surgical resection with oncologic margins of the head and neck NC (usually a locally advanced sinonasal primary) may be associated with a significant postoperative morbidity and a severe alteration of quality of life (such as orbital clearance, total maxillectomy or skull base resection). The benefit-risk balance of this local treatment has to be well evaluated in correlation to external radiotherapy *[Level V; Grade A]*.

Considering RT, as a general principle, careful treatment planning is necessary. The use of three-dimensional highly conformal intensity modulated techniques is highly recommended. Considering the age of the patients, the high dose required, and the location of the tumors, proton therapy may have advantages *[Level V; Grade A]*. There is no consensus regarding radiation doses due to the rarity of the disease. Radiotherapy dose needs to be adapted based upon extent of local disease (margins, extracapsular extension, perineural & intravascular extension) as in adult patients with epithelial carcinomas. Consequently, high doses between 50 to 70 Gy are generally reported[15,65] *[Level IV; Grade B]*. Irradiation is recommended on the extended primary tumor site and any involved lymph node area *[Level V; Grade B]*. In case of exclusive radiotherapy, 65-70 Gy on primary tumor and adenopathy is recommended, with 50 – 54 Gy for elective node irradiation *[Level V; Grade B]*[15]. Protontherapy could be used provided that this technics will not delay the beginning of the RT.

A systematic lymph node irradiation should be considered in the absence of a pathologically confirmed N0 situation following complete cervical lymph node exploration for head and neck tumors *[Level V; Grade C]*. Depending on the location of the tumor, the elective lymph nodes should include bilateral retropharyngeal and the level II-V lymph nodes.

4.1.4 Targeted therapy

In the context of poor response to conventional treatment, several targeted therapies have been suggested, based on a better understanding of the underlying molecular disorders in NC and preclinical data reporting objective responses to BRD or HDAC inhibitors, by inducing epithelial differentiation and arrest of proliferation of NC cells[23–25,66–69]. Mechanisms of action of BRD and HDAC inhibitors are schematized in *Appendix 1*[70,71].

Several phase I/II trials have been conducted with BRD inhibitors[72]. As for example:

- RO6870810 (NCT01987362): partial response in 2/8 patients with NC, stable disease in 5/8. Median Progression Free Survival was 94 days (range, 15-783)[73]
- Molibresib GSK525762 (NCT01587703): partial response in 2/19 patients with NC[28,74].
- Birabresib OTX105/MK-8628 (NCT02259114): partial response in 3/10 patients with NC, stable disease in 3/10 with duration of 1.4 to 8.4 months [75,76]
- ODM-207(NCT03035591): Four patients with NC with no tumor response. One out of four patients with NMC had SD as their best response (during 16 weeks), the other patients had clinical progression prior to their first disease assessment.[77]
- BI 894999 (NCT02516553): study reached its completion date on Nov23, 2021; publication of results is expected in 2022.

Fimepinostat (CUDC-907), a dual HDAC and PI3K inhibitor, has been evaluated in a recent phase I trial (NCT02307240), but the results are not yet available. However, several case reports have shown transient responses[23,78]. Several trials have argued for a potential benefit to a combination strategy using HDAC inhibitors and other anti-tumor agents in various tumor types[79]. Thus, despite the unconvincing results with only transient responses reported in single-agent treatment for patients with NC, a combination of BET inhibitors and conventional chemotherapy or other targeted therapy could be of interest with potential clinical benefit, and further studies investigating such strategies are needed[80]. Considering very poor prognosis observed with conventional treatment alone, such a combination could be considered as the first-line treatment *[Level V; Grade C]*.

At present, data supporting the role of BET inhibitors in NC are too preliminary, and these targeted therapies preferably should be mainly reserved for patients with relapsed/refractory disease unless evaluated as part of a trial together with conventional chemotherapy *[Level V; Grade B]*.

5. Assessments

Patients should undergo clinical and radiological evaluation before, during and after the end of the treatment. Depending on the primary tumor location, CT scan with contrast enhancement or MRI is usually adopted. Assessment should be performed to evaluate response to chemotherapy every 2 to 3 cycles, before surgery to plan the operation procedure and after surgery to evaluate the possible residuals, before RT to plan dose and volume of irradiation and after irradiation to assay the tumor status before adjuvant chemotherapy *[Level V; Grade B]*.

In the case of initial metastatic disease, imaging evaluation should include all known metastatic sites. Other imaging studies should be considered depending on clinical evaluation *[Level V; Grade B]*.

