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ORIGINAL PAPER

NUT Midline Carcinoma: A Series of Five Cases, Including One with Unusual Clinical Course

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Abstract NUT midline carcinomas (NMCs) are rare, poorly differentiated tumors with aggressive biological behavior and a characteristic molecular signature. Availability of NUT antibody has facilitated diagnosis of NMC without molecular testing. We report a series of head and neck NMCs diagnosed using NUT IHC at our institute, including one case with an unusual course. Immunohistochemistry for NUT was performed in nasal and sinonasal tumors with diagnoses of undifferentiated carcinoma, poorly differentiated squamous cell carcinoma and malignant neoplasm, not otherwise specified, to identify cases of NMC. Clinicopathological features were reviewed. Five cases of NMC were identified, accounting for 9.6% of poorly differentiated/undifferentiated carcinomas of the sinonasal region. These patients had a sex ratio of 2:3, and ranged in age from of 10 to 31 years (mean: 25.2 years). Patient 4 had previously been diagnosed with basal cell carcinoma arising in left nasolacrimal duct, and inverted papilloma of nasal cavity. She presented to us with a left lacrimal fossa mass extending into nasal cavity, which was diagnosed as NMC. NMC is a rare neoplasm, the awareness of which is imperative for pathologists to identify cases in which NUT IHC should be ordered. NUT IHC should be performed in all cases of a poorly differentiated carcinoma, particularly those with foci of squamous differentiation, irrespective of patient age and unusual tumor location, as seen in one of our cases. Although considered a highly aggressive and lethal neoplasm, NMC can have a more prolonged clinical course on occasion.

Keywords NUT · Poorly differentiated carcinoma · Squamous cell carcinoma · Sinonasal · Lacrimal fossa · Head and neck

Introduction

NUT midline carcinomas (NMCs) are rare, novel, poorly differentiated tumors with aggressive biological behavior. Initially considered as neoplasms seen predominantly in children and young adults, more recent reports show that they occur in a wider age group [1, 2].

Although they lack specific clinical or histomorphological features and resemble poorly differentiated squamous cell carcinomas, NMCs are characterized by a molecular signature, viz. chromosomal rearrangement involving nuclear protein in testis (*NUT*) gene, located on chromosome 15q14. The *NUT* gene most frequently fuses with BRD4 gene located on 19p13.1; however, other fusion partner genes have been identified in tumors termed NUT variant carcinomas [3–5]. The recent availability of a commercially available NUT antibody for immunohistochemistry has facilitated the diagnosis of NMC by providing an economical and robust method to distinguish it from other poorly differentiated carcinomas.

These tumors typically occur in midline structures of the head and neck, and in the mediastinum; however, they have been described in various other organs, including more lateral structures such as lung and parotid gland [1, 6, 7].

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Increasing awareness of NMC brings new sites and facts about this tumor into the literature [8, 9]. We report a series of head and neck NMCs diagnosed using NUT IHC at our institute, including one case arising in the lacrimal fossa, which has been described in only a single case report previously [10]. Moreover, in this case, NMC developed 3 years after diagnosis of basal cell carcinoma at the same site.

Methods

We searched our Departmental archives using the following search items in the sinonasal and nasal region: undifferentiated carcinoma, poorly differentiated squamous cell carcinoma, malignant neoplasm, not otherwise specified (NOS), which were diagnosed between 2009 and 2017. Histopathological and immunohistochemical features were reviewed. Immunohistochemistry for NUT was performed on formalin-fixed paraffin-embedded tumor sections for confirmation of diagnosis of NMC, using a rabbit monoclonal primary antibody against NUT (clone C52B1; Cell Signaling Technologies Inc., Danvers, MA) in a dilution of 1:50. Sections from non-neoplastic testis were used as positive controls. Strong, diffuse ($\geq$ 50% of cells), speckled nuclear staining was considered as positive, as described previously [11]. Approval was obtained from the Institute Ethics Committee to conduct this study on patient tumor samples.

Results

We retrieved 52 cases of poorly differentiated/undifferentiated carcinomas, poorly differentiated squamous cell carcinomas and malignant neoplasm, NOS. NUT IHC revealed five cases with diffuse nuclear immunoreactivity, accounting for 9.6% of poorly differentiated/undifferentiated carcinomas of the sinonasal region. These patients had a sex ratio of 2:3, and ranged in age from of 10 to 31 years (mean: 25.2 years). Histopathological features of the cases are summarized in Table 1.

