

# **NUT Carcinoma of the Salivary Glands.**

## **Clinicopathologic and Molecular Analysis of Three Cases and a Survey of NUT Expression in Salivary Gland Carcinomas**

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## SUMMARY

NUT carcinoma (NC) represents a rare subset of highly aggressive poorly differentiated carcinomas characterized by rearrangement of the *NUT* (aka *NUTM1*, nuclear protein in testis) gene, most commonly fused to *BRD4*. Originally described as a mediastinal/thymic malignancy, NC has been reported at a variety of anatomic regions including the upper and lower aerodigestive tract. To date, only seven NC cases of probable salivary gland origin have been reported. We herein describe three new cases (all affecting the parotid gland) in two women (39 and 55 years old) and one man (35-years old). Histological examination showed poorly differentiated neoplasms composed of poorly cohesive small to medium-sized cells with variable squamoid cell component that was focal and abrupt. Immunohistochemistry showed uniform expression of p63 and distinctive punctate expression of the NUT antigen in the tumor cell nuclei. Review of the reported salivary gland NC cases (total: 10) showed a male: female ratio of 1.5:1 and an age range of 12 to 55 years (median: 29). Site of the primary tumor was the parotid (7), sublingual (2) and submandibular (1) glands. All presented as rapidly growing masses treated by surgery followed by adjuvant radio-/chemotherapy. **Initial nodal status was positive in 8/10.** At last follow-up (1 to 24 months; median: 5), 7/10 patients died of disease at a median of 5.5 months (1-24 months). Of 9 cases with genetic data, the fusion partner was *BRD4* (n=7), non-*BRD4/3* (n=1) or undetermined (n=1). None of 306 salivary gland carcinomas spanning the spectrum of salivary carcinoma types screened by NUT IHC was positive. This is the first small series on salivary NC highlighting the importance to include this rare disease in the differential diagnosis of poorly differentiated salivary gland carcinomas and in cases of presumable poorly differentiated carcinoma of unknown origin.

**Key words:** salivary glands; carcinoma; NUT carcinoma; midline carcinoma; poorly differentiated carcinoma; squamous cell carcinoma; BRD4; FISH.

## INTRODUCTION

NUT carcinoma (NC) represents a rare subset of highly aggressive poorly differentiated epithelial neoplasms defined by rearrangement of the *NUT* gene, which is most commonly fused to *BRD4* to form the *BRD4-NUT* oncogene.<sup>1</sup> Originally described in 1991 as a mediastinal/thymic malignancy<sup>2</sup>, NC has been recently reported from a variety of anatomic regions including in particular the upper and lower aerodigestive tract, but rarely intra-abdominal organs as well.<sup>3-7</sup> Affected patients are mainly young adults but the age range varies greatly from the new-born to the elderly.<sup>3-7</sup> Although originally considered strictly a neoplasm related to midline structures, recent case reports described this rare disease in lateralized organs such as the lungs and parotid gland. For this reason, the World Health Organization (WHO) classification of tumors has changed the name from NUT midline carcinoma to NUT carcinoma.<sup>8-10</sup> A thorough review of the English literature uncovered a total of 7 single case reports of NC of probable or possible salivary gland origin.<sup>11-17</sup> We herein describe three new NC cases originating in the parotid gland and review previously reported cases.

## MATERIALS AND METHODS

The three NC cases were retrieved from the consultation files of three of the authors (A.A., I.F. and C.F.). They have been diagnosed in the years 2010, 2015 and 2017.

Immunohistochemistry (IHC) was performed on 3- $\mu$ m sections cut from paraffin blocks using a fully automated system ("Benchmark XT System", Ventana Medical Systems Inc, 1910 Innovation Park Drive, Tucson, Arizona, USA) and the following antibodies: pankeratin (clone AE1/AE3, 1:40, Zytomed, Berlin, Germany), CK7 (OV-TL, 1:1000, Biogenex), p63 (SSI6, 1: 100, DCS), S100 protein (polyclonal, 1:2500, Dako), CD56 (clone MRQ-42, 1:100, CELL MARQUE), chromogranin A (clone LK2H10, 1:500, Beckman-

Coulter GmbH), synaptophysin (clone SY38, 1:50, Dako), SMARCB1 (MRQ-27, 1:50, Zytomed), and anti-NUT antibody (clone C52B1, 1:45, Cell Signaling). Interpretation of results of the NUT IHC was based on published data showing distinctive granular (punctate or dusty) nuclear immunoreactivity present in > 50% of neoplastic cell nuclei.<sup>18</sup> Epstein Barr virus (EBV) in-situ hybridization (EBER 1/2 probes, ZytoVision, Bremerhaven, Germany) was performed according to the manufacturer guidelines. Positive and negative controls were used throughout. Normal testicular tissue was used as a positive control for the NUT IHC.<sup>5,6</sup>

