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NUTM1 Gene Fusions Characterize a Subset of Undifferentiated Soft Tissue and Visceral Tumors

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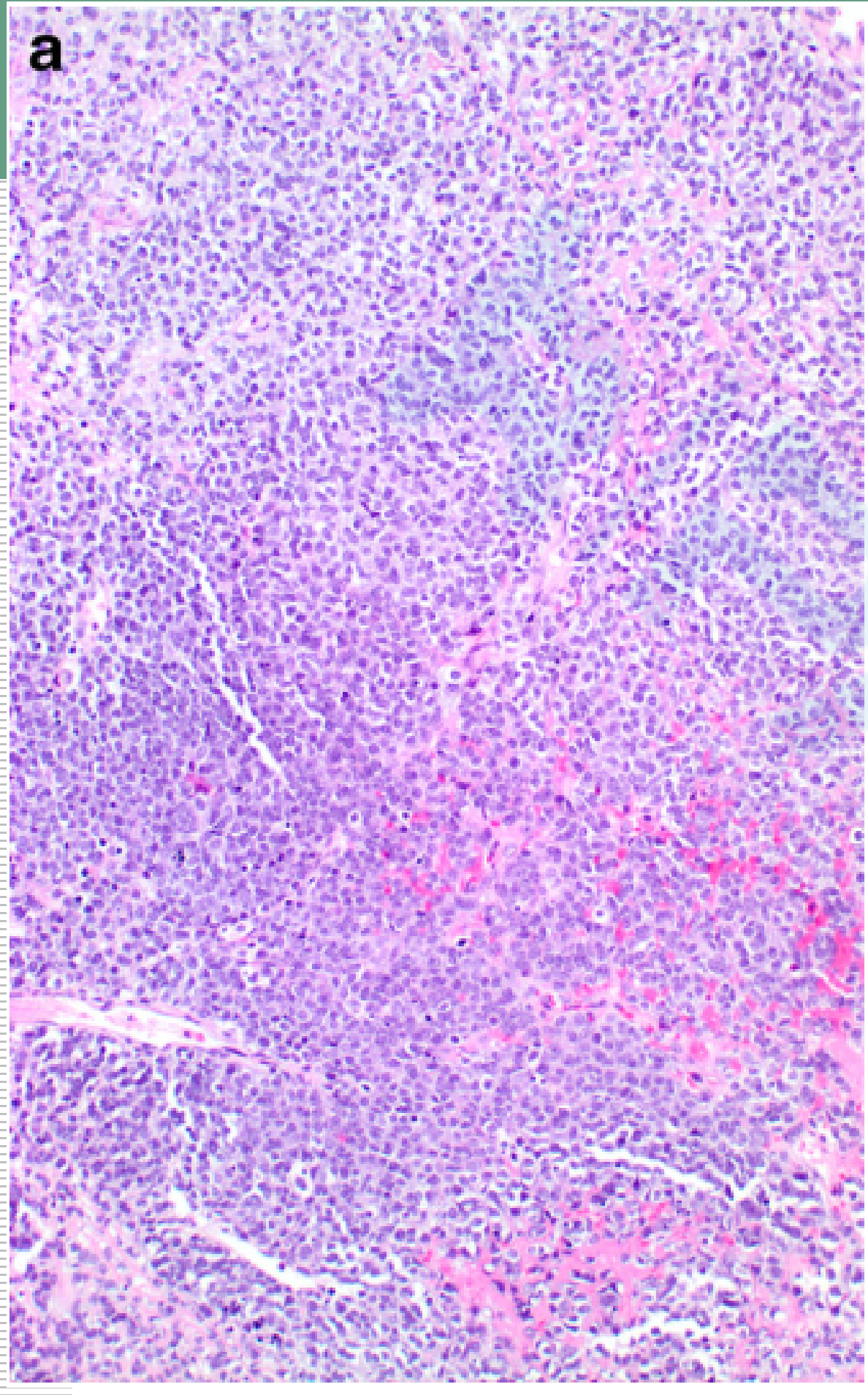
NUT carcinoma

- NUT carcinoma is a poorly differentiated carcinoma(often with evidence of squamous differentiation) defined by the presence of nuclear protein in testis (NUT) gene (NUTM1) rearrangement
- ICD-O code: 8023/3
- NUT carcinoma is a rare tumour in the upper aerodigestive tract. Due to its rarity, the true incidence is unknown. In the largest series reported(n=40), the median patient age was 21.9 years,but people of all ages were affected(range: 0.1-82 years). A slight predominance of females was seen, wit 55% of the cases occurring in females