Case 1

A 30-year-old male presented with complaints of proptosis for 1 month, and diminished vision for 15 days. On examination, a left sinonasal and orbital mass was identified, from which a biopsy was taken. Histopathological examination showed an infiltrative tumor with undifferentiated basaloid cells arranged in sheets and trabeculae. Many neutrophils were seen interspersed between tumor cells. Focally, abrupt squamous differentiation was noted. Frequent mitoses, and foci of necrosis were present. This was reported as poorly differentiated squamous cell carcinoma. On review, tumor cells were immunopositive for cytokeratin, NUT, and p40; p16 staining was seen in approximately 20% of tumor cells, and CD34 was negative.

Case 2

A 31-year-old lady presented with complaints of nasal obstruction for 3 months, diminished vision for 2.5 months, and proptosis and episodes of epistaxis for 1 month. Imaging revealed a large mass involving the left nasal cavity, and ethmoid, sphenoid and maxillary sinuses, with extension into the left orbit and anterior cranial fossa. A biopsy had been performed elsewhere, which had been reported as sinonasal undifferentiated carcinoma. Two cycles of neoadjuvant chemotherapy were administered (Carboplatin 25 mg/m^2 and Etoposide 100 mg/m^2), which was followed by left lateral rhinotomy and excision

Table 1 Histopathological features of five cases of NUT midline carcinoma

Features	Case 1	Case 2	Case 3	Case 4	Case 5
Margins	Infiltrative	Infiltrative	Infiltrative	Infiltrative	Infiltrative
Pattern of arrangement of tumor cells	Solid, trabecular	Solid, trabecular	Solid	Solid, trabecular	Solid
Undifferentiated basaloid cells	Present	Present	Present	Present	Present
Squamous eddies	Present	Present	Absent	Present	Absent
Peripheral palisading	Absent	Absent	Absent	Present focally	Absent
Spindling	Absent	Absent	Absent	Present focally	Absent
Inflammatory cells interspersed within tumor cells	Neutrophils	Neutrophils	Neutrophils	Eosinophils	Neutrophils
Necrosis	Present	Present	Present	Present	Present
Mitosis	Frequent	Frequent	Frequent	Frequent	Frequent
Cystic change	Absent	Absent	Absent	Yes, with many foamy histiocytes, eosinophils and foreign body giant cells	Absent
Calcification	Absent	Absent	Absent	Present within central necrotic areas	Absent

of the sinonasal mass and left orbital exenteration. Sections showed features resembling Case 1 (Fig. 1a), and was reported as poorly differentiated carcinoma with focal squamous differentiation. Resection margins were positive, and the patient was planned for post-op CT, but she defaulted, and died within 2 months of surgery. NUT IHC was later found to be positive (Fig. 1c), along with p40 (Fig. 1b) and focal p16 (20–25% of tumor cells). CD34 was also focally positive (Fig. 1d).

Case 3

A 25-year-old male presented with complaints of nasal obstruction and epistaxis for 1 month. On examination, a mass was identified in the right nasal cavity, from which a biopsy was taken. Histopathological examination showed an infiltrative tumor composed of medium sized, poorly differentiated cells having scant cytoplasm, monomorphic round to ovoid nuclei and prominent nucleoli. Tumor cells were immunopositive for cytokeratin and negative for synaptophysin, and it was diagnosed as undifferentiated carcinoma. The patient did not follow up at our institute after the biopsy. Subsequently, tumor cells were found to be immunopositive for NUT and p40. On review of HE sections, focal individual cell keratinization was identified. However, well defined squamous islands were not seen. p16 staining was noted focally (30% of tumor cells).