## **FISH**

To detect gene translocation involving the *NUT* gene locus, FISH was performed on sections cut from formalin-fixed paraffin-embedded tissue blocks using the ZytoLight *SPEC NUT* Dual Color Break Apart Probe (ZytoVision, Bremerhaven, Germany) with standard protocols according to the manufacturer's instructions. The presence of two pairs of fused green and orange signals was considered normal findings. On the other hand, translocation-positive nuclei showed one fused orange/green signal and one separate orange and green signal. 200 nuclei were counted in four different areas of each tumor. Eighty percent positive interpretable nuclei were defined as positive for a rearrangement.

To identify the *BRD4-NUT* chromosomal translocation by FISH, *BRD4* and *NUT* dual color split-apart FISH on formalin-fixed, paraffin embedded sections was performed as described<sup>4</sup>, using the following probes for *NUT* split-apart: NUT 5' centromeric probes, RP11-368L15 and RP11-1084A12 (biotin labeled, red) and NUT 3' telomeric probes, RP11-1H8 and RP11-64o3 (digoxigenin labeled, green); and the following probes for *BRD4* split-apart: BRD4 5' centromeric probes, RP11-207i16 and RP11-3055m5 (biotin

labeled, red), and BRD4 3' telomeric probes, RP11-319010 and RP11-681D10 (digoxigenin labeled, green).

Case 3 was in addition evaluated by conventional karyotyping studies. Chromosome metaphases of tumor cells were obtained from short term primary cultures as previously described.<sup>19</sup> Chromosomes were GTG-banded, and the karyotype was established according to the ISCN rules (2016).

### **Immunohistochemical screening for NUT expression in salivary gland carcinomas**

A total of 306 salivary gland carcinomas were stained with the monoclonal anti-NUT antibody (281 using tissue microarrays and the remainder on conventional slides). Histological subtypes according to the most recent WHO classification<sup>20</sup> were summarized in table 1.

## **RESULTS**

### **Clinical history**

#### Case 1

A 39 year-old female without significant past medical or family history presented with a rapidly enlarging, infiltrative tumor of the right parotid gland and right-sided lymphadenopathy, which was excised by radical parotidectomy and right levels II-III neck dissection at her local hospital. This was diagnosed locally as likely high-grade mucoepidermoid carcinoma. The patient underwent adjuvant chemoradiotherapy (66Gy 33# with weekly cisplatin). Approximately three months after excision, imaging showed disseminated metastatic disease to the bone (to sacrum, third lumbar vertebra, left neck of femur and rib), lymph nodes and lungs. She went on to receive palliative radiotherapy to the lumbosacral spine (30 Gy 10#). She was referred to our center to discuss further

potential treatment options. Pathology review showed NUT carcinoma. The patient was given palliative radiotherapy, and three months later commenced on an investigational targeted therapy, but died of progressive disease four months after this.

### **Case 2**

A 35-year-old man underwent total parotidectomy and neck dissection for an infiltrative parotid mass. Histological evaluation (performed abroad) was reported as undifferentiated large cell carcinoma of the parotid corresponding to pT3 pN2b, grade 3, R0. Postoperative investigations (three months later) showed multiple osseous metastases. A biopsy was obtained from the pelvic bone. The initial blocks/slides could not be retrieved for review. Palliative chemotherapy was initiated.

### **Case 3**

A 55-year-old female noted a right parotid mass that rapidly grows to a size of 9 cm in greatest dimension. She underwent superficial parotidectomy in another institution. Three months later, she presented with a recurrence at the parotidectomy scar and underwent total parotidectomy with facial nerve excision and a radical neck dissection (at this time a pT3pN1) complemented with chemo- and radiotherapy. Six months after initial diagnosis, she developed lung, liver and bone metastasis and died of her progressive disease seven months after primary surgery.

### **Pathological findings**

Pathological features were essentially comparable and are described herein together. All tumors were located within the salivary gland parenchyma (Fig. 1A). They were disposed in cords, nests and/or diffuse poorly cohesive sheets of undifferentiated but relatively uniform small to medium-sized polygonal cells (Fig. 1B-E). The tumor cell

nuclei showed variable chromatin condensation with inconspicuous (Fig. 1C) to variably recognizable nucleoli (Fig. 1F) and were surrounded by a moderate rim of pale-eosinophilic to clear cytoplasm. Superficial resemblance to poorly differentiated squamous cell carcinoma was most evident in Case 1 (Fig. 1D, E). The latter showed acutely inflamed stroma with prominent stromal and intraepithelial granulocytosis (Fig. 1F). Prominent single cell necrosis/apoptosis and extensive areas of necrosis were frequent. Islands of abrupt squamous differentiation were noted in all three cases including the bone metastasis from case 2 (Fig. 2A, B). The background stroma varied from edematous slightly myxoid to fibrous. Entrapment of native ductules was a prominent feature in all cases (Fig. 2A, C), occasionally with entrapment of single goblet cells, likely of ductal origin (Fig. 2A). Other unusual features included variable myxohyaline stromal changes with entrapped basaloid neoplastic nests that might be mistaken for preexisting pleomorphic or basal cell adenoma (Fig. 2D).