- The etiology is unknown. There is no association with HPV, EBV, other viral infection; smoking or other environmental factors.
- Predilection site: the head and neck or mediastinum and lung
- Clinical feature: nonspecific symptoms caused by a rapidly growing mass
- Prognosis is poor, with a median overall survival of 9.8 months. Some evidence suggests that patients with NUT-variant carcinoma may have a longer servival than do BRD-NUT carcinoma patients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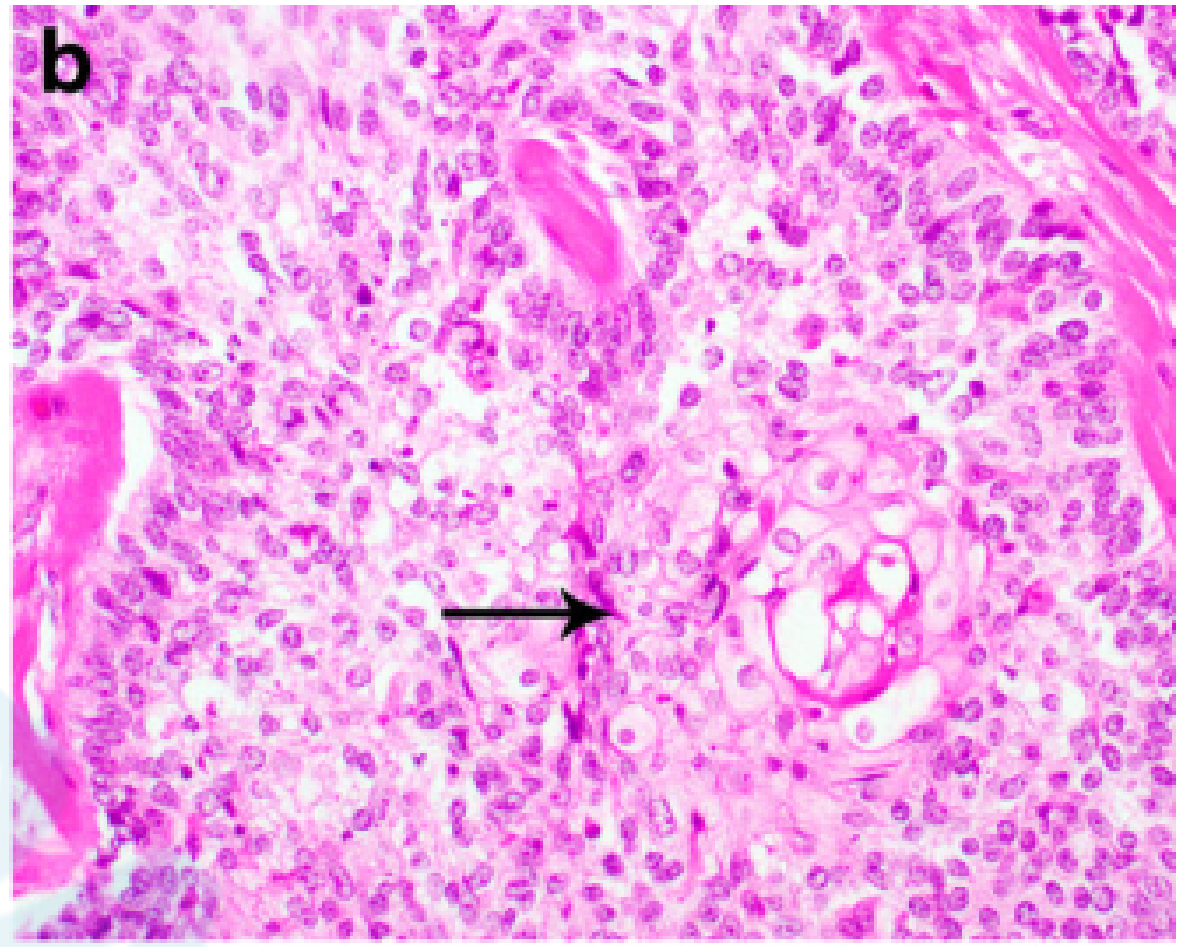
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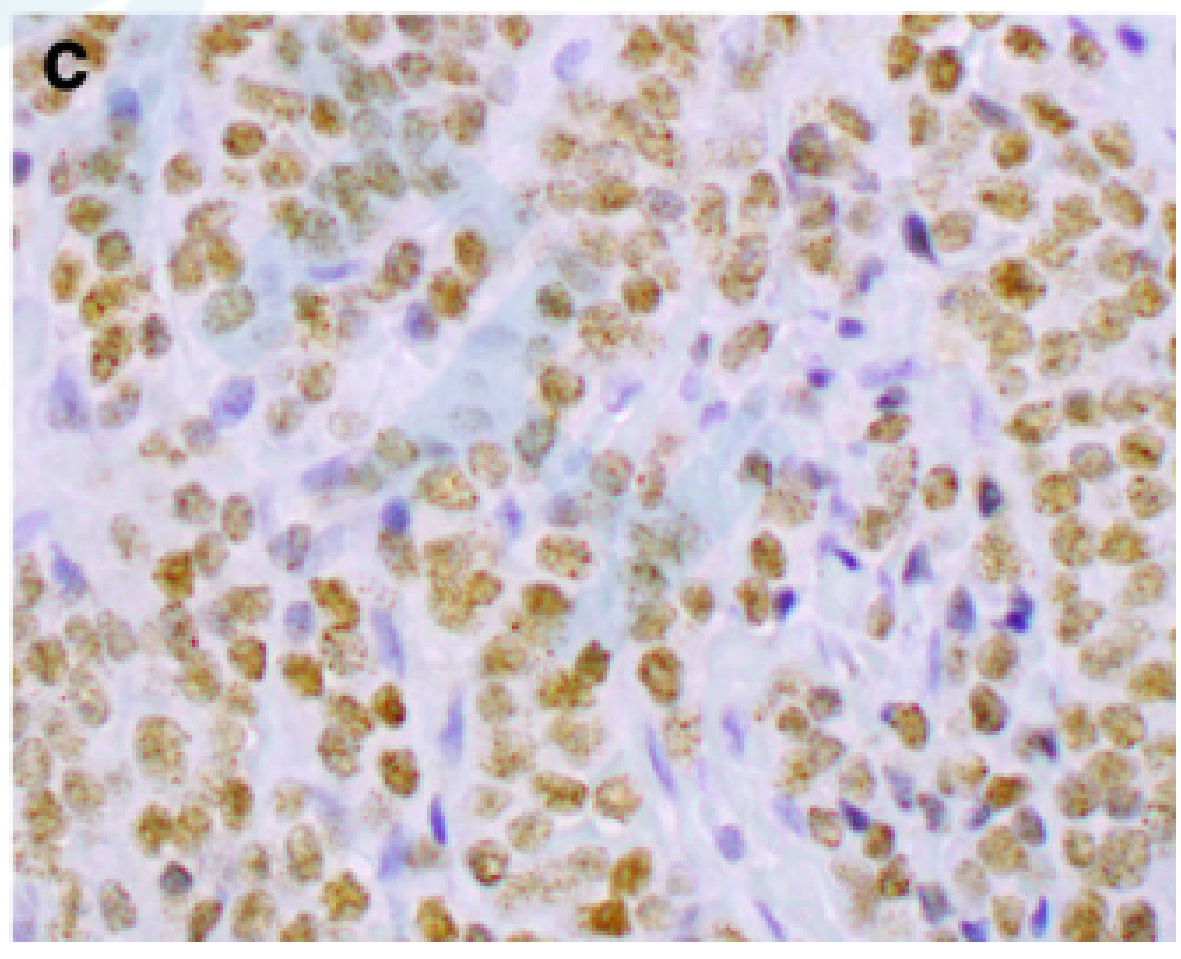
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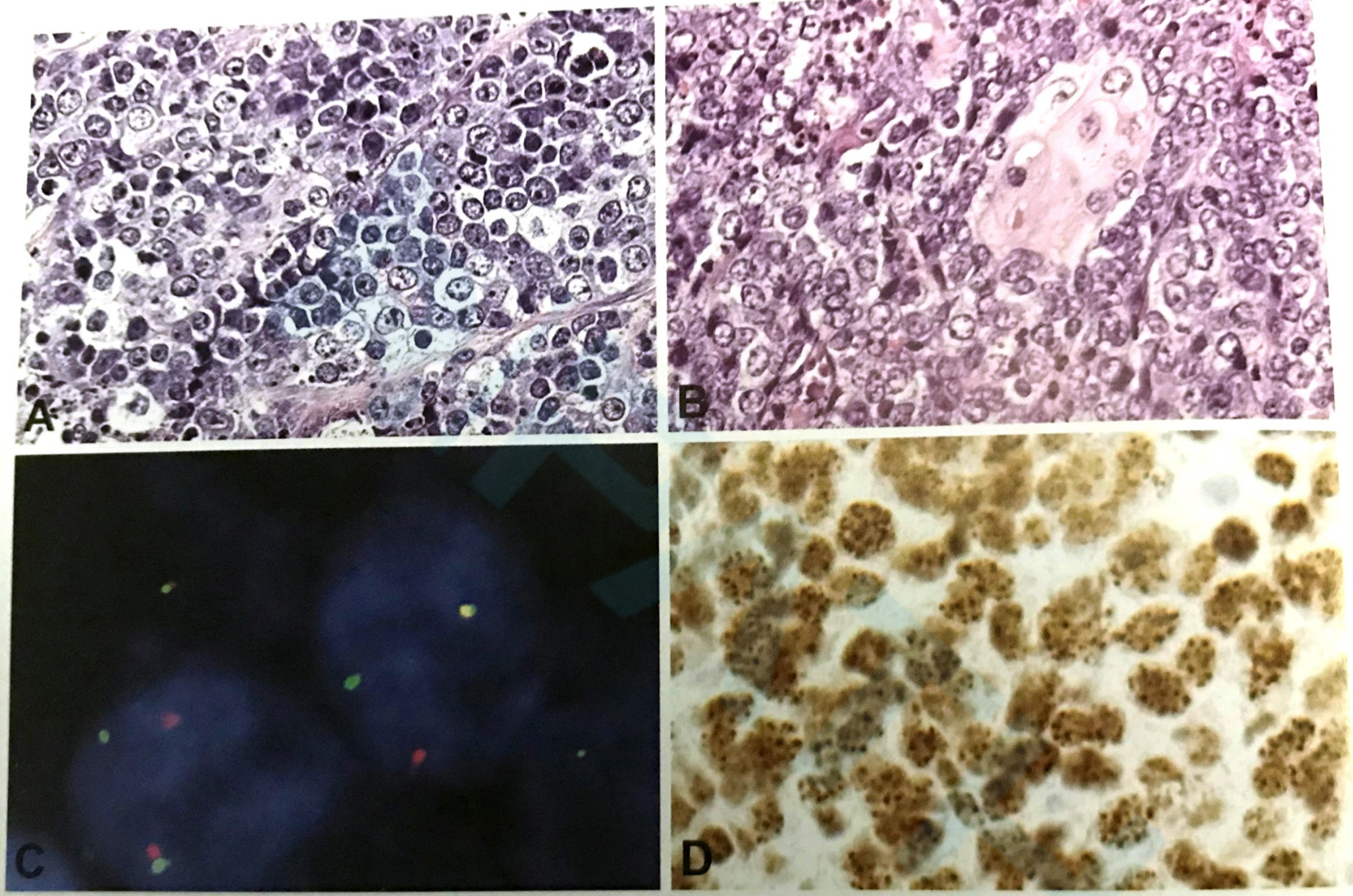
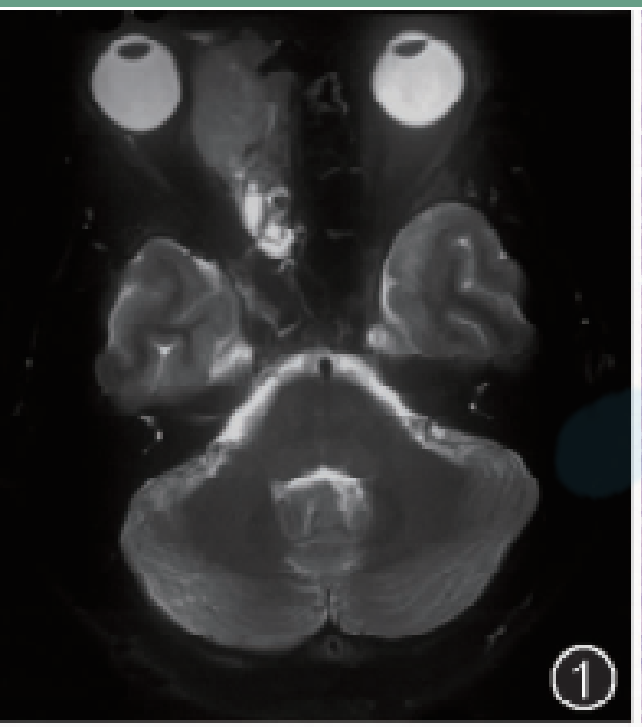
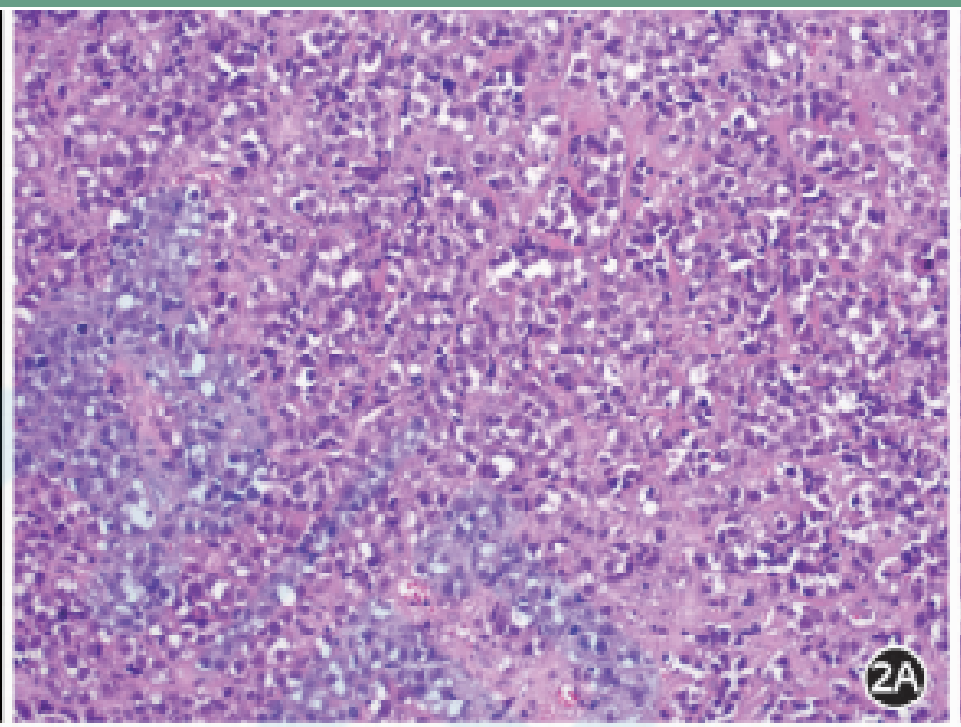
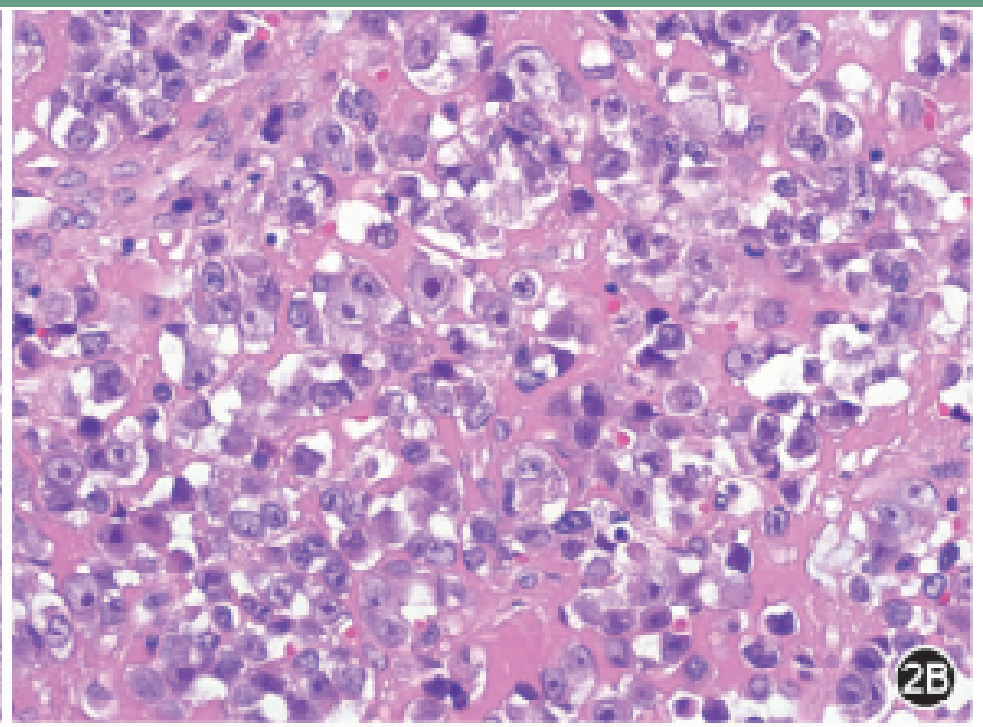
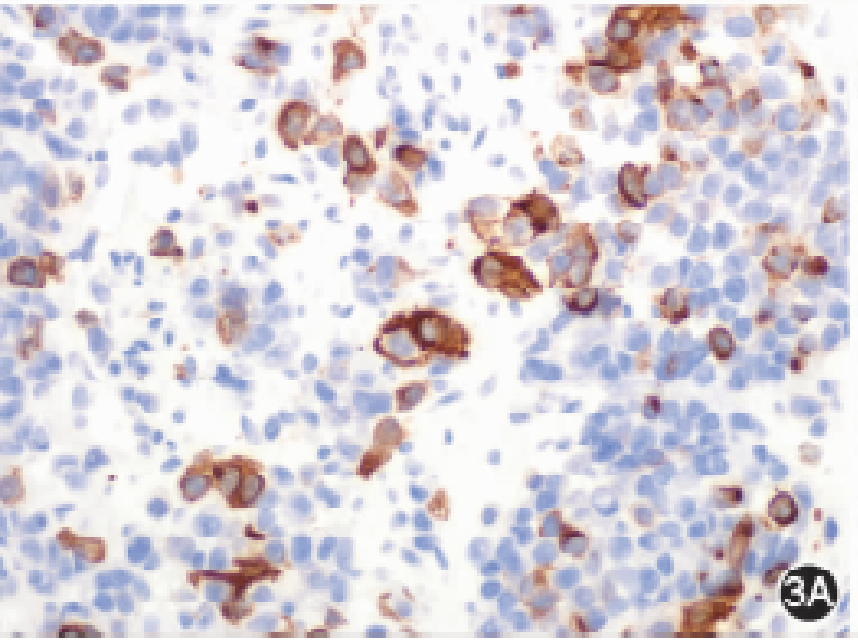


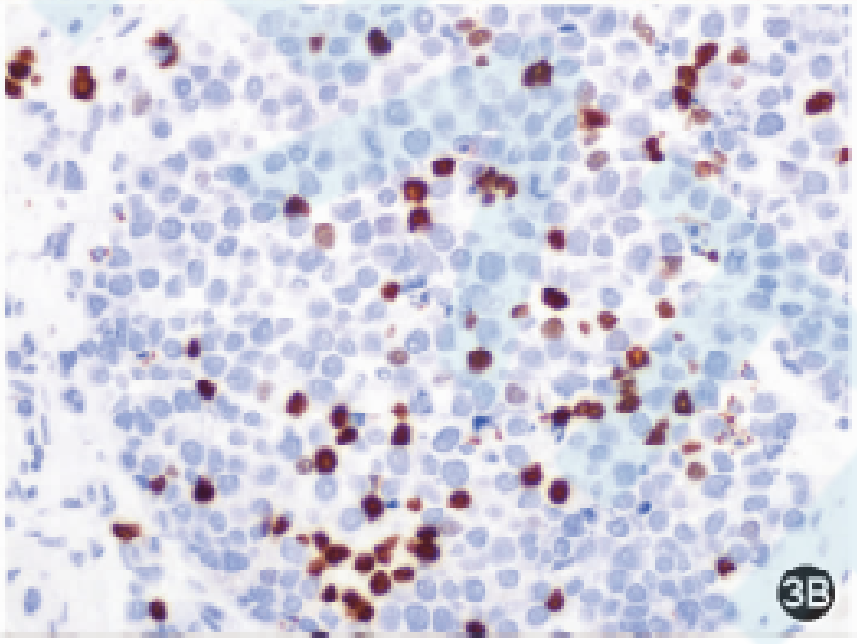
Fig. 1.09 NUT carcinoma. **A** Sheets of moderate-sized monomorphic poorly differentiated epithelioid cells have pale to clear glycogenated cytoplasm; the intervening stroma is scant, and necrosis and mitoses are invariably present. **B** Abrupt keratinization can appear as a discrete island within a sea of poorly differentiated cells. **C** FISH demonstrates *NUT* rearrangement when red and green probes flanking the *NUT* locus are split apart; the red and green signals together are the normal *NUT* allele. **D** Diffuse, nuclear immunohistochemical staining with the NUT antibody is diagnostic of NUT carcinoma; the speckled pattern is characteristic but not always this distinct.











