Summary: Nuclear protein of the testis (NUT) midline carcinoma can present in the head, neck, and mediastinum. In general, it presents in young adult men and has a poor prognosis. We report on a case of NUT midline carcinoma of the mediastinum in a man 27 years of age without any prior malignancy. Due to the location of the tumor, mediastinal lymphoma and germ cell tumor were initially considered; however, immunohistochemistry was performed using NUT antibody that revealed it to be NUT midline carcinoma. Although guidelines exist for squamous cell carcinoma of the head, neck, and mediastinum, no such specific guidelines are available for NUT midline carcinoma, which looks morphologically similar to squamous cell carcinoma but behaves more aggressively and carries a poor prognosis.

Background
Nuclear protein of the testis (NUT) midline carcinoma is a rare, aggressive, and fatal carcinoma that most often occurs in the midline of the body, which includes the head, neck, and mediastinum. It is characterized by undifferentiated morphological features immunoreactive to NUT and defined by NUT rearrangement. It is a rare subtype of squamous cell carcinoma. Previous reports cite an identical chromosomal translocation, suggesting that NUT midline carcinoma is also known as carcinoma with chromosomal translocation 15:19.1-4 NUT encodes a protein at the chromosome 15 breakpoint that demonstrates testis-restricted expression and nuclear localization signals. This type of carcinoma typically affects young adults (median, 15 years of age). In general, NUT midline carcinoma is restricted to the mediastinum, head, and neck areas, but rare cases have also involved gynecological structures.

Case Report
A man 27 years of age who had never smoked presented with cough and hemoptysis. Initially, he presented at an urgent care clinic where radiography of his chest was obtained, the results of which were concerning for mediastinal mass and lymphadenopathy (Fig 1A).

The patient later developed severe headaches and facial swelling and flushing. He was diagnosed with a mediastinal mass, and his health care team also suspected that he might have superior vena cava syndrome; therefore, he was referred to the H. Lee Moffitt Cancer Center & Research Institute (Tampa, FL) for further workup.

Computed tomography (CT) of his chest was performed and showed a large mediastinal mass and lymphadenopathy (Fig 1B–C). CT of his abdomen and pelvis revealed a right adrenal nodule 3.0 cm in size that appeared to be adrenal adenoma. Findings on ultrasonography of his scrotum and testicles were unremarkable.

Based on these findings, we suspected that the large mediastinal mass in the patient was either a germ cell tumor or lymphoma.

Fiberoptic bronchoscopy, bronchoalveolar lavage, and biopsies of the tumor in the left main stem bronchus were performed. The results showed diffusely thickened mucosa throughout the right bronchial tree and left main stem bronchus. The tumor extended into and moderately obstructed the left upper lobar bronchus, extending into and severely obstructing the left lower lobar bronchus. Specimens taken from the biopsies of the left main stem bronchus were sent for frozen section analysis. They were interpreted as a poorly differentiated malignant neoplasm. The final pathology results were deferred to permanent sections to perform the necessary immunohistochemistry (IHC) and flow-cytometry studies.

Papanicolaou smear and Romanowsky-stained slides showed cellular, loosely cohesive, and isolated cells (Fig 2A–B). The cells were 3 times larger in diameter than that of a small lymphocyte. The nuclei were round to oval in shape and had slightly irregular contours; some cells had prominent nucleoli. The
nuclear chromatin was dense and finely granular in most of the cells, and vesicular open chromatin was observed in occasional cells. No overt keratinization or dyskeratosis was seen. Glandular structures were not observed.

His case was initially determined to be a germ cell tumor of the mediastinum. Another small tissue fragment was submitted for lymphoma workup. Ancillary studies were performed on the tissue obtained from biopsy of the left main stem bronchus, fragments of which showed squamous mucosa with invasive, malignant, epithelioid cells in a background of extensive necrosis, acute inflammation, and reactive changes. Small foci of the tumor showed vague squamous features (Fig 2C–F).

Immunoperoxidase stains were performed and revealed that the tumor cells were strongly reactive for pankeratin (AE1/AE3/CAM5.2), p40, and cytokera-

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Fig 1A–C. — (A) Horizontal view of radiography of the chest and (B–C) computed tomography show a large mediastinal mass with lymphadenopathy.