Case 4

This 10-year-old girl initially presented to an eye clinic in Nepal in June 2014 with complaints of excessive watering from the left eye. On evaluation, nasolacrimal duct blockage was identified, and external dacryocystorhinostomy was performed. Intraoperatively, a fleshy mass was seen from which a biopsy was taken. On histopathological examination, the tumor was composed of basaloid cells with scant cytoplasm and round to oval, hyperchromatic nuclei arranged in nests and lobules. Peripheral palisading and frequent mitoses were noted. A diagnosis of basal cell carcinoma (BCC) was rendered. She was referred to another hospital for further management, where nasal endoscopy revealed a nasal mass. Excision biopsy was performed, which showed nests of keratinized squamous cells infiltrating stroma rich in neutrophils, lymphocytes and plasma cells. Focal nuclear hyperchromasia, necrosis and few mitotic figures were identified, and the case was diagnosed as inverted papilloma. The patient was then referred to our institute, where a contrast enhanced CT scan showed a small infiltrative left medial canthus lesion measuring 10 mm × 5 mm, which was abutting uveal-scleral rim of eyeball, with nasal bone destruction and extension to anterior ethmoids. A frontoethmoidectomy was planned but the patient did not follow up.

Presently, in March 2017, the patient visited the OPD with complaints of a swelling near medial canthus for 3 months, which progressively increased in size. On examination, a

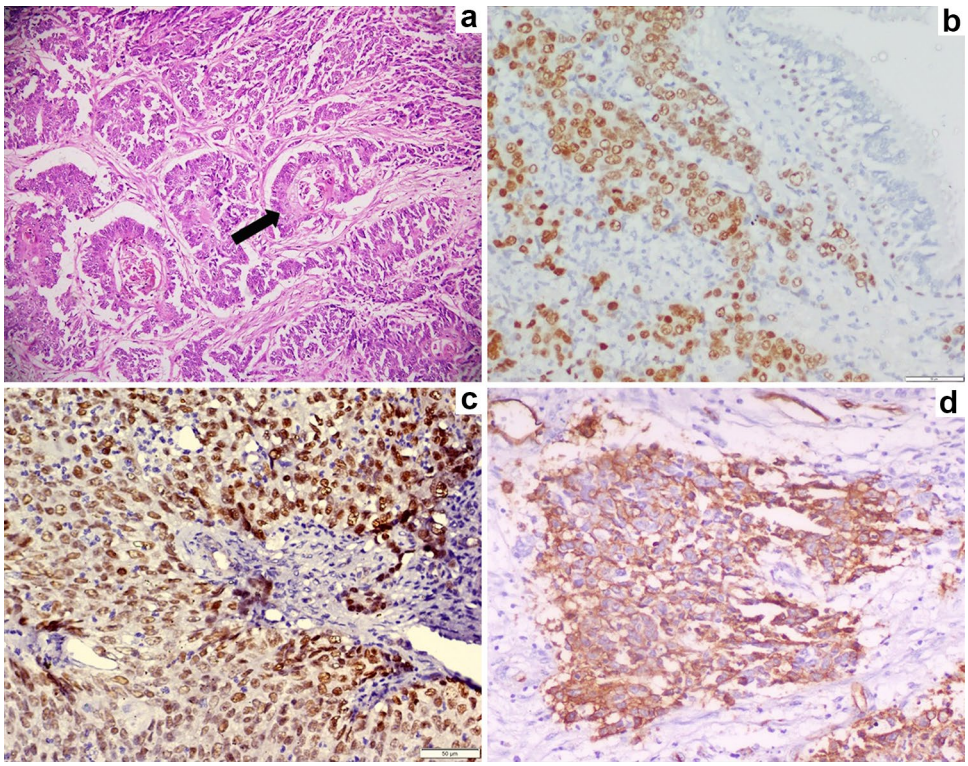

Fig. 1 Photomicrographs from Case 2 show basaloid tumor cells arranged in trabeculae with central squamous differentiation (arrow) at places (**a**; HE, ×100); Tumor cells show nuclear immunopositivity for p40 (**b**; IHC, ×100) and NUT (**c**; IHC, ×200), and focal CD34 positivity (**d**; IHC, ×200)

bluish, firm, cystic mass was seen just below the medial canthus (Fig. 2a). In addition, a pale friable mass was seen filling the left nasal cavity. CT image is shown in Fig. 2b. Patient underwent a lateral rhinotomy. Intraoperatively, there was a 3.5 × 3 cm cystic mass in the left lacrimal fossa, with inferior oblique muscle attached to it. Lacrimal crest and lateral nasal bone were dehiscent, with a solid tumor filling the anterior nasal cavity. The lacrimal fossa cyst and nasal tumor mass were excised.