Immunohistochemistry showed uniform expression of p63 (Fig. 3A), variable reactivity for pancytokeratin and lack of neuroendocrine marker expression. In areas lacking strong pancytokeratin expression, the latter highlighted the foci of abrupt squamous differentiation. All three tumors showed distinctive punctate nuclear expression of the NUT protein (Fig. 3B, C). EBER ISH was negative in all cases.

FISH analysis using the *NUT* probe showed break apart signals in >90% of the neoplastic cell nuclei in all three cases (Fig. 3D). *BRD4* was the fusion partner in case 1 (determined by FISH (Fig. 3E) and Case 3 (conventional karyotyping studies revealed a 43~46, XX, t(15;19)(q14;p13) karyotype corresponding to a *NUT/BRD4* translocation). In Case 3, the fusion partner could not be determined due failure of FISH testing as a result of poor tissue preservation.

## **NUT IHC in unselected salivary gland carcinoma types**

None of 306 salivary gland carcinomas across the spectrum of salivary carcinoma types screened with the NUT IHC was positive (table 1).

## **Clinicopathologic features of all known salivary NCs**

Including our three cases, a total of 10 salivary NC cases have been reported (table 2).<sup>11-</sup>

<sup>17</sup> They affected mainly males (M: F ratio = 1.5: 1) with an age range of 12 to 55 years (median: 29). Nine patients were under 40 years at the time of diagnosis and 3/10 cases were children. Site of origin of the primary tumor was the parotid (n=7), the sublingual (n=2) and the submandibular (n=1) glands. Regarding laterality, six tumors were on the right and three on the left side (one of unknown laterality). All tumors presented as rapidly growing masses. **Regional lymph nodes were initially involved in 8/10 cases.**

Treatment was surgery followed by adjuvant radio-/chemotherapy in all cases. The majority of patients developed rapid postoperative disseminated disease. At last follow-up (1 to 24 months; median: 5), **6/10 patients died of disease** at a median of 5.5 months (1-24 months). One patient was alive with metastatic disease under palliative treatment while only two patients were alive without evidence of disease at 7 and 14 months.

All eight tumors stained with the anti-NUT antibody showed positive nuclear staining and all 9 cases assessed by genetic methods (FISH and/or karyotyping) showed *NUT* rearrangement. The fusion partner was *BRD4* (n=7), non-*BRD4/3* (n=1) or unknown due to FISH failure (n=1). As illustrated in table 3, NC of salivary glands showed frequent expression of pancytokeratin (8 of 9 cases) and consistently expressed p63 (9 of 9 cases) similar to their non-salivary counterparts.<sup>4,5</sup> Neuroendocrine markers were largely negative except for focal minor expression of synaptophysin (2 of 9 cases) and variable reactivity for CD56 (2 of 9 cases) observed in two cases each, respectively. All cases have been consistently negative for EBV ISH. Our cases were stained with SMARCB1; all three



showed intact expression. The original diagnosis was stated in 9 cases. A diagnosis of undifferentiated or poorly differentiated SCC/carcinoma/tumor was rendered or suggested in 6 cases. The remaining three cases were interpreted as probably representing high-grade variants of myoepithelial carcinoma, adenoid cystic carcinoma or mucoepidermoid carcinoma.

## DISCUSSION

Since its first description as a mediastinal (midline) malignancy<sup>2</sup>, NC has been reported from diverse sites with about one third-to-one half of cases originating in the head and neck area.<sup>3,5,6</sup> More than half of head and neck cases affected the sinonasal cavities.<sup>5,6</sup> While this rare disease probably is still significantly under-recognized, even at these characteristic (mediastinal and sinonasal) sites, NC originating at unusual sites such as the salivary glands is likely under-recognized as well. This is mainly due to the superficial similarity of this neoplasm to conventional squamous cell carcinoma (SCC) and the uniform reactivity for p63 as a marker of squamous cell differentiation. Review of the English literature uncovered 7 single case reports describing NC in or closely associated with salivary glands.