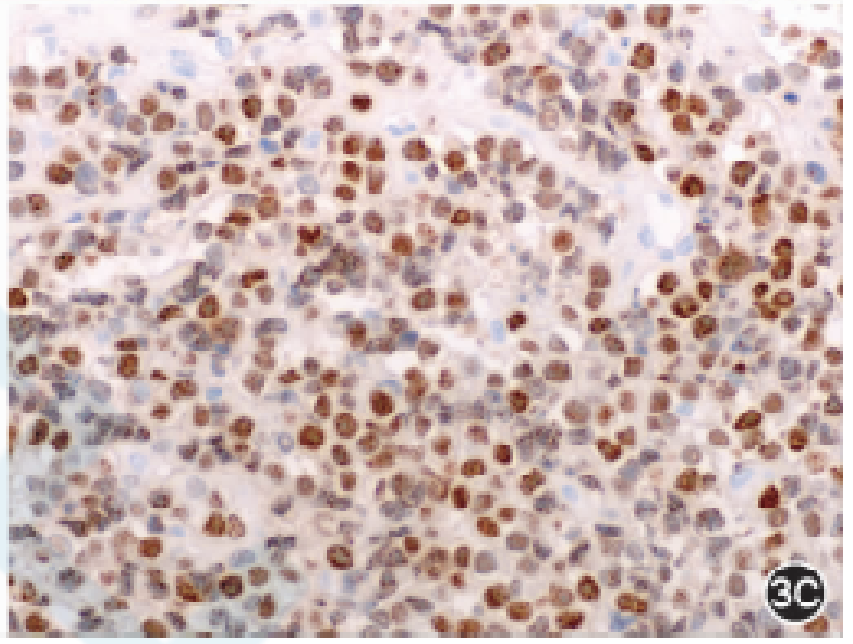


图 1　患者 MRI 表现，显示肿瘤呈侵袭性生长，破坏右侧眼眶内壁骨质　**图 2**　NUT 中线癌的 HE 镜下表现；A 示肿瘤细胞由片状或巢状排列的低分化或未分化细胞构成　HE　中倍放大；B 示肿瘤细胞的黏附性差，胞质较少，染色质细腻或呈颗粒状，核仁明显，核分裂象易见，胞质嗜酸性或透亮　HE　高倍放大　**图 3**　NUT 中线癌免疫组织化学结果；A 示肿瘤细胞呈 AE1/AE3 的散在表达　EnVision 法　高倍放大；B 示散在的肿瘤细胞 p63 阳性　EnVision 法　高倍放大；C 示肿瘤细胞 NUT 弥漫核点状阳性　EnVision 法　高倍放大

- Immunohistochemistry
  - Commonly positive include p63, p40, and cytokenatins
  - Occasionally(in 55% of cases) expresses CD34
  - Occasional positivity for neuroendocrine markers, p16, and TTF1 has also been described
- Genetic profile: NUTM1 is fused with RBD4(70%)、RBD3(6%) or NSD3; undefined fusion partner(s), which is referred to as NUT-variant carcinoma

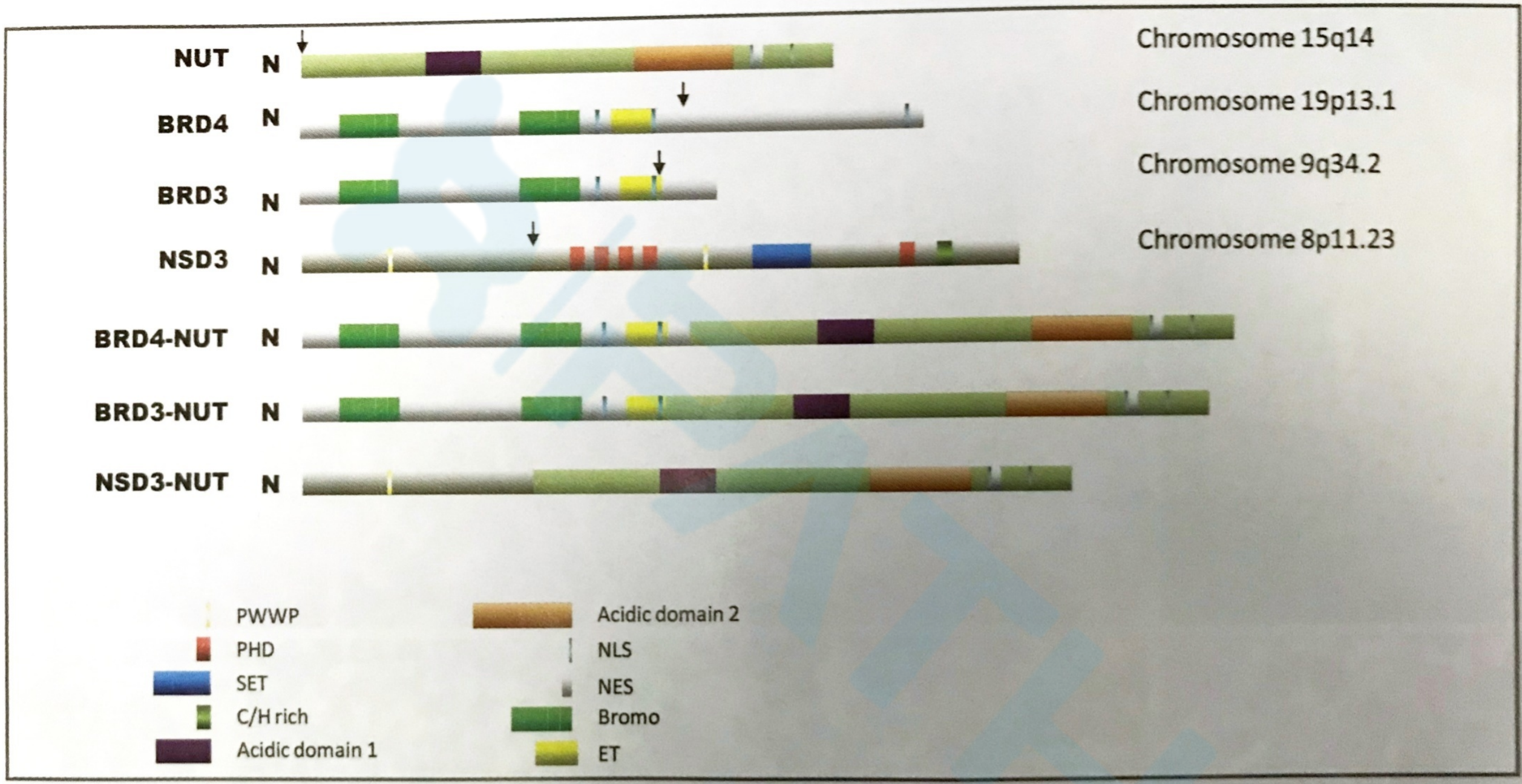


Fig. 1.10 NUT carcinoma. Schematic illustration of the various translocations that occur in NUT carcinoma between *NUT* genes and *BRD4*, *BRD3*, and *WHSC1L1* (also called *NSD3*); the arrows indicate breakpoints. Nearly the entire NUT transcript is preserved in every known translocation. PWWP, PWWP domain; PHD, plant homeodomain; SET, SET domain; C/H rich, Cys/His-rich domain; NLS, nuclear localization signal sequence; NES, nuclear export signal sequence; Bromo, bromodomain; ET, extraterminal domain.

Differential diagnosis

- Poorly differentiated squamous cell carcinoma
- Ewing sarcoma/PNET
- Sinoasal undiffernetiated carcinoma
  - SMARCB1-deficient sinonasal carcinoma
- Lymphoma
- Germ cell tumour
- Olfactory neuroblastoma

- NUT midline carcinoma tend to be distributed along the midline axis with a predilection for the head and neck, and mediastinum and lung
- In addition, there are rare reports of primary bladder, breast, endometrium, kidney, and orbit involvement
- Following discovery of an index case of an undifferentiated soft tissue tumor containing NUTM1 rearrangement, we sought to investigate the incidence of NUTM1-related fusions among undifferentiated tumors in the soft tissue and viscera

MATERIALS AND METHODS

- Case Selection
  - The index case revealed NUTM1 rearrangement
  - Undifferentiated soft tissue and visceral tumors by the 2 senior authors (B.C.D./C.R.A.; 2007 to 2017)
  - Cases was purported to represent the site of primary disease were pulled for rereview
- Immunohistochemistry
  - keratins (pancytokeratin, high-molecular and low-molecular weight keratins), claudin-4, p63, S100, GFAP, CgA, syn, actin, desmin CD34, and NUT
  - 0, no staining; 1+, <5%; 2+, 5% to 25%; 3+, 26% to 50%; 4+,51% to 75%; and 5+, 76% to 100%
- RNA Sequencing
- Fluorescence In Situ Hybridization

RESULTS

TABLE 1. Clinical Attributes of 6 Cases of NUT-associated Tumor of Soft Tissue and Viscera

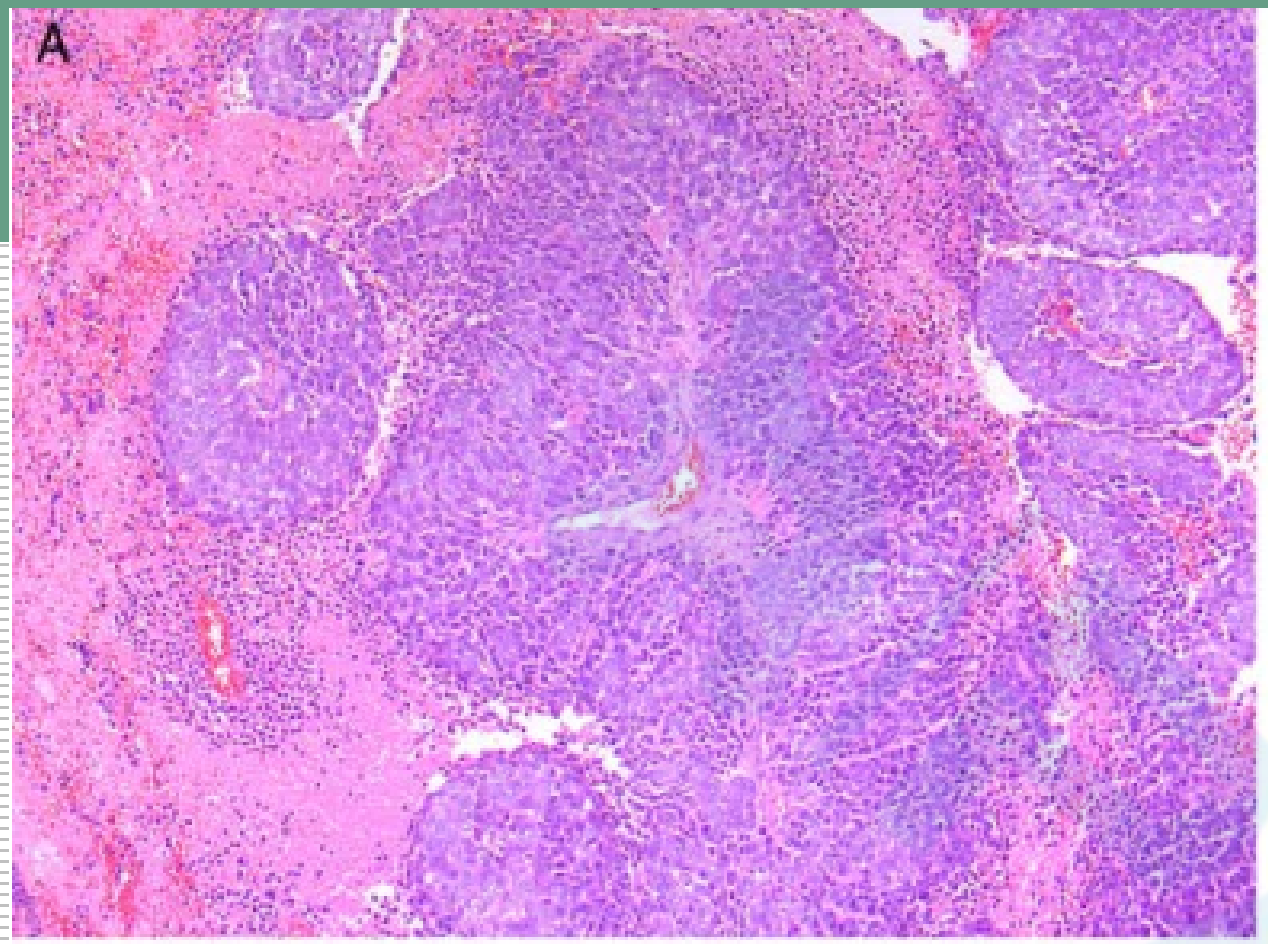
Case	Age (y)	Sex	Site	Treatment	Clinical Course	Status (mo)
1*	61	M	Thigh, proximal, L	Biopsy	LN metastases	DOD 3
2	45	M	Upper arm, L	Surgery, chemo, rads	LN, lung, soft tissue metastases	DOD 48
3	39	F	Stomach wall	Surgery, chemo	Peritoneal dissemination, LN, liver, spleen metastases	AWD 108
4	3	M	Brain, parietal, L	Surgery, chemo	NA	DOD 12
5	71	F	Kidney, L	Biopsy	Lung metastases	DOD 2
6	36	F	Kidney, R	Nephrectomy	Lung metastases	DOD 6

*Index case.
AWD indicates alive with disease; DOD, dead of disease; F, female; L, left; LN, lymph node; M, male; NA, not available; R, right.