Sections examined showed an infiltrative tumor with areas of necrosis and cystic change. Undifferentiated basaloid tumor cells were arranged in lobules and trabeculae in a fibrovascular stroma rich in inflammatory cells, particularly eosinophils (Fig. 3a). Foci of squamous differentiation were seen in the form of pale to clear cells forming eddies located centrally within the solid islands (Fig. 3b). The presence of polygonal cells with clear cytoplasm raised the possibility of sebaceous carcinoma, due to the orbit being a common site for this tumor. However, Oil red O staining did not reveal any intracytoplasmic lipid. Alcian blue—PAS stain for mucin was also negative in these clear cells. Focal peripheral palisading of tumor cells was identified, along with spindling and ovoid shape of nuclei, suggestive of basal cell carcinoma (Fig. 3c). Areas of cystic change contained many foamy histiocytes, eosinophils and foreign body giant cells. Foci of necrosis with central calcification were seen. Strong NUT immunopositivity confirmed the diagnosis as NMC (Fig. 3d). p16 immunopositivity was seen in approximately 60% of tumor cells (Fig. 3e). HPV testing performed on DNA extracted from FFPE tissue by High risk HPV Hybrid Capture II was negative for High risk HPV, with cut-off ratio of 0.56 (> 1 is considered positive). The postoperative period was uneventful, and the patient is scheduled to receive radiotherapy at a dose of 64 Gy/32 cycles over 6.5 weeks with concurrent weekly Cisplatin at a dose of 40 mg/m^2. She is yet to be registered in the NMC registry.

Case 5

This 30-year-old lady presented with complaints of nasal obstruction for 3 months and diminished vision for 1 month. Endoscopic examination revealed a polypoidal mass in middle meatus of left nasal cavity, extending to left maxillary sinus and left orbit. A biopsy was performed which showed an undifferentiated carcinoma composed of medium to large cells with pale eosinophilic cytoplasm, vesicular nuclei and prominent nucleoli. No evidence of squamous differentiation was identified. Numerous neutrophils were admixed with the tumor cells. Tumor cells were immunopositive for pancytokeratin, p40, and NUT, while they were negative for synaptophysin, and EBV-LMP1. p16 staining was seen focally.

Discussion

NMC is a newly described, genetically defined neoplasm occurring in midline structures, particularly the head and neck and the mediastinum. It is considered a subtype of squamous cell carcinoma due to presence of histological evidence of abrupt squamous differentiation, as well as immunohistochemical expression of p63 and p40, markers of squamous cells. It has been postulated that these tumors arise from epithelial progenitor cell rests that are seen early in life, in the first two to three decades. This hypothesis has been further supported by the frequent immunoexpression of CD34, a marker of stem cell phenotype, in cells from this tumor, as also seen in one of our cases [4].

The incidence of NMC remains unknown due to its rarity, its lack of pathognomonic morphological features, and due to the requirement for molecular diagnostic methods such as fluorescence in situ hybridization or reverse transcriptase-PCR, which are not available in most laboratories the world over [2]. The recent development of an