As reflected in the initial diagnostic suggestions, NC was not considered in the vast majority of cases and the diagnosis was mainly made after molecular testing. Thus, this carcinoma variant might be more common in the salivary glands than the current literature suggests, as diagnosis is only feasible if NC is included in the differential diagnosis and NUT IHC performed. Otherwise, a diagnosis of poorly differentiated SCC, sebaceous carcinoma or undifferentiated/anaplastic carcinoma unclassified would have been rendered, based on the appearance of the neoplasm and presence or absence of squamoid areas.

The prognosis of NC is poor with the majority of patients succumbing to their disease within a few months of diagnosis.<sup>21,22</sup> This is mainly the consequence of aggressive nature of NC with a high rate of regional and distant disease dissemination usually shortly after diagnosis and to the lack of response to conventional chemotherapeutic regimens. As a consequence of global transcriptional repression and blockade of cell differentiation likely resulting from the underlying genetic aberration, NC frequently displays undifferentiated phenotype by conventional histology.<sup>23</sup>

**Recognition of NC is mandatory for providing important prognostic information specific to this entity and is essential for optimizing patient's therapy.** In addition, emerging new therapeutic strategies targeting the underlying gene fusion (bromodomain inhibitors and histone deacetylase inhibitors) might be a promising option for this orphan disease in the future.<sup>24,25</sup> Thus recognition of NC is encouraged for a better characterization of this lethal disease.

Although NC is considered a variant of SCC by some authors, its relationship to conventional SCC is unclear. Lack of other molecular alterations in NC in contrast to the highly heterogeneous molecular profile of conventional SCC argues for the *BRD-NUT* fusion being the sole driver of this rare disease.<sup>2,3</sup> The frequency of NC among salivary gland cancer is unknown. In the sinonasal tract, NC accounted for 10% of poorly differentiated/undifferentiated carcinomas.<sup>26</sup> Lack of NUT immunoreactivity among 306 salivary gland carcinomas across the spectrum of salivary carcinoma types screened in this study suggests the rarity of NC among consecutive salivary gland carcinomas which is in line with the rarity of this entity in other organs as well. However, as *NUT* rearrangements are not expected among specific differentiated subtypes of salivary

gland carcinomas other than poorly differentiated SCC and undifferentiated carcinomas, the exact frequency of NC among poorly differentiated/ undifferentiated primary salivary gland carcinomas remains to be assessed in future studies. Thus, it is recommended to include NUT IHC in the workup of undifferentiated carcinomas and in SCC in the salivary glands with features suggestive of NC as reported above. However, blind screening of salivary gland carcinomas that are fitting the well characterized carcinoma types seems to be useless. The NUT IHC is considered sufficient for diagnosis based on the high specificity (almost 100%) and sensitivity (87%) of the antibody.<sup>5,6,18</sup> While genetic testing is generally not necessary for diagnosis, it is recommended to determine the fusion partner for better understanding and characterization of this rare aggressive disease. There is preliminary evidence suggesting prognostic difference between *BRD4-NUT* and *NUT*-variant carcinomas with the latter possibly associated with longer overall survival.<sup>4</sup>

Salivary NUT carcinoma should be distinguished from a variety of primary and metastatic neoplasms including poorly differentiated SCC, myoepithelial carcinoma, high-grade mucoepidermoid carcinoma, basal cell adenocarcinoma and epithelial myoepithelial carcinoma. While most of these salivary gland carcinoma entities can be excluded by well-established diagnostic criteria, some secondary morphological features entrapment of reactive ducts and distorted native ductal epithelium (including goblet cells) might be a source of confusion. It is possible that these entrapped normal tissue elements might explain some of the original diagnoses in previously reported cases including high-grade mucoepidermoid carcinoma and adenoid cystic carcinoma. Furthermore, heterologous cartilaginous differentiation as reported in a previous salivary NC should be distinguished from carcinoma ex pleomorphic adenoma.<sup>11</sup>

Likewise, presence of cells with voluminous clear cytoplasm (abrupt squamous differentiation) should not be mistaken for sebaceous differentiation. Based on these differential diagnostic considerations and given the heterogeneous and non-specific NUT carcinoma morphology, inclusion of NUT carcinoma in the differential diagnosis and use of the NUT immunohistochemistry in the work-up of unclassified or atypical looking carcinomas is encouraged.

In summary, this is the first small series on salivary NC highlighting the importance to include this rare disease in the differential diagnosis of poorly differentiated salivary gland carcinomas, in particular in cases of poorly differentiated SCC of presumable salivary gland origin or of unknown origin and in high-grade myoepithelial carcinomas.