TABLE 2. Immunohistochemical and Molecular Findings in 6 Cases of NUT-associated Tumor of Soft Tissue and Viscera

Pt	PanCK	HMWK	LMWK	C-4	p63	S100	GFAP	Syna	Chro	Actin	Desmin	CD34	NUT	Molecular
1	5+	5+	5+	1+	2+	–	5+	2+(w)	–	–	–	–	5+	*BRD3-NUTM1*
2	–	–	–	–	–	–	–	–	–	1+(w)	–	–	–	*BCORL1-NUTM1*
3	2+(w)	–	2+(w)	–	–	–	3+(w)	–	–	–	–	–	–	*MXD1-NUTM1*
4	–	–	–	–	–	NA	2+	1+	–	NA	NA	NA	5+	*BRD4-NUTM1*
5	1+(w)	–	1+(w)	NA	–	–	1+(w)	–	–	–	–		5+	*BRD4-NUTM1*
6	5+*	2+	NA	4+	1+	NA	NA	–	–	NA	–	NA	3+ (w)	*BRD4-NUTM1*

*Diffuse strong immunoreactivity for CK7.[8]
– indicates negative; C-4, Claudin-4; Chro, chromogranin; HMWK, high-molecular weight keratin; LMWK, low-molecular weight keratin; NA, not assessed; panCK, pancytokeratin; Pt, patient number; Syna, synaptophysin; w, weak.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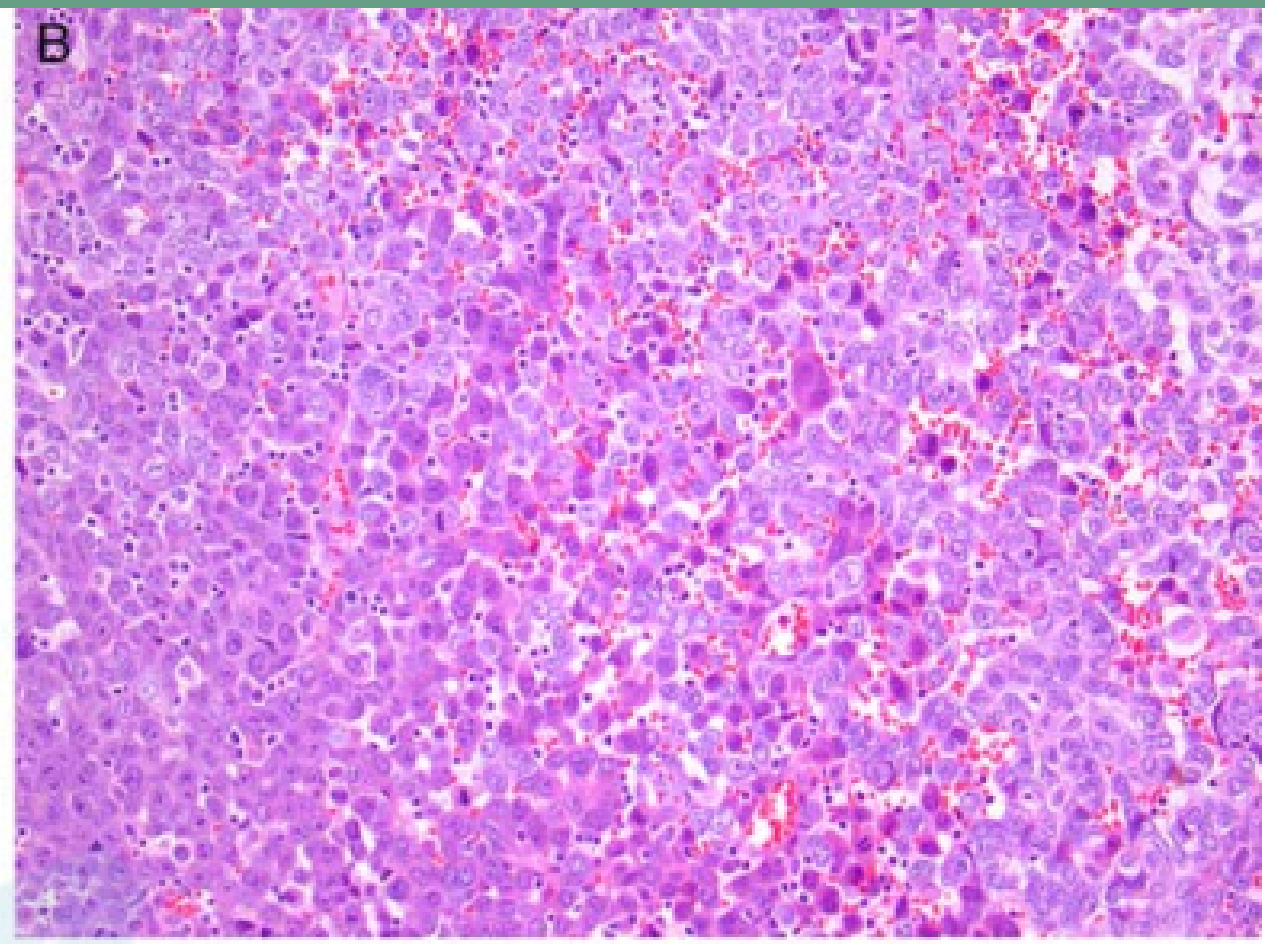
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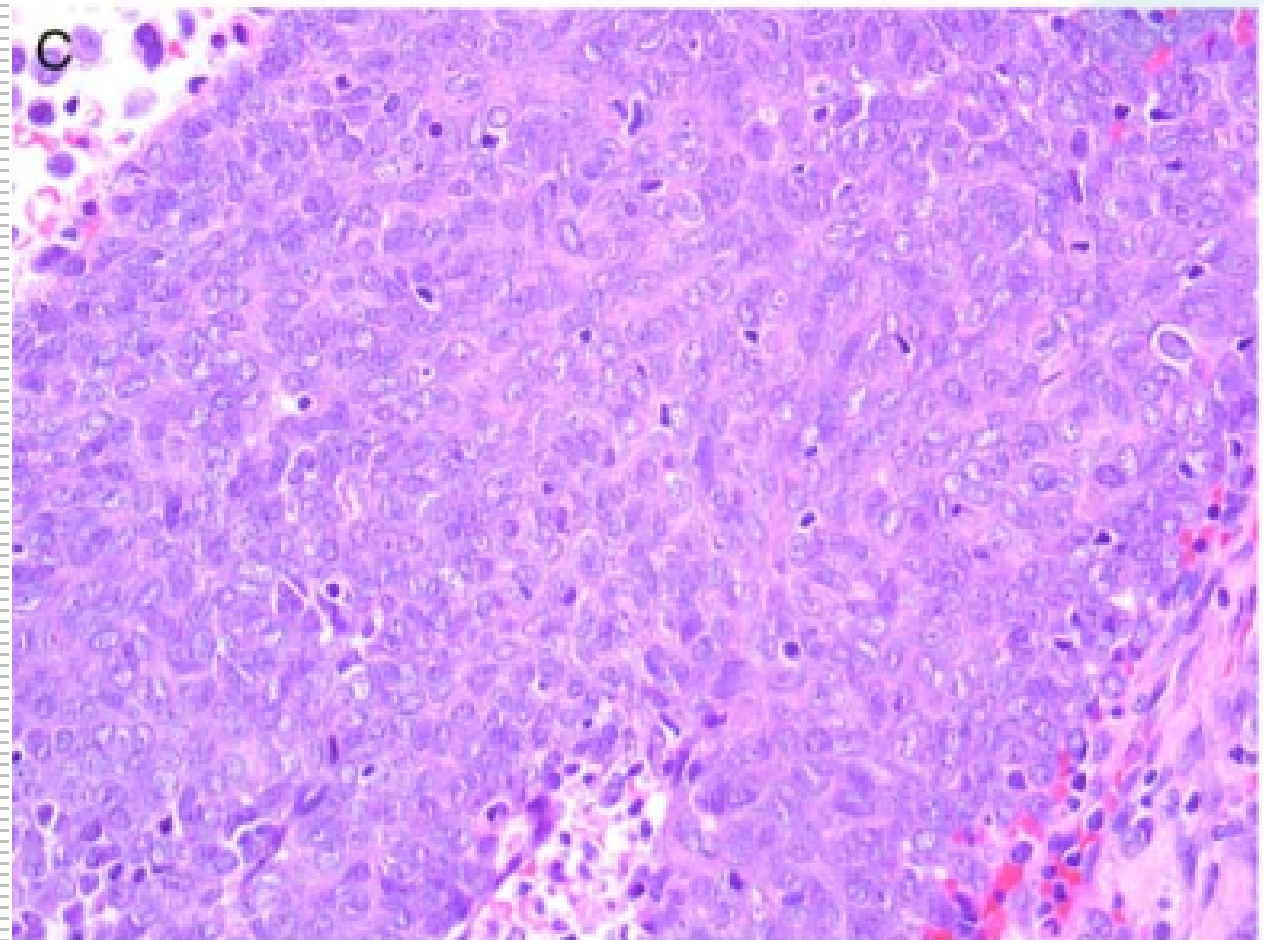
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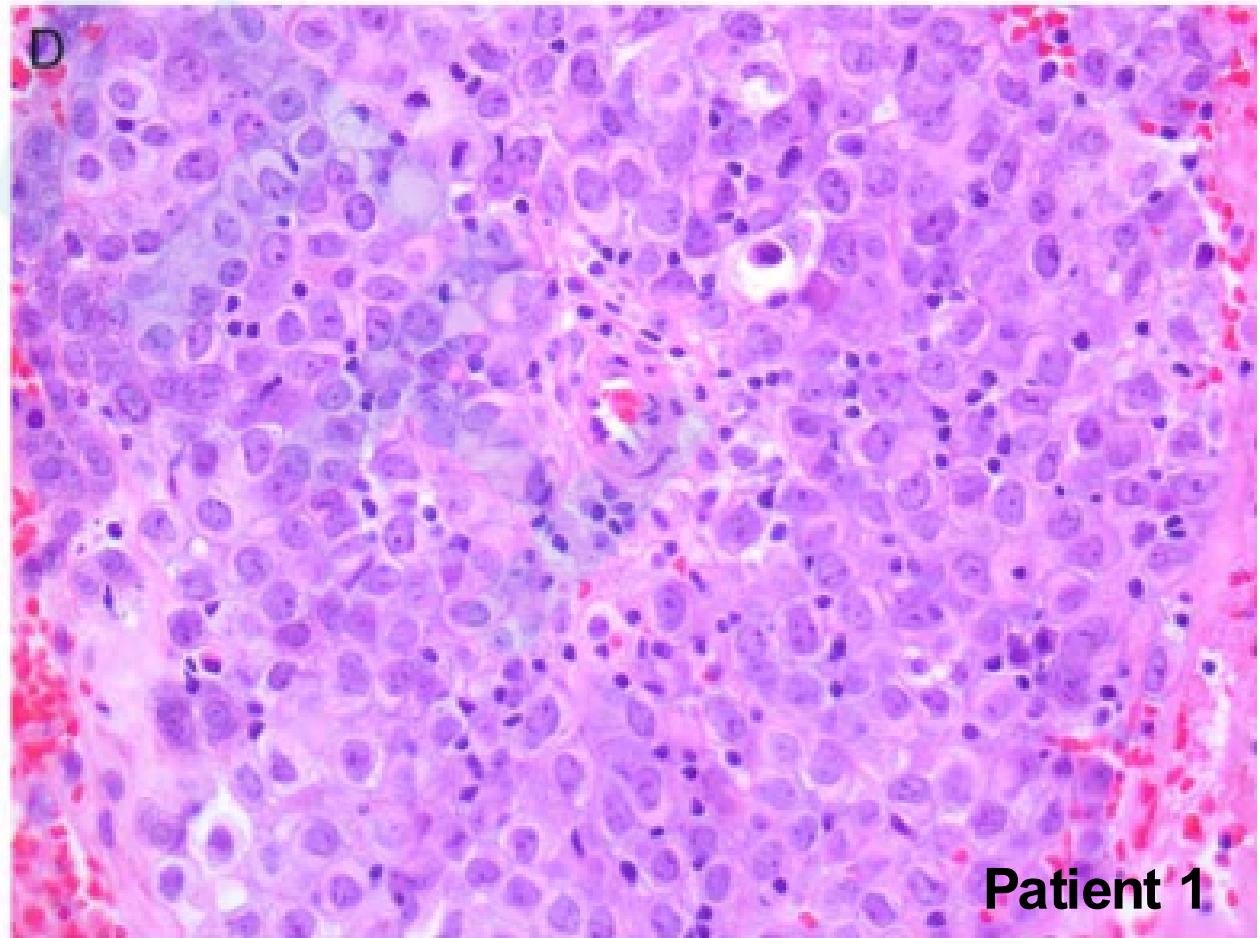
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Patient 1