6. Summary of known adverse events associated with treatment recommendation

Type of treatment	Main side effects
Surgery	*Depending on the tumor location and extent of the surgical procedure* General post-surgical risks including post-operative pain, hemorrhage, infection… Specific post-surgical complications depending on the tumor site
Chemotherapy (depending on chemotherapeutic agents used)	– General side effects: fatigue, risk of infection, nausea and vomiting, hair loss, loss of appetite, hematological toxicity… – Vincristine: peripheral neuropathy, constipation… – Actinomycin-D: hepatic toxicity… – Ifosfamide: renal and bladder toxicities (tubulopathy, hemorrhagic cystitis), central neurotoxicity, gonadotoxicity, cardiotoxicity… – Doxorubicin: cardiotoxicity… – Cyclophosphamide: bladder toxicity, second malignancy, gonadotoxicity… – Cisplatinum: renal, hearing, gonadotoxicity – Etoposide: allergic reaction, second malignancy…
Radiotherapy (depending on the tumor location, dose and volume of irradiation)	- Acute side effects that may persist for a longer time: pain, mucositis, fatigue, hematological side effect, skin reactions. - Late side effects, which may arise after a long delay from treatment completion: *For thoracic location:* musculoskeletal growth retardation (in growing children), osteoradionecrosis and fractures, pulmonary sequelae (lung fibrosis), cardiotoxicity (especially in the case of irradiation after anthracycline exposure), endocrine dysfunction …

	For head and neck location: facial and dental developmental defect, osteoradionecrosis and fractures, trismus, functional damages including hearing loss and vision impairment, cognitive deficit, endocrinopathy…

6. Supportive treatment

After surgery, general supportive post-operative treatment (i.e., scar nursing, analgesic therapy…) are recommended *[Level V; Grade A]*.

In the case where chemotherapy is needed, central venous catheter insertion should be considered and is strongly recommended before chemotherapy administration *[Level V; Grade B]*. Anti-emetic treatment is administered in addition to chemotherapeutic agents. Early nutritional status evaluation +/- nutritional intervention if needed are recommended. Hyper-hydration and Uromitexan-Mesna© to prevent bladder toxicity is required with ifosfamide/cyclophosphamide administration *[Level V; Grade A]*. Dexrazoxane with anthracycline administration could be used in particular if thoracic radiotherapy is expected *[Level IV; Grade C].*

In the cases where RT is considered, other supportive treatment may be necessary depending on the potential acute side effects (analgesic therapy, skin care, nutritional support …) *[Level V; Grade A]*.

Pneumocystis prophylaxis should be proposed according to local procedures *[Level V; Grade A]*.

7. Genetic considerations

As mentioned, no genetic predisposition has been reported to date[12,13]. In this context, there is no specific need of genetic counselling for pediatric NC, but this option may be discussed and should thus be proposed on an individual basis depending on the family history and preferences *[Level IV; Grade B]*.

8. Follow-up

NC harbors very poor prognosis, and reported long-term survivorship are extremely rare in the literature, generally presented with head and neck primary tumor without metastases and managed by multimodal treatment[14,62,63]. Therefore, it should be considered that in case of tumors that are refractory to treatment or progress during therapy or thereafter, palliative care should be offered to the patient and the family, so that quality-of-life directed therapeutic decisions can be undertaken in such cases.

Due to the possibility of long-term toxicities after intensive multimodal strategy including RT or invasive surgery, a strict follow-up more than 5 years is highly recommended for these patients. Surveillance

should focus on both the risk of recurrence (locoregional and/or metastatic) and potential long-term side effects, including surgical complications, radiation-related effects depending on the dose and volume of irradiation and post-chemotherapeutic side effects depending on the chemotherapeutic agents used *[Level IV; Grade A]*.

There is no standardized follow-up planning available, and it should be evaluated case-by-case depending on tumor characteristics and treatment received. As a general principle, close clinical and imaging evaluation (i.e., every 3 months) is recommended in the first two years after treatment, then every 4 to 6 months for the next 3 years. Imaging studies should assay every tumor site (primary tumor ± metastatic sites), and additional evaluation should be proposed according to clinical symptoms *[Level V; Grade C]*.