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Table 1: Summary of the salivary gland tumors tested for NUT immunoexpression in this study

Tumor type	+/Total number of cases tested
Adenoid cystic carcinoma (7 high-grade)	0/50
Mucoepidermoid carcinoma	0/41
Salivary duct carcinoma*	0/62
high-grade adenocarcinoma/NOS*	0/35
Acinic cell carcinoma	0/32
Poorly differentiated SCC**	0/28
Myoepithelial carcinoma	0/17
polymorphous carcinoma***	0/13
Undifferentiated/rhabdoid carcinoma	0/05
Basal cell adenocarcinoma	0/07
Oncocytic carcinoma	0/04
Epithelial myoepithelial carcinoma	0/04
Primary malignant mixed tumor	0/04
Poorly differentiated neuroendocrine carcinoma	0/02
Cystadenocarcinoma	0/02
Total	0/306

\*= this group includes also some carcinoma ex pleomorphic adenoma cases.

\*\*= this group includes tumors without detectable other primary at time of clinical workup but many likely represent metastases from others, e.g. previous skin carcinomas, occult primaries, etc..

\*\*\*=all cases belong to the old category of polymorphous low-grade adenocarcinoma (PLGA).

Table 2: Clinicopathologic Features of Reported NUT Carcinomas of the Salivary Glands (n=10)

References	Age (y)/ Gender	Site	Size (cm)	Symptoms/ Duration	Initial nodal /M Status	Treatment	MTS/Duration	Outcome
den Bakker et al <sup>11</sup>	15 M	Right parotid gland	1.5 (6 at surgery 8 mo later)	Mass	N+M0	Surgery+aRCT	No	ANED (7 mo)
Ziai et al <sup>12</sup>	15 M	Left submandibular gland	2.5	Mass and pain, 3 mo	N+M0	Surgery+aRT	No	ANED (14 mo)
Park et al <sup>13</sup>	12 M	Right parotid gland	2.4	Mass	N+M0	Surgery+aRCT	2 recurrences within 12 mo	DOD (24 mo)
Vulsteke et al <sup>14</sup>	32M	Left parotid gland	3.8	Rapidly growing mass	N0M0	Surgery+aRT, then CT for MTS	Disseminated bone MTS (1 mo)	DOD (4 mo)
Andreasen et al <sup>15</sup>	40 F	Right sublingual gland	4	Swelling, meal-related pain (1 mo)	N0M0	Surgery, palliative RCT	Wide-spread bone +cervical nodes (1 mo)	DOD (5.5 mo)
Klijanienko et al <sup>16</sup>	21 F	Left parotid gland	5	Mass	N+M0	Surgery, RT	Subcutaneous and liver (2 mo)	DOD (2 mo)
Seim et al <sup>17</sup>	26 M	Right sublingual gland	3	Pain, swelling, otalgia	N+M0	Surgery, palliative RCT	Extensive lung +bone MTS (2 wk)	DOD (1 mo)
Current series	39 F	Right parotid gland	3.6	Rapidly growing mass	pN2b M0	Surgery+aRCT BET inhibitor	Disseminated bone, nodes and lung (3 mo)	DOD (7 mo)
Current series	35 M	Parotid gland	NA	Mass	N+M0	Surgery, then palliative CT	Bone (postoperative)	AWD (3 mo)
Current series	55 F	Right parotid gland	9	Mass	N+M0	Surgery, then RCT	Live, lung and bone (6 mo)	DOD (7 mo)