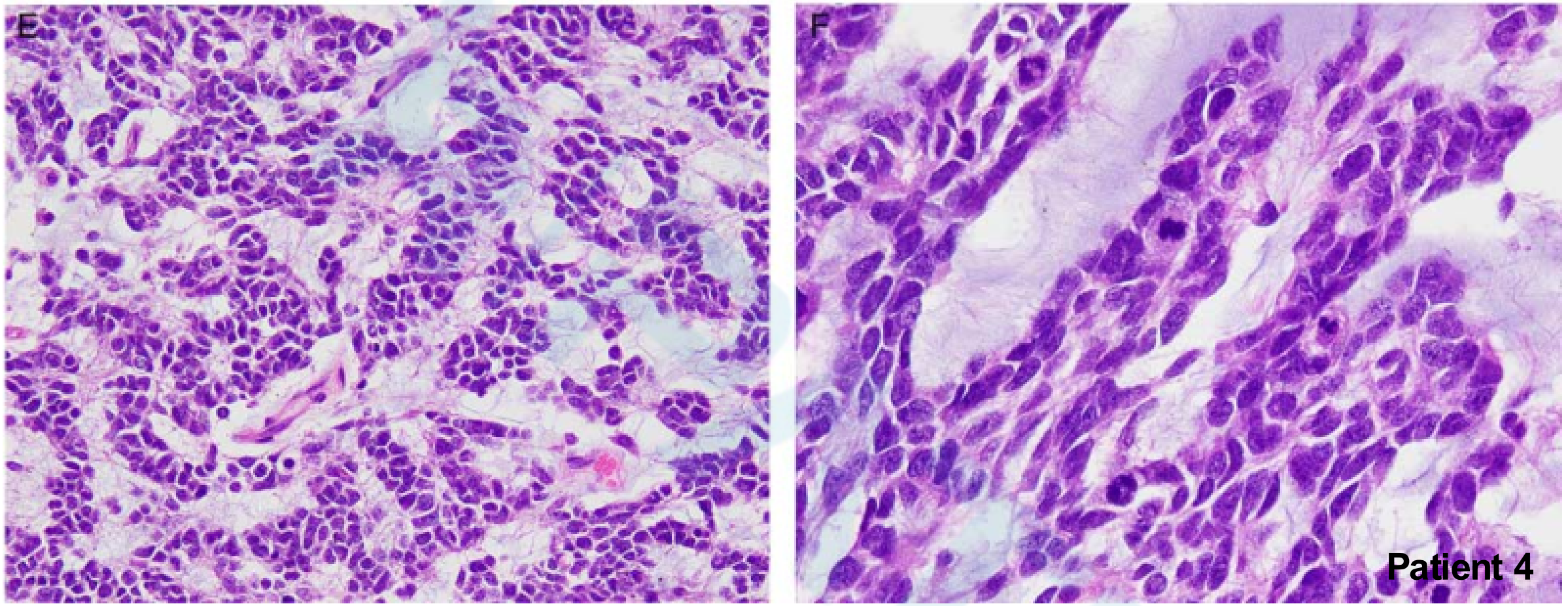


FIGURE 1. Patient 1: an intramuscular NUT-associated tumor. A, Sheets of predominantly large epithelioid cells radiating from a vessel with an abrupt transitioning into areas of necrosis. B, Juxtaposition of epithelioid-polygonal cells with differing cytoplasmic and nuclear characteristics. C, Area of ovoid cells with syncytial pattern. D, Sheets of epithelioid-rhabdoid cells with prominent nucleoli. Patient 4: a parietal lobe NUT-associated tumor. E, Epithelioid-polygonal cells with a reticular-alveolar pattern and prominent myxoid stroma. F, Higher magnification highlighting the nuclear molding, speckled chromatin, and conspicuous mitotic activity.

A

B

C

D

Patient 2

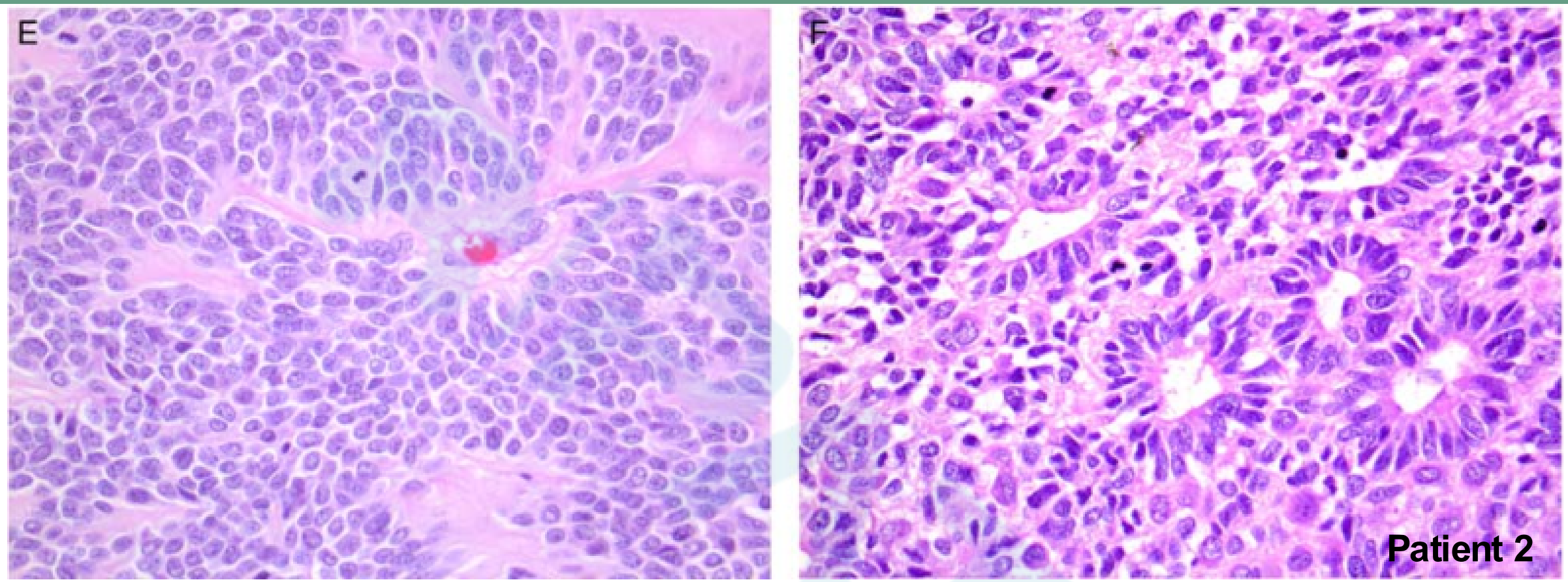
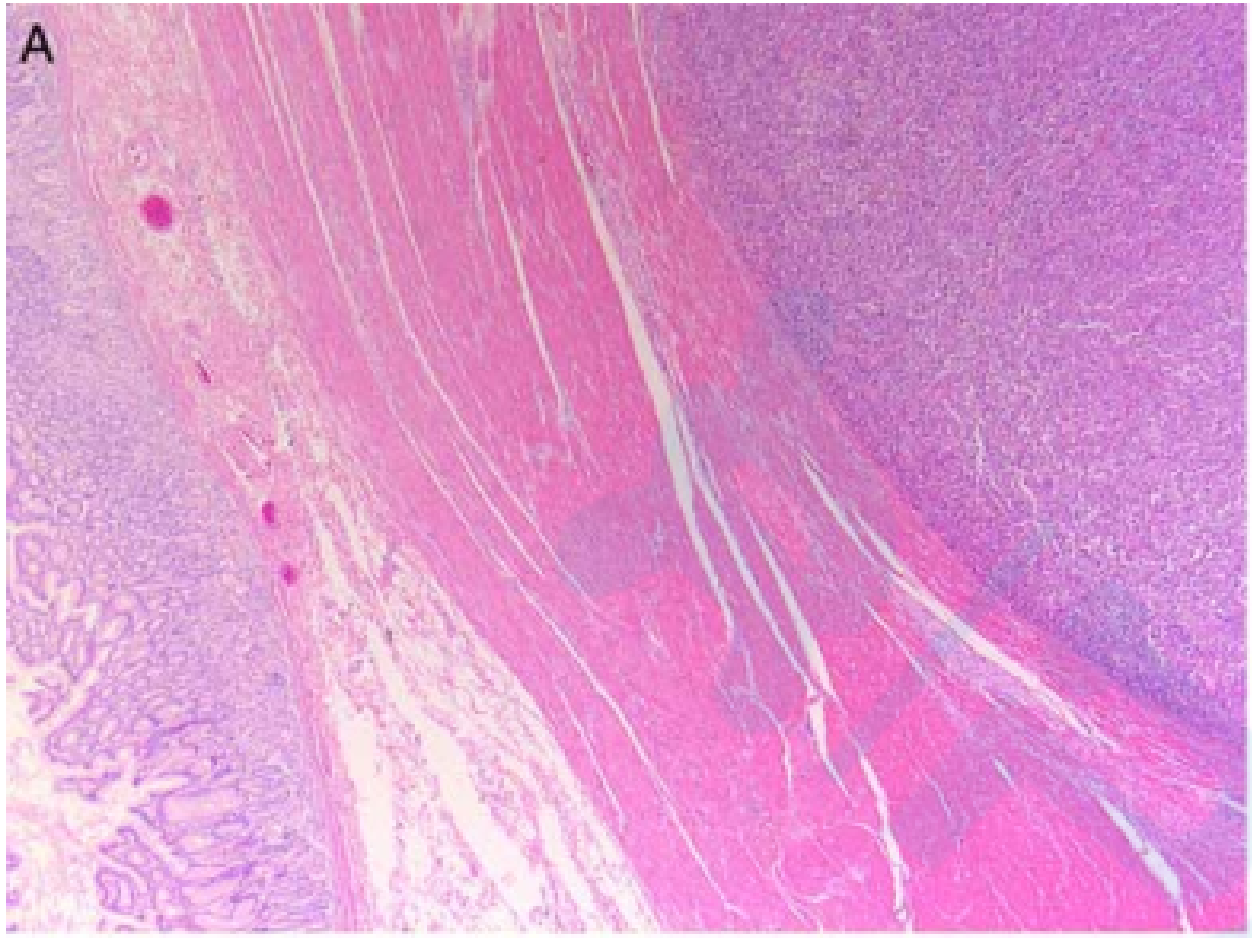