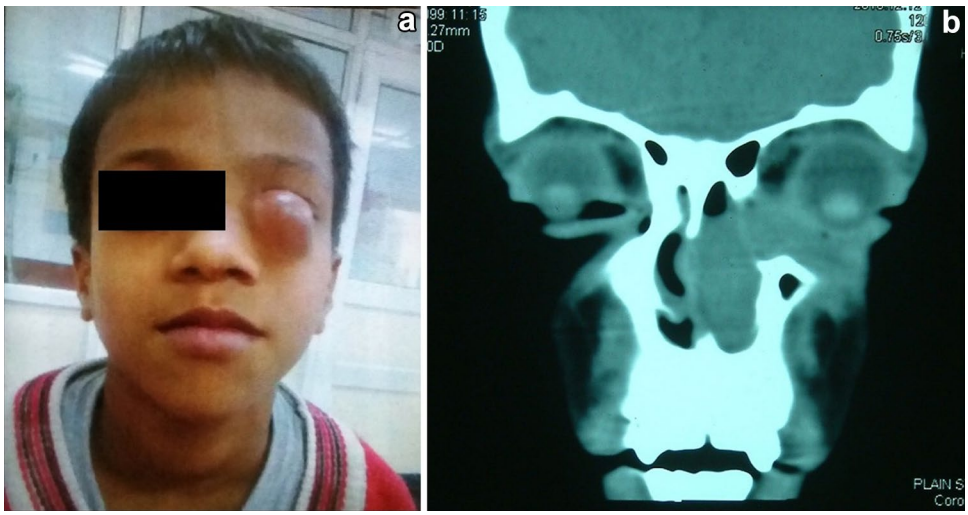

Fig. 2 Patient 4 with a cystic lesion near medial canthus (**a**); Coronal CT image shows a mass lesion in the left lacrimal sac area with erosion of nasal bone and extension into left nasal cavity; laterally, the mass is abutting the medial surface of the anterior half of left eyeball (**b**)