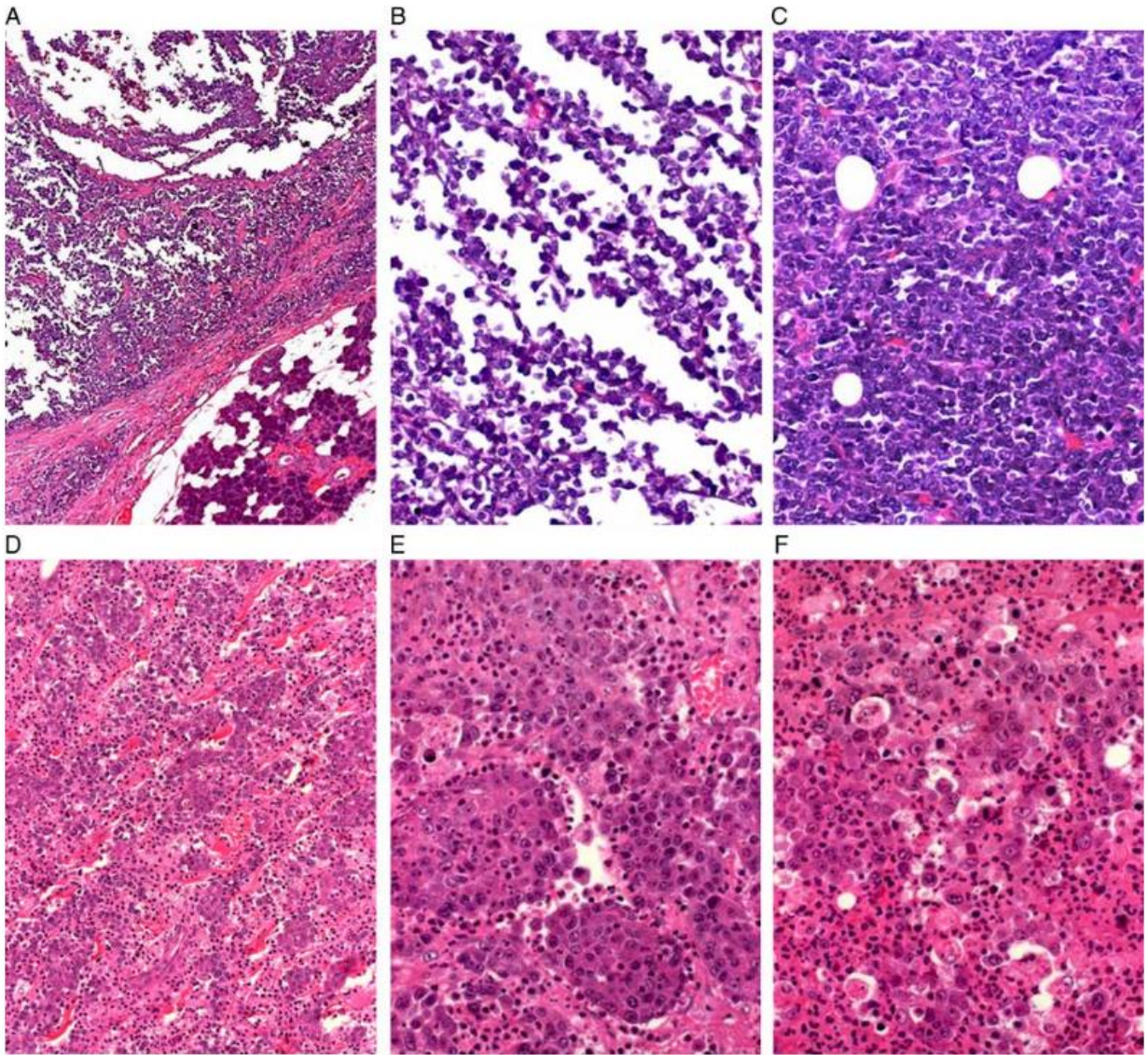
a indicates adjuvant; ANED, alive with no evidence of disease; AWD, alive with disease; CT, chemotherapy; DOD, died of disease; F, female; NA, not available; M, male; mo, months; MTS, metastasis; RCT, radiochemotherapy; RT, radiotherapy.

Table 3: Histopathological, immunohistochemical and molecular findings in salivary NUT carcinomas (n=10).

Case	Histological pattern	unusual features	Original diagnosis	Pan-CK	p63	CK7	SYN	Ch-A	CD56	INI1	NUT IHC	Genotype
1	Poorly cohesive basaloid cells, squamoid islands	Foci of cartilage	Poorly differentiated carcinoma (FNA)	+	+	NA	-	-	+	NA	+	<i>NUT-BRD4</i>
2	Poorly cohesive basaloid cells, squamoid islands	No	Nots specified	+	+	NA	-	-	-	NA	NA	<i>NUT</i> -variant (other than <i>BRD4/BRD3</i> )
3	Basaloid with desmoplasia, no squamous islands	No	Poorly differentiated SCC	+	+	NA	-	-	-	NA	+	NA
4	Poorly cohesive large cells	No	Myoepithelial carcinoma with neuroendocrine features	+	+	NA	+F	NA	+	NA	+	<i>NUT-BRD4</i>
5	Poorly cohesive basaloid cells, squamoid islands	No	Poorly differentiated basaloid SCC	+	+	+	<1%	-	-	NA	+	<i>NUT-BRD4</i>
6	Poorly cohesive basaloid cells	No	Poorly differentiated AdCC (FNA)	+	+	NA	-	-	-	NA	+	<i>NUT-BRD4</i>
7	Poorly cohesive large cells	No	Undifferentiated carcinoma	NA	NA	NA	NA	NA	NA	NA	NA	<i>NUT-BRD4</i>
8	Poorly cohesive large cells, squamoid islands	Prominent granulocytes	High-grade tumor, favor MEC	+	+	+	-	-	-	intact	+	<i>NUT-BRD4</i>
9	Poorly cohesive basaloid cells, squamoid islands	No	Undifferentiated large cell carcinoma	+	+	NA	-	-	-	intact	+	<i>NUT</i> , partner unknown (FISH failed)
10	Poorly cohesive basaloid cells, squamoid islands	No	Undifferentiated tumor	-	+	-	-	-	-	intact	+	<i>NUT-BRD4</i>

AdCC, adenoid cystic carcinoma; Pan-CK, pancytokeratin; Ch-A, chromogranin A; FNA, fine needle aspiration; F, focal; IHC, immunohistochemistry; NA, not available; MEC, mucoepidermoid carcinoma; SSC, squamous cell carcinoma; SYN, synaptophysin.