FIGURE 2. Patient 2: an intramuscular NUT-associated tumor with a markedly varied morphology. A, Nests and cords of polygonal cells set within hyaline stroma, resembling myoepithelial carcinoma. B, Spindle cells with a herringbone pattern. C, Cords of epithelioid cells separated by wiry collagen, vaguely resembling sclerosing epithelioid fibrosarcoma. A soft tissue metastasis that occurred 2 years later, which retained many of the initial features; however, there was overall greater cellularity, atypia, and mitotic activity. D, Spindle-epithelioid cells with areas of chondromyxoid stroma. E, Cellular nests of polygonal cells radiating around delicate blood vessels. The nuclei are large with delicately speckled chromatin. F, Area of rosette formation.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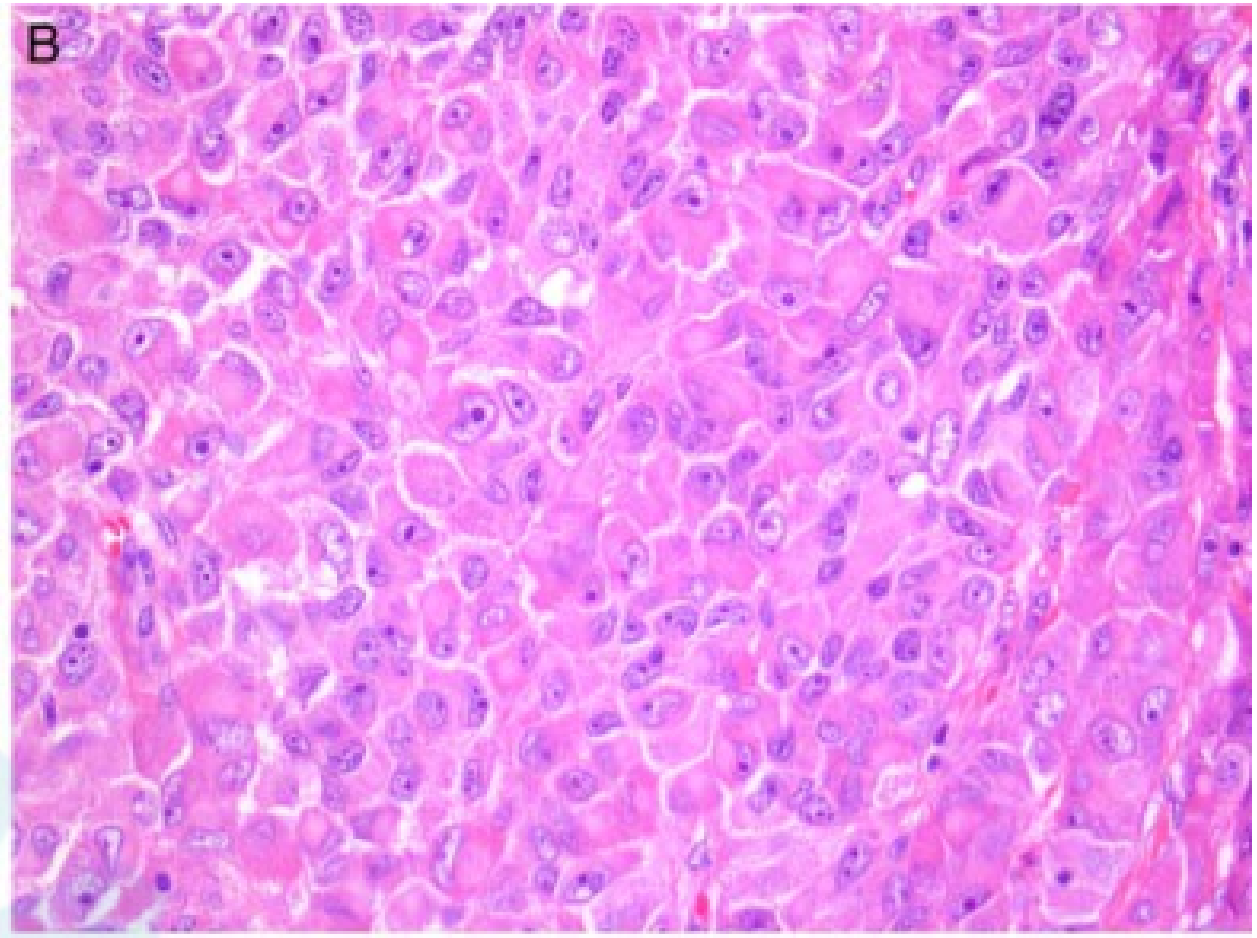
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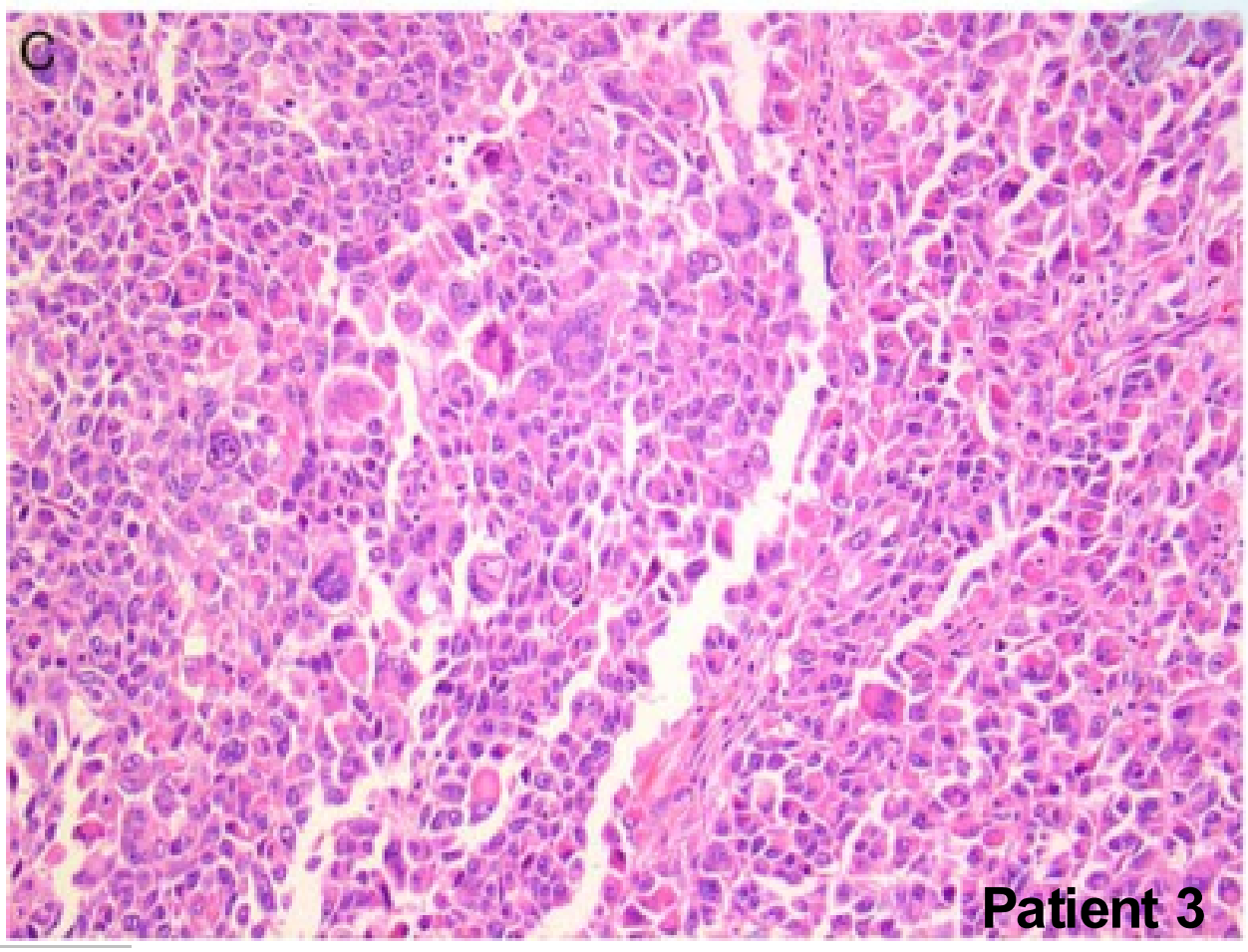
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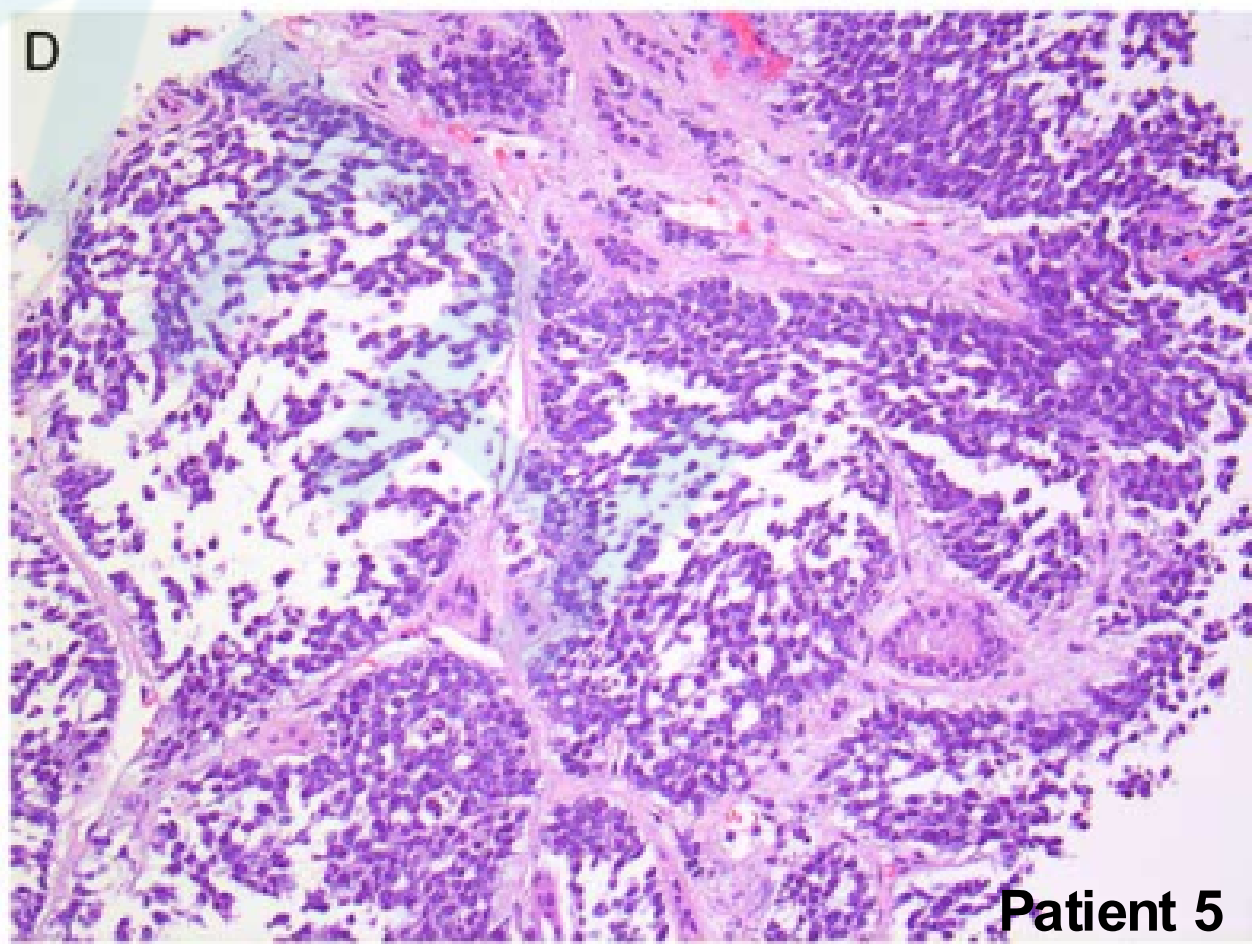
Patient 3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Patient 5

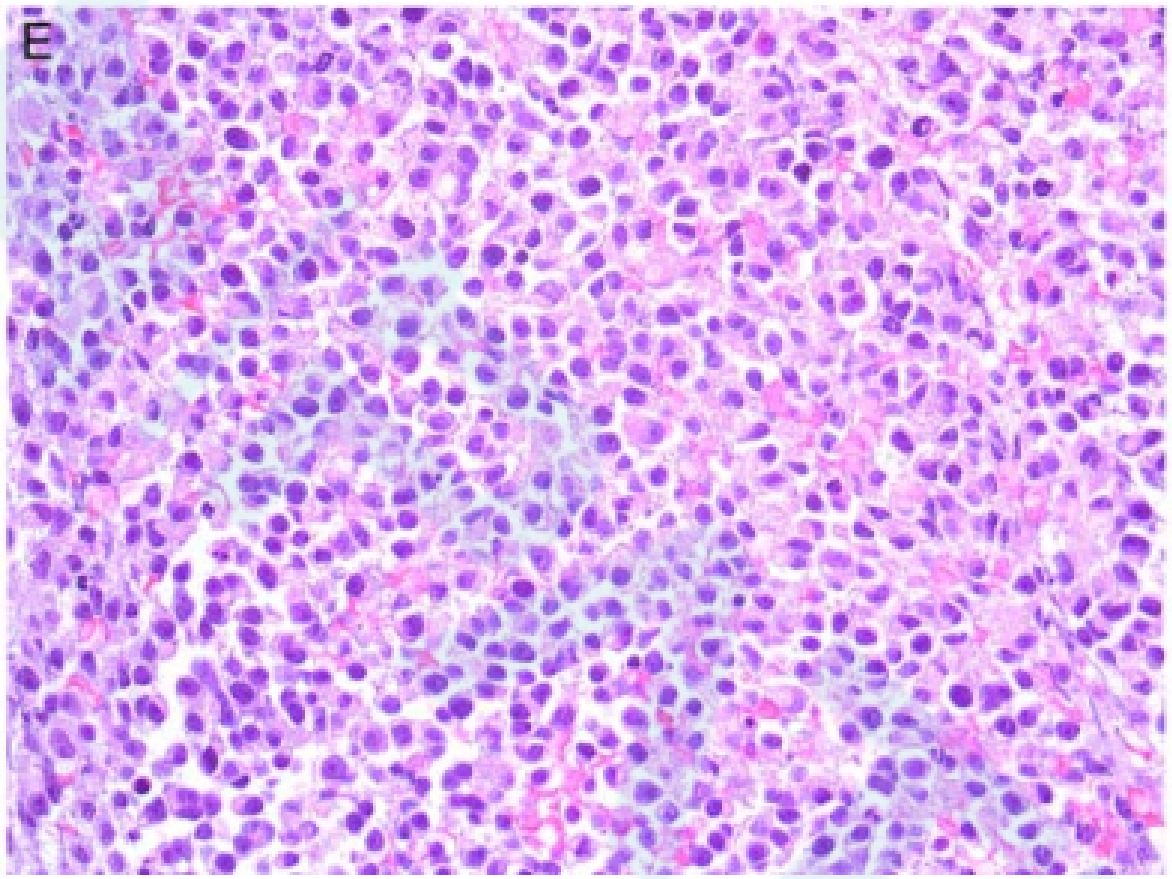
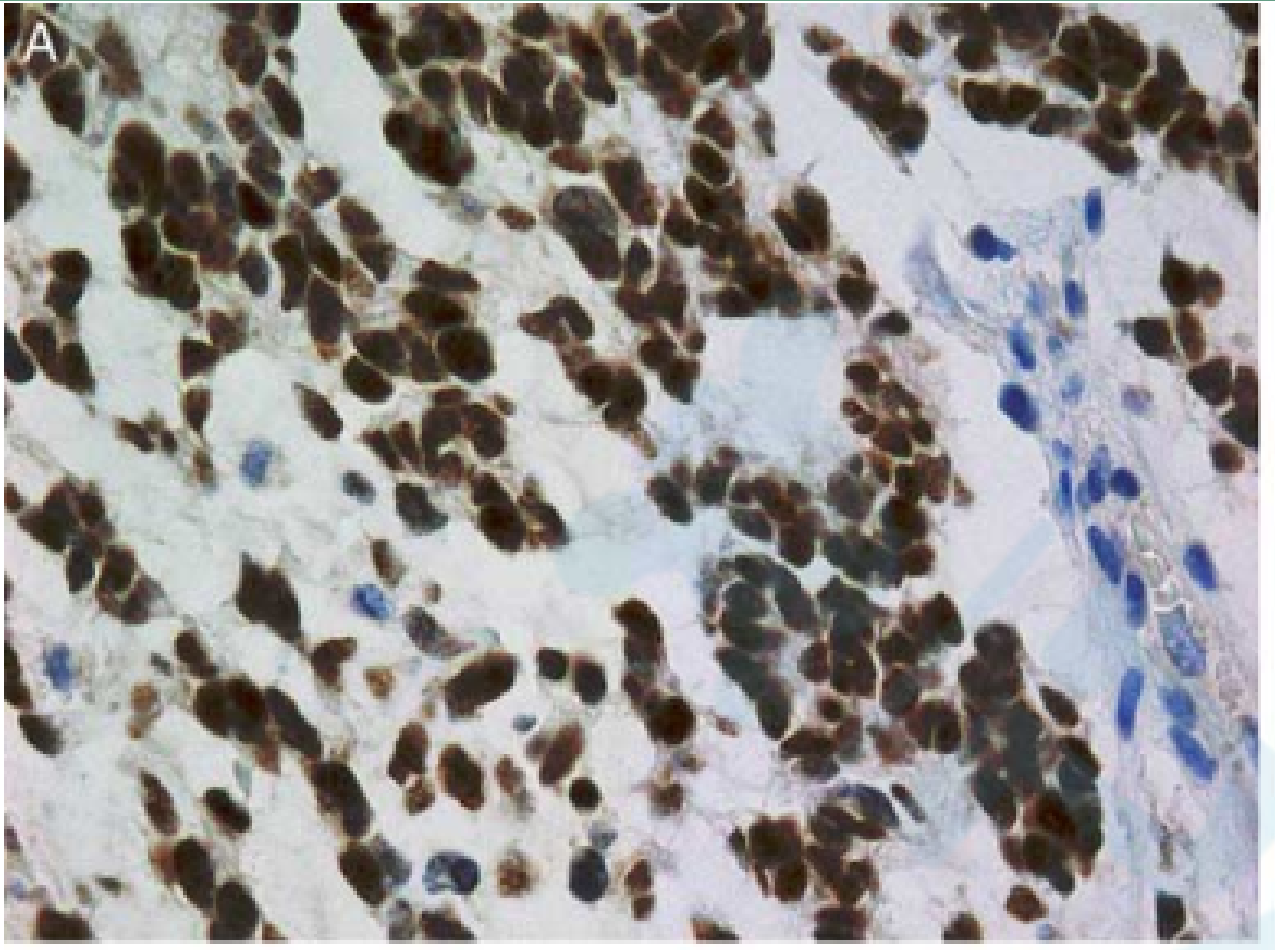