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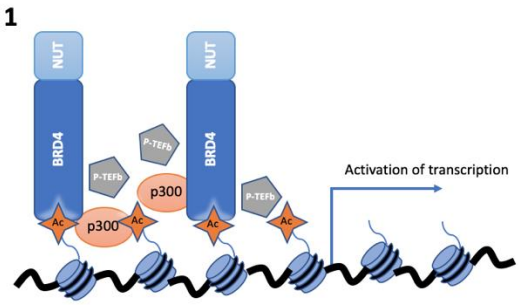
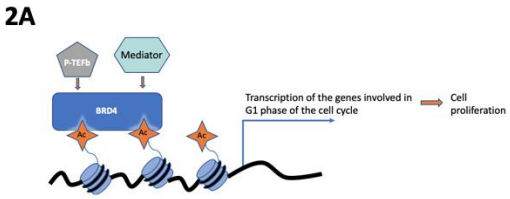
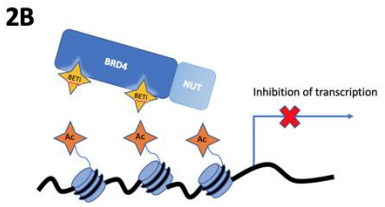
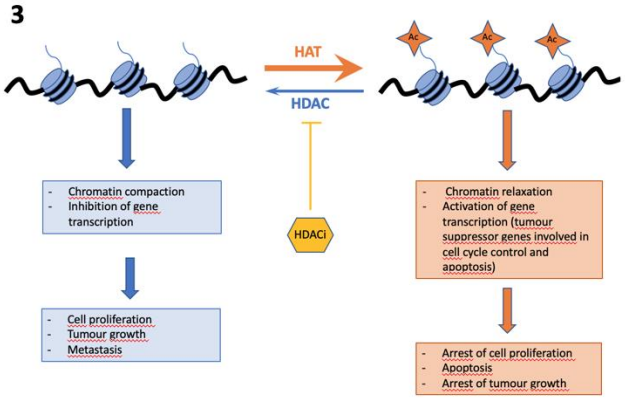
APPENDIX 1 – Molecular mechanisms of NUTM1 and BRD4









Activation of transcription

Transcription of the genes involved in phase G1 of the cell cycle

Cell proliferation

Chromatin compaction
Inhibition of gene transcription

Chromatin relaxation
Activation of gene transcription
(Tumor suppressor genes involved in cell cycle control and apoptosis)

Inhibition of transcription

Cell proliferation
Tumor growth
Metastasis

1) Mechanisms of oncogenesis associated with NUT carcinoma. The BRD4-NUTM1 oncoprotein binds to chromatin on acetylysines on histone tails via its bromodomain and recruits P300, a histone acetyl-transferase, which acetylates the adjacent histones, in turn recruiting BRD4-NUTM1. P-TEFb, a transcription factor, is also recruited. This hyperacetylated domain leads to activation of transcription. *Legend: Ac: acetylated,* (diagram adapted from Alekseyenko *et al., Genes Dev.*, 2015)[81].

2) Mechanism involving BRD4 protein: A) under physiological conditions, the BRD4 protein binds to chromatin by attaching to the tails of acetylated histones via its bromodomain, and recruits the transcription factor P-TEFb and the mediator complex, a co-activator, allowing gene transcription. B) In the presence of a BRD inhibitor, this inhibitor binds to the bromodomain, prevents binding to chromatin and inhibits transcription. *Legend: Ac: acetylated, BRDi: bromodomain inhibitor* (diagram adapted from Jimenez *et al.*, *Pediatr Blood Cancer*, 2016)[1].

3) Mechanism of acetylation and deacetylation of histones and role of HDAC inhibitors (HDACi). *Legend: HAT: histone acetyltransferase, HDAC: histone deacetylase, Ac: acetylated* (diagram adapted from Manal *et al.*, *Bioorganic Chemistry,* 2016)[82].

APPENDIX 2 – Diagnosis and treatment flowchart – EXPeRT group proposal

Suspected diagnosis of aggressive NUT carcinoma

Diagnostic work-up :
- **Biopsy of primary site** and biopsy/cyto-aspiration of any **suspicious enlarged lymph nodes**
- **Primary tumor work-up**: head and neck MRI ± CT or chest/abdominal CT according to the primitive site
- **Expert pathology review** with immuno- histochemistry and systematic **molecular characterization**
- **Metastases**: chest CT and 18-FDG PET-CT ± brain MRI in patients with metastases
- Inclusion in **protocol** or **registry** if available