**Figure 1.** Salivary NCs were centered within salivary parenchyma (A) and displayed poorly cohesive sheets of small-sized to medium-sized cells arranged into pseudoalveolar (B), solid (C), corded (D), or nested (E) pattern. The nucleoli ranged from inconspicuous (C) to prominent (F), note extensive granulocytosis in F (A, B, C from case 3; D, E, F from case 1).



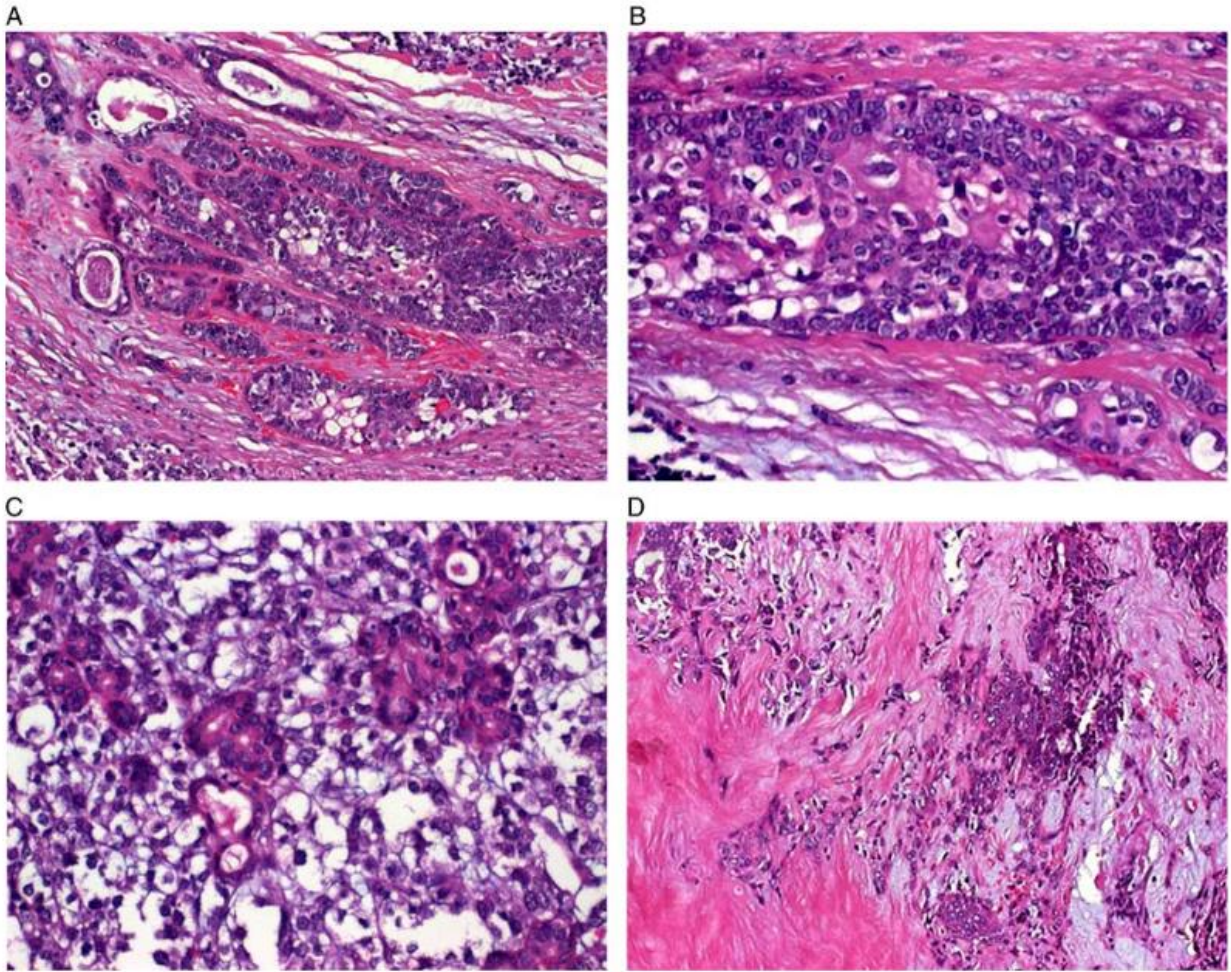


Figure 2. Foci of abrupt (clear cell) keratinization were seen in all 3 cases. Potentially misleading features included florid ductular proliferations (A, C) with entrapped of rare goblet cells (A, midlower field) and myxohyaline stromal changes reminiscent of pleomorphic adenoma (D). A to D from case 3.