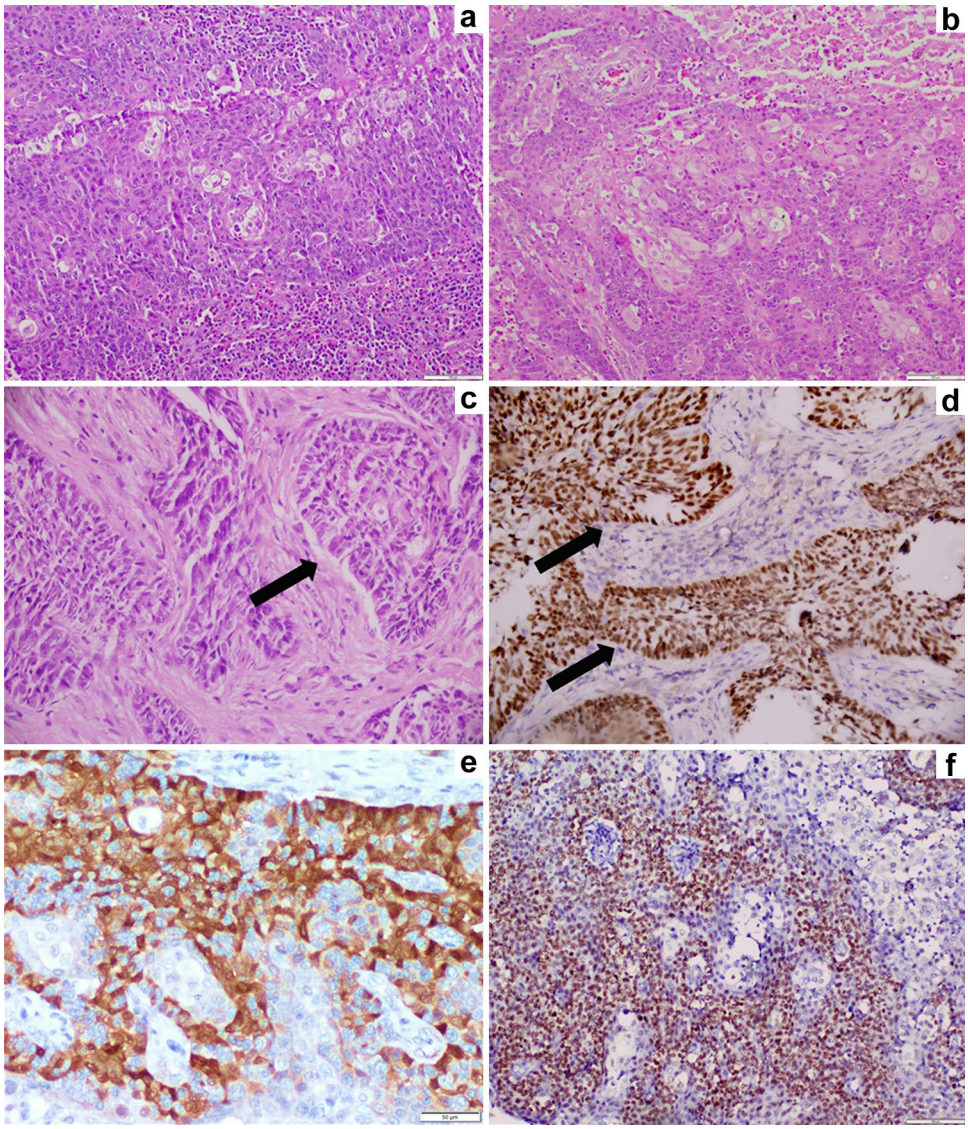

Fig. 3 Photomicrographs from Case 4 show undifferentiated basaloid tumor cells arranged in sheets and trabeculae in an eosinophil-rich stroma (**a**; HE, ×100). Foci of squamous differentiation show cells with pale to clear cytoplasm (**b**; HE, ×100); areas mimicking basal cell carcinoma with stromal retraction and peripheral palisading are seen (**c**; HE, ×400), highlighted by NUT immunostaining (**d**; IHC, ×400). Focal p16 (**e**; IHC, ×400), and diffuse p40 (**f**; IHC, ×200) positivity is present

immunohistochemical stain for NUT which has been found to be sensitive and specific for the diagnosis of NMC, along with increasing awareness of this entity, have led to an increase in the number of cases being diagnosed [2, 11]. We report our experience with five cases of head and neck NMCs diagnosed on NUT immunohistochemistry.

In this series, we report a case of NMC arising from the nasolacrimal duct, which is an extremely rare site, having been reported only once previously [10]. A frequent malignant tumor at this location, i.e. the medial canthus of the eyelid, is sebaceous carcinoma, which can pose a significant hurdle in arriving at the diagnosis of NMC [12]. Clear and keratinized cells of NMC can closely resemble sebaceous cells which also have clear cytoplasm [13]. In addition, sebaceous carcinoma can also show the presence of basaloid undifferentiated cells along with clear cells, leading to misdiagnosis. However, the clear cytoplasm of sebaceous cells is due to the presence of multiple fine lipid vacuoles, while NMC cells contain glycogen, which can be demonstrated histochemically [14]. Other differential diagnoses at this location include squamous cell carcinoma (SCC) and adenoid cystic carcinoma (ACC). Conventional SCCs show greater degree of nuclear pleomorphism as compared to NMC, and the squamous differentiation is not abrupt. While ACCs show p63 immunopositivity, they are usually negative for p40 unlike NMC. Ultimately, a high index of suspicion is necessary in poorly differentiated carcinomas lacking evidence of lineage differentiation apart from squamous, particularly in children and adolescents, to raise the diagnostic consideration of NMC, followed by the judicial use of NUT IHC in the absence of molecular testing.

Patient 4 was previously diagnosed with BCC and inverted papilloma. Focal peripheral palisading as seen in Case 4, although unusual, has been described in NMC,

and may cause confusion with BCC [15, 16]. The lesion had first presented with a mass causing obstruction of the nasolacrimal duct, and not as a skin nodule with ulceration, which is the usual presentation for BCC. Further, BCC is extremely rare in children. Thus, it is probable that this patient had NMC from the initial presentation. The second possibility is that the current NMC arose in the background of a BCC. As we were unable to procure the previous biopsy slides and tissue for NUT IHC, we can only speculate on the same.

The prognosis of NMC is uniformly poor. All 11 cases in the series reported by French et al. metastasized via the hematogenous route, and all but one patient succumbed to disease despite aggressive management strategies, emphasizing the lethal behavior of this neoplasm [4]. Subsequently Bauer et al. published the survival data of 54 NMC patients, the largest series to date [2]. They reported progression free survival rates at 1 and 2 years as 15 and 9%, while 1-year and 2-year overall survival rates were 30 and 19%, respectively; median overall survival time was 6.7 months. Among our patients, one expired within 2 months of surgery, and three were lost to follow-up. However, a short clinical history with a rapidly progressive tumor mass was present in all but one case, highlighting the aggressive nature of this tumor.

Conclusion

NMC is a rare, recently described neoplasm, the awareness of which is imperative for pathologists to identify cases in which NUT IHC should be ordered. Use of NUT IHC has great diagnostic and prognostic relevance, which may extend to providing predictive/therapeutic information in the future. Thus, NUT IHC should be performed in all cases of a poorly differentiated carcinoma with foci of squamous differentiation irrespective of patient age and unusual tumor location, as seen in one of our cases. Lastly, although considered a highly aggressive and lethal neoplasm, NMC can have a more prolonged clinical course on occasion.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed on patient tumor samples in this study were in accordance with the ethical standards of the Institute Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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