FIGURE 3. Patient 3: gastric NUT-associated tumor. A, The tumor was centered in the muscularis propria and extended to serosa. B, Sheets and nests of polygonal-rhabdoid cells with dense eosinophilic cytoplasm. C, Scattered nuclear atypia and multinucleated giant cells were present in several tumors. Patient 5: renal NUT-associated tumor. D, Infiltration of renal parenchyma by round-rhabdoid cells. E. Sheets of discohesive cells with areas of degeneration. This tumor was predominantly necrotic.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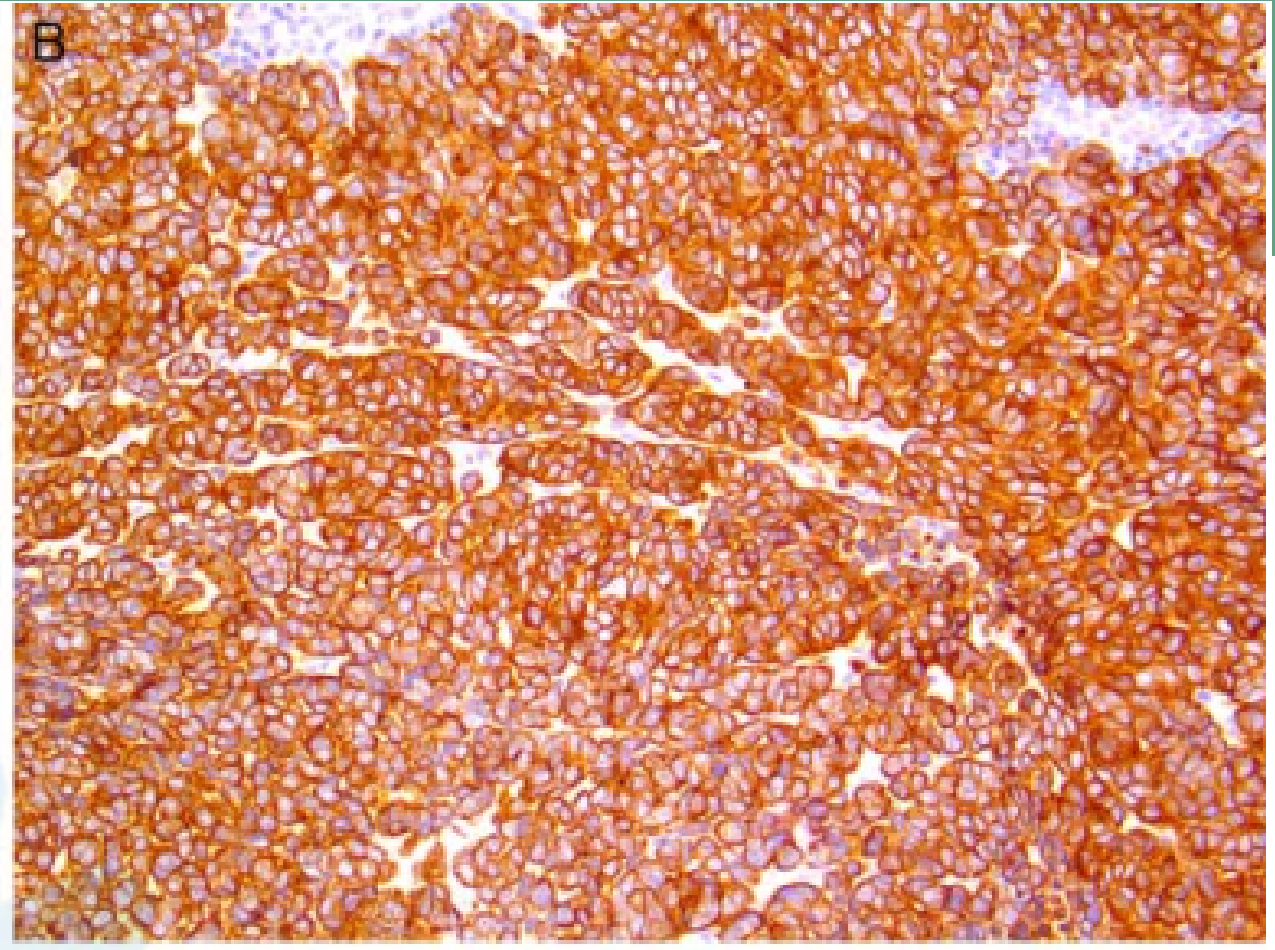
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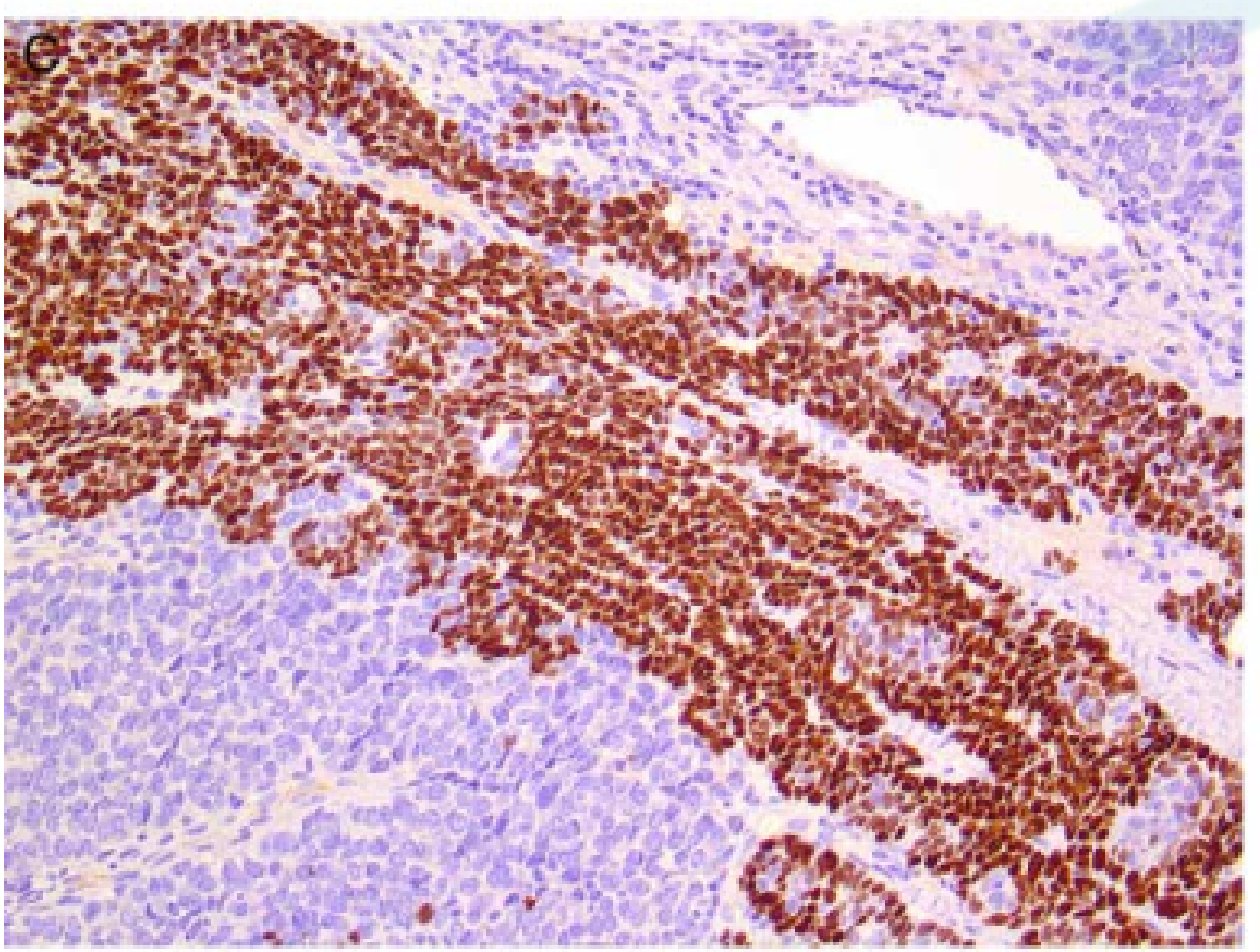
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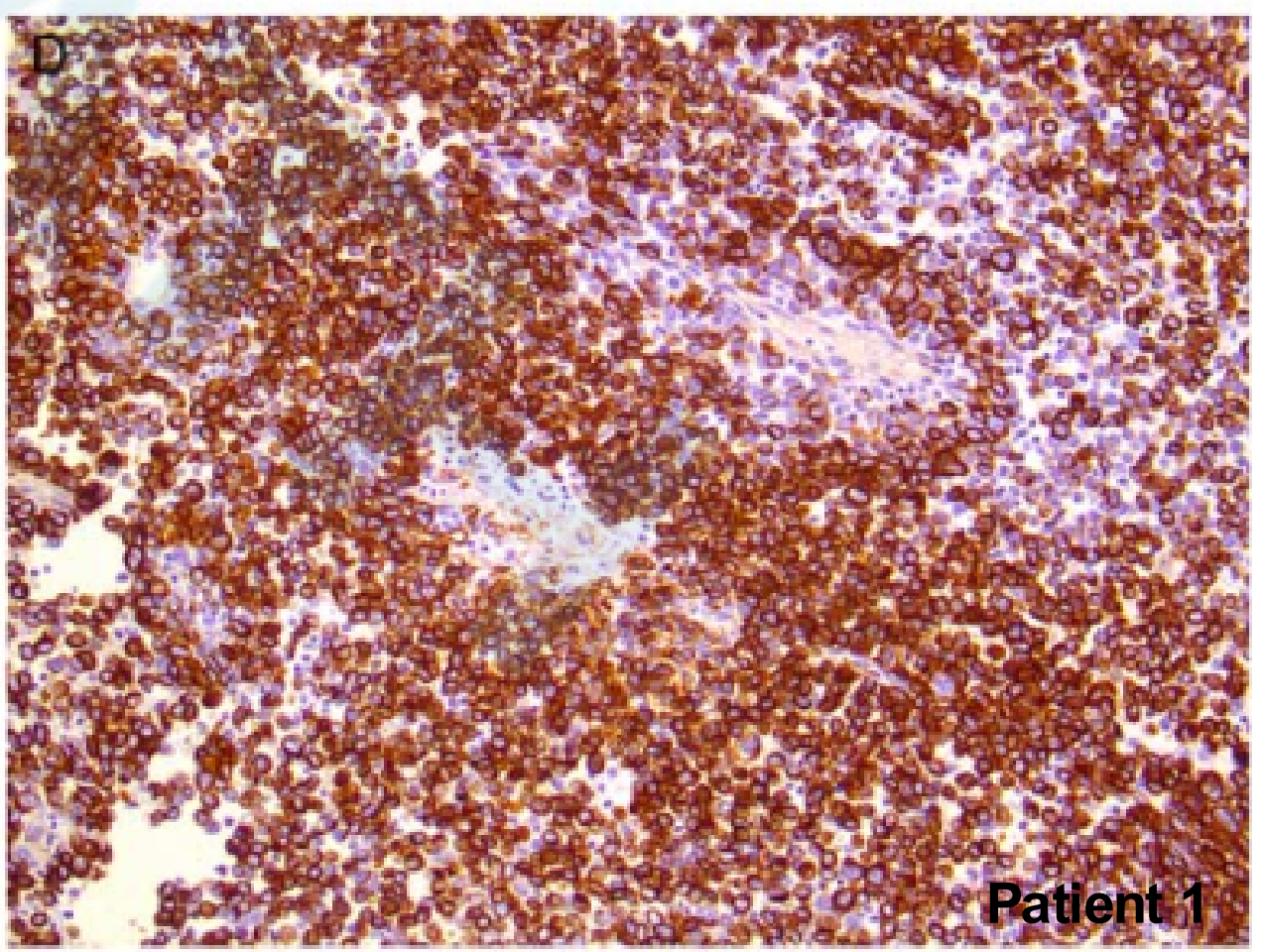
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Patient 1