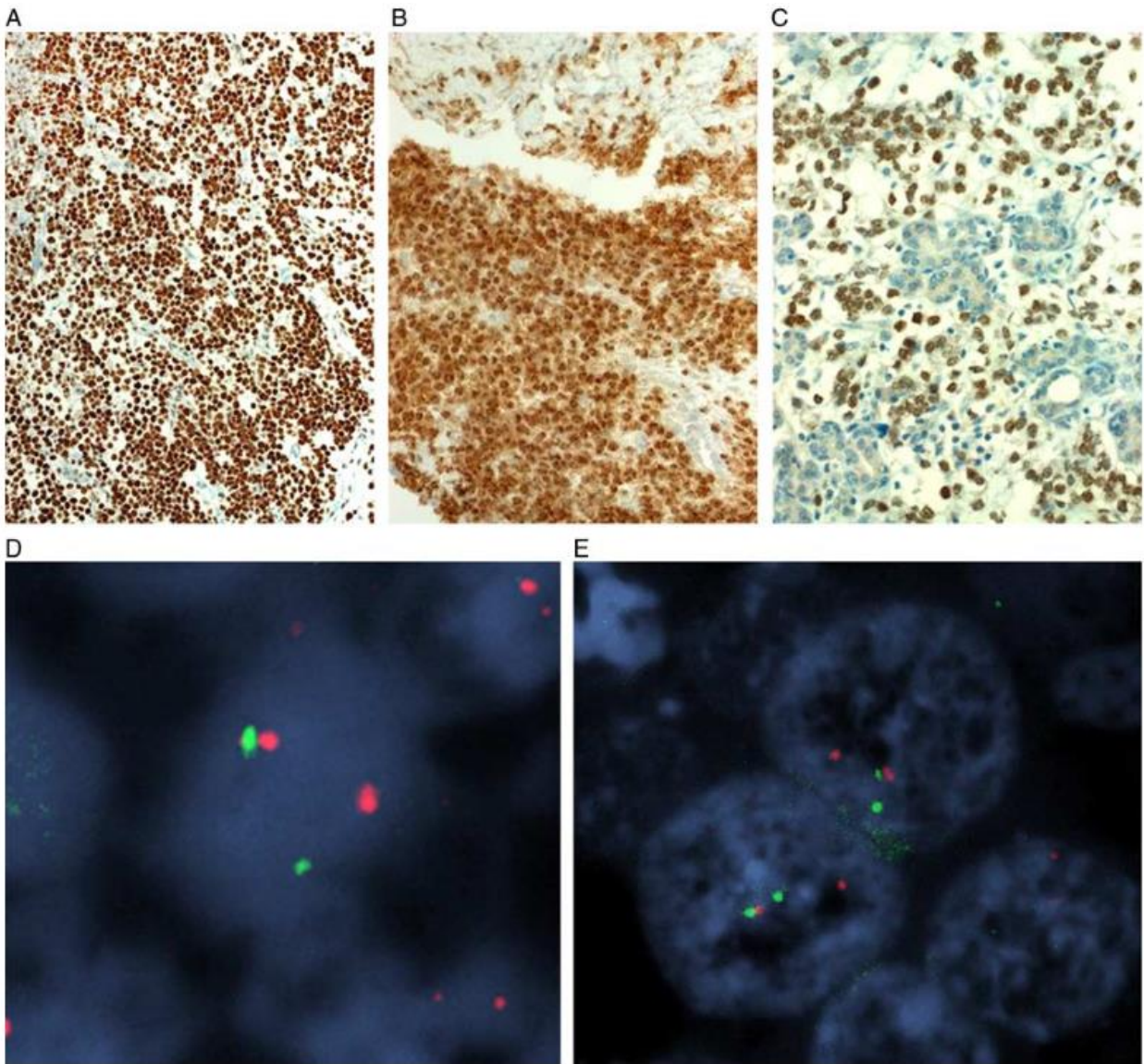


Figure 3. IHC showed consistent expression of p63 (A, case 3) and NUT protein (B, case 2). The NUT immunostain highlighted the neoplastic cells amid native salivary tissue (C, case 3). FISH analysis using the NUT probe (D) and BRD4 probe (E) showed break-apart signals indicating a NUT/BRD4 translocation (image from case 1)