EXPeRT

NUT carcinoma – loco-regional extension

Multidisciplinary discussion

Resectable tumor: 2 options

Neo-adjuvant chemotherapy

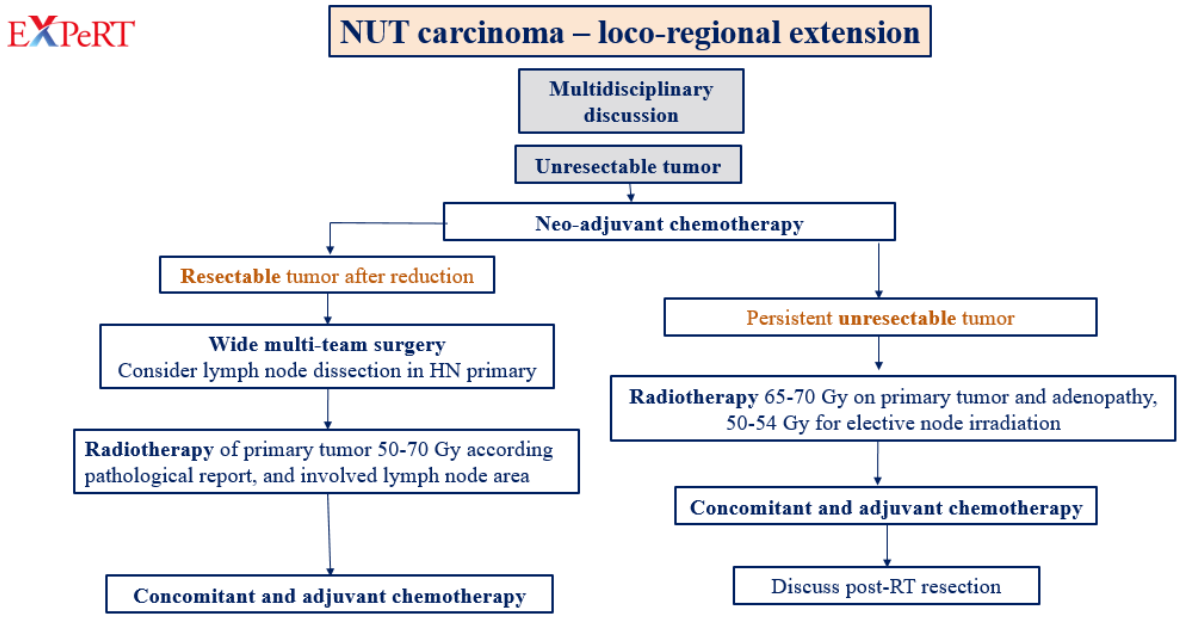
Early wide multi-team surgery
Consider lymph node dissection in HN primary

Radiotherapy of primary tumor (50-70 Gy)*
And involved lymph node area

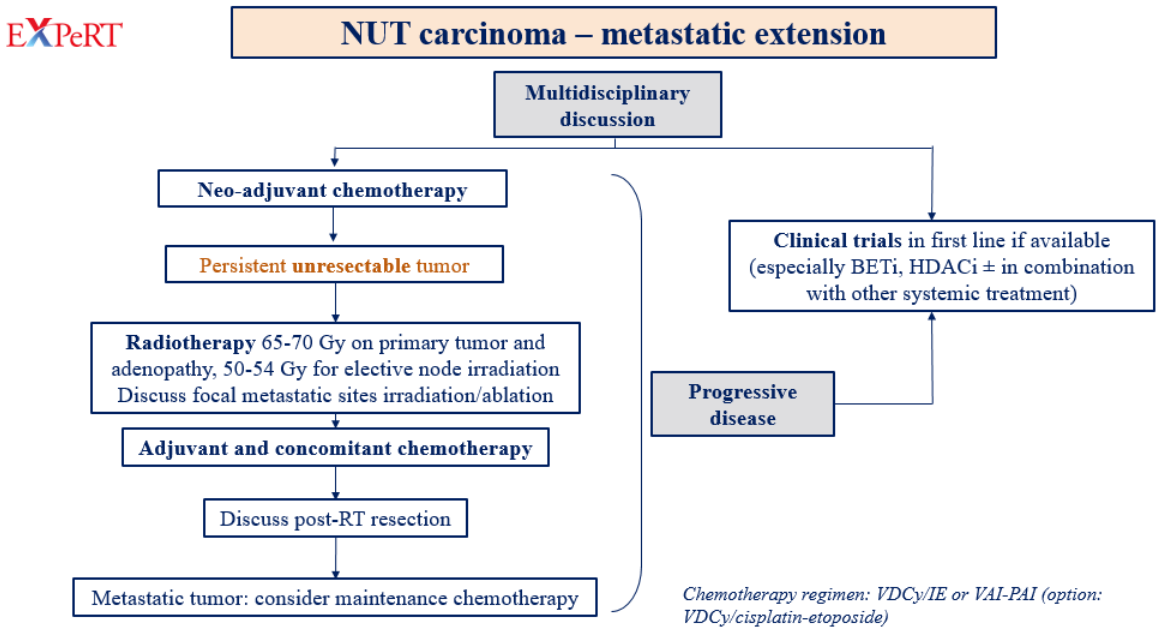
Concomitant and adjuvant chemotherapy

*Cf. text, for dosage

Chemotherapy regimen: VDCy/IE or VAI-PAI (option: VDCy/cisplatin-etoposide)



Chemotherapy regimen: VDCy/IE or VAI-PAI (option: VDCy/cisplatin-etoposide)



Chemotherapy regimen: VDCy/IE or VAI-PAI (option: VDCy/cisplatin-etoposide)
RT, radiotherapy; BETi, BET inhibitors; HDACi, HDAC inhibitors