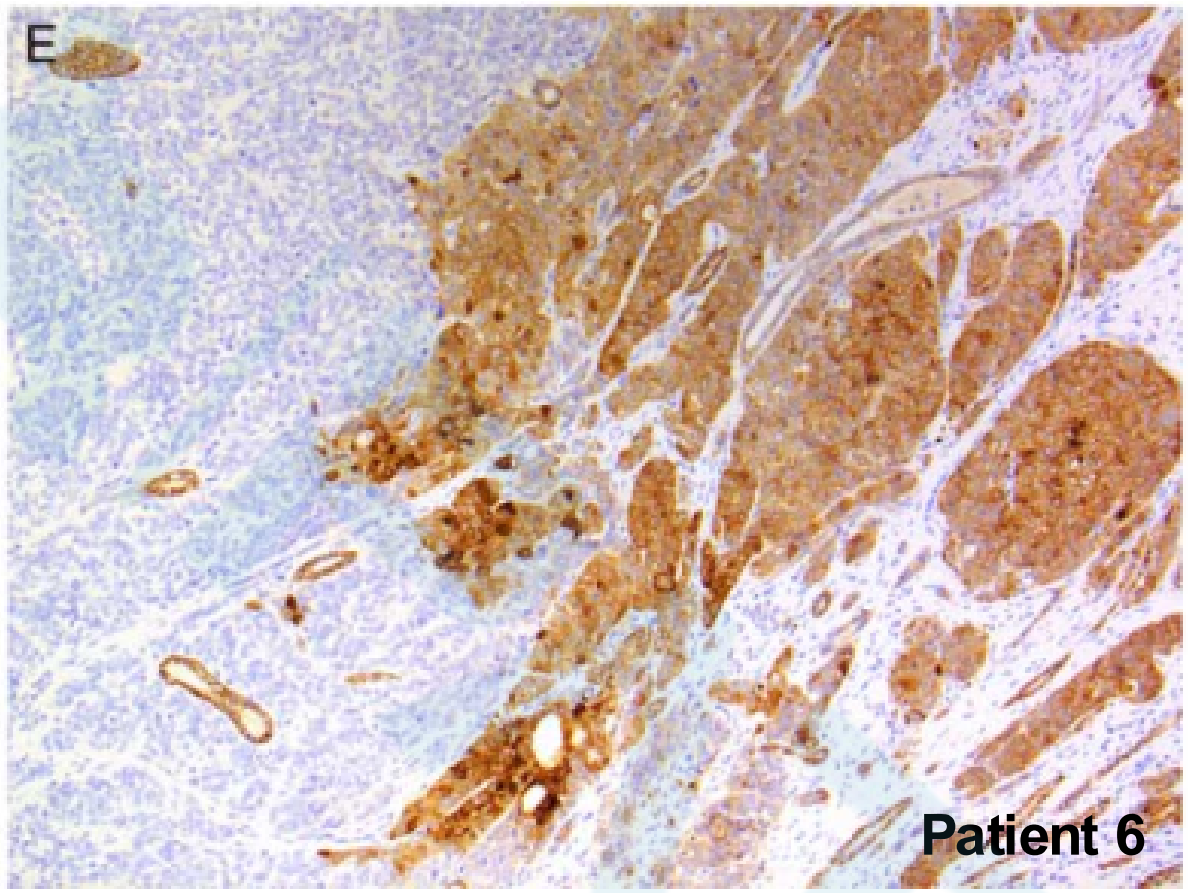
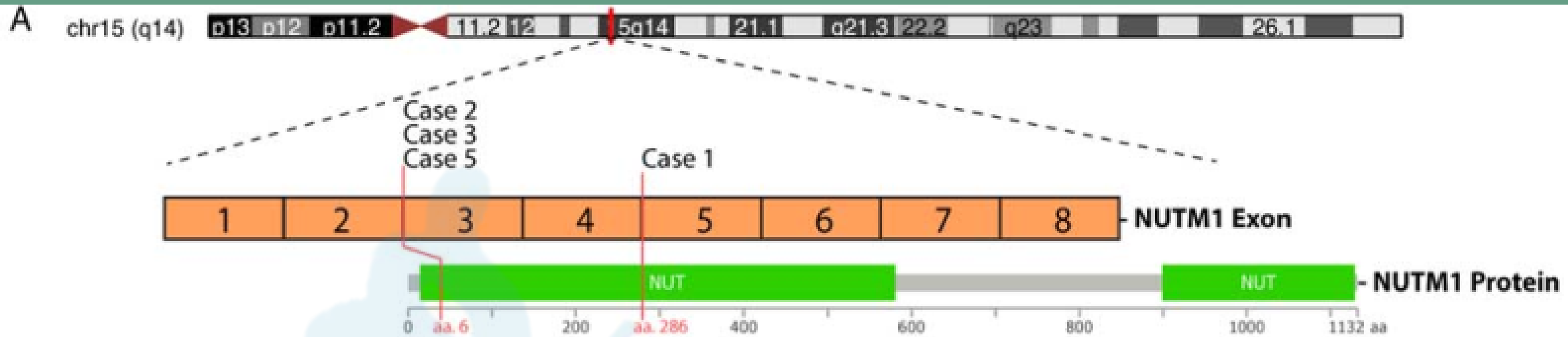


FIGURE 4. Patient 1: immunohistochemistry highlighting (A) diffuse expression of NUT, (B) pancytokeratin, (C) patchy p63, (D) glial fibrillary acid protein, and (E) Immunohistochemisty for claudin-4 in Patient 6; slightly more than half of this case was immunopositive (note entrapped tubules).



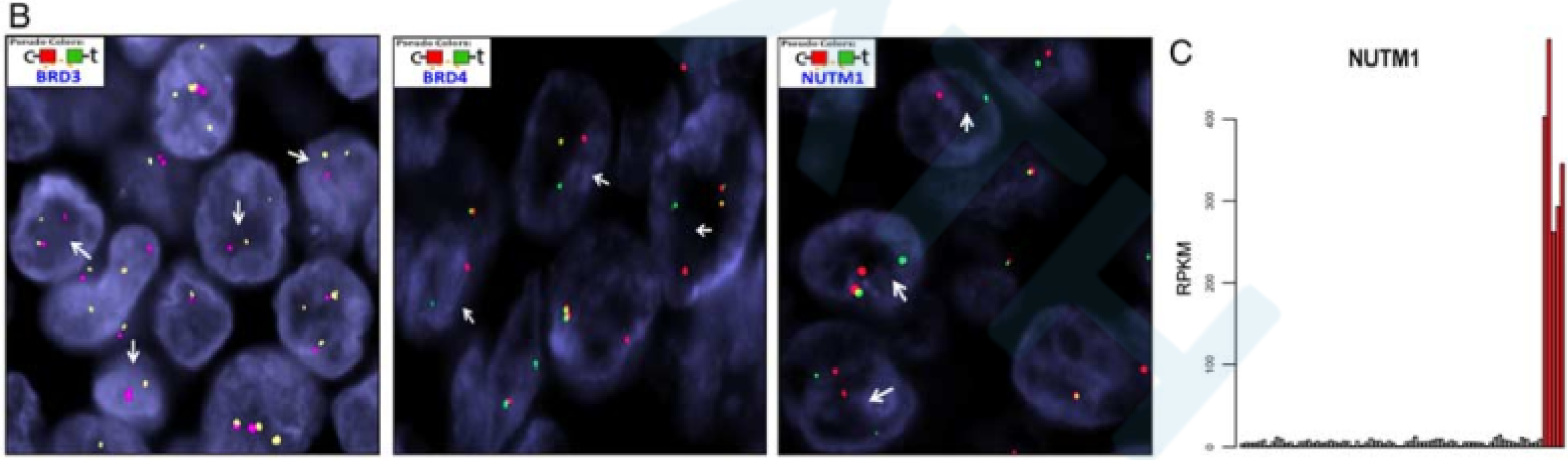


FIGURE 5. A, Illustration of the *NUT* gene, and corresponding protein, highlighting the location of the gene breakpoint locations, and fusion gene sequences. B, Representative photomicrographs of fluorescence in situ hybridization break-apart probes for *BRD3*, *BRD4*, and *NUTM1* split signal indicative of gene rearrangement (arrows). C, Bar chart illustrating mRNA expression profiles of *NUTM1* (cases 1 to 5).

DISCUSSION

- Our study cohort was characterized by a markedly heterogenous phenotype, which lacked defining morphologic and/or immunohistochemical attributes
- Notably, 2 of the cases in our series were negative for NUT by immunohistochemical testing
- Interestingly, the 2 negative cases in our series corresponded to the 2 novel NUTM1 fusion partners (BCORL1-NUTM1 and MXD1-NUTM1)
- By illustrating the presence of high levels of mRNA expression in all cases examined our findings raise the possibility that, in a subset of cases, a posttranscriptional mechanism may account for the absence of NUT staining by immunohistochemistry

- The sole pediatric patient in our series, presenting as a left parietal lobe tumor in a 3-year-old male, deserves further discussion
- We considered whether this unusual lesion might be related to the neoplasms recently described as CNS Ewing Family Tumors with CIC-NUTM1 fusions
- On methylation profiling, however, this neoplasm did not cluster with tumors of the CNS Ewing Family Tumor CIC group or with any other embryonal or other neuroepithelial tumors included in the array algorithm

- On the basis of frequent keratin expression it has been concluded NUT midline carcinoma represent a carcinoma
- Admittedly, keratin expression alone does not establish a diagnosis of carcinoma and it is a relatively consistent feature of several sarcomas (eg, epithelioid sarcoma, desmoplastic small round cell tumor, and synovial sarcoma)
- Undifferentiated small round cell tumors such as Ewing sarcoma, and those with BCOR rearrangement have also been reported to show keratin, epithelial membrane antigen, and p63 expression

- The relationship between NUT-associated tumors of soft tissue, or viscera, and NUT midline carcinoma remains unclear
- Our cases possessed NUTM1 breakpoints similar to those previously reported; nevertheless, given differences in anatomic distribution, morphology and immunophenotype-and uncertainty regarding underlying histogenesis-we feel it prudent to classify the neoplasms in this series as a NUT associated tumor, rather than NUT midline carcinoma

CONCLUSION

- In summary, we report 6 cases of primary undifferentiated tumors occurring in the soft tissue and viscera (brain, kidney, stomach wall) associated with NUTM1 rearrangement, and 2 novel NUTM1 fusion partners (BCORL1 and MXD1)
- Despite some overlap with NUT midline carcinoma, the tumors in this series differed in several regards, including: anatomic distribution, morphology, and immunophenotype; of particular relevance is the fact that definitive evidence of epithelial differentiation could not be established among many of our cases

- As a result, we have tentatively labeled these malignant neoplasms a NUT associated tumor. It is conceivable this may represent an overarching category that also includes NUT midline carcinoma
- Tumors harboring NUTM1 gene fusions are presumably underrecognized, and the extent to which they account for undifferentiated mesenchymal, neuroendocrine, and/or epithelial neoplasms is unclear
- Moreover, the relationship, if any, between NUT-associated tumors in soft tissue and/or viscera, and conventional NUT carcinoma, remains to be elucidated

- The authors recently encountered a third case of primary soft tissue NUT-associated tumor arising in a 16-year old male as a large, deep-seated thigh mass (21 cm), encasing the distal femur with areas of intramedullary involvement
- Morphologically, the tumor showed predominantly an epithelioid/rhabdoid phenotype, with focal areas of round/primitive cell features
- Immunohistochemically, the tumor was negative for cytokeratins and NUT
- Targeted RNA sequencing, further confirmed by FISH, showed the presence of a NSD3-NUTM1 fusion, as previously reported

Thank You !