Fig 2A–F. — (A–B) Romanowsky stain shows a cellular cytology specimen with rare groups composed of loosely cohesive large cells and many isolated tumor cells. (C–F) Findings on left main stem bronchus reveal a nonspecific sheet of medium- to large-sized, slightly dyscohesive tumor cells with occasional prominent nucleoli and high mitotic activity.
tins 5 and 6 (Fig 3A–B). Triple stain for PIN4 (AMACR, CK903, p63) showed the tumor cells strongly positive for p63. The tumor cells were nonreactive for CD45, CD20, placental alkaline phosphatase, Sal-like protein 4, glypican 3, α-fetoprotein, c-Kit (CD117), endosomal membrane protein, synaptophysin, CHG, and Wilms tumor 1, thus excluding lymphoma and germ cell tumor from the differential diagnosis. The morphological and immunoperoxidase findings were suggestive of a malignant tumor with squamous differentiation.

Based on the tumor histomorphological findings of the poorly differentiated carcinoma, the location of the tumor, and the patient’s age, NUT midline carcinoma was added to the differential diagnosis. Additional testing for NUT IHC was positive (see Fig 3). Thus, a diagnosis of NUT midline carcinoma was made.

The patient was also experiencing left temporal/retro-orbital and back pain, which necessitated additional radiological studies. Magnetic resonance imaging of the brain revealed bony metastatic disease involving the left greater wing of the sphenoid, the lateral wall of the left orbit, and the floor of the left middle cranial fossa with minimal intracranial, extradural tumor extension. Imaging findings also showed lytic lesions in the thoracic and lumbar spine. The patient received chemotherapy (carboplatin/paclitaxel) along with radiation to the mediastinum (30 Gy in 10 fractions) and sphenid/skull base (30 Gy in 5 fractions).

The patient underwent left temporalis biopsy, the findings of which revealed metastatic squamous carcinoma. In situ hybridization testing for high-risk human papillomavirus (types 16, 18, 31, 33, and 51) was positive, indicating the presence of at least 1 of the high-risk types. Targeted, next-generation sequencing was also performed. A gene panel showed no clinically relevant results. He was enrolled in a phase 1 clinical trial. While participating in the trial, he had initial, significant resolution of diffuse bone metastases; however, positron emission tomography/CT for staging purposes showed bone disease progression.

The patient eventually developed thrombocytopenia and spontaneous tension pneumothorax without evidence of pulmonary embolism seen on CT of the chest. He was started on vancomycin, cefepime, and levofloxacin; in addition, his dose of steroids was increased. However, the patient acutely decompensated; he developed pericardial effusion, which continued to worsen, and he was exhibiting early signs of tamponade.

The health care team discussed the long-term goals of care with the patient and his family, who together decided to pursue comfort measures alone. He was then given patient-controlled analgesia (hydromorphone). Due to increasing breakthrough agitation, the patient was prescribed intravenous lorazepam. Following a discussion with the supportive care team, his family decided to pursue palliative sedation and intravenous phenobarbital. Five months after undergoing the diagnostic biopsy, the patient died.

Discussion

NUT midline carcinoma is a rare and aggressive carcinoma that usually presents as widely metastatic and unresectable disease.1 The mean survival rate is approximately 9 months.7-11 Typically, it affects boys aged 15 years, but it may present in older persons.1,7,12,13 The majority of cases occur in the midline of the body, including the head, neck, and the mediastinum.1 It may involve the nostrils, epiglottis, orbits, sinuses, bladder, and the right iliac bone.8,13

Sixty-two cases have been reported of NUT midline carcinoma; of those, only 2 have presented in gynecological structures.6 Unlike most solid tumors,
NUT midline carcinomas are not classified according to tissue or site of origin; instead, they are genetically defined.\textsuperscript{1,6} NUT was initially described in 1991. Its symbol comes from the protein coded at the chromosome 15 breakpoint.\textsuperscript{2-5}

The undifferentiated morphological features of NUT midline carcinoma are immunoreactive to NUT and are defined by rearrangement involving the NUT locus at 15q14. This generates a specific fusion transcript with a member of the bromodomain-containing family, such as BRD4, which is located on chromosome 19p13.1. It is also known as carcinoma with chromosomal translocation 15:19.\textsuperscript{1}

In approximately two-thirds of NUT midline carcinoma cases, NUT was discovered to structure a steady fusion oncogene with BRD4, creating chimeric genes that encode BRD–NUT fusion proteins.\textsuperscript{5} The other one-third of cases of NUT midline carcinomas in which NUT was fused with an unidentified oncogene were designated as a NUT-variant carcinoma other than BRD4.\textsuperscript{5}

Historically, a diagnosis of NUT midline carcinoma was made by showing NUT rearrangement by dual color, split-apart fluorescence in situ hybridization, or by demonstration of a BRD4–NUT fusion transcript by reverse transcriptase polymerase chain reaction. Haack et al\textsuperscript{8} changed this by developing a particular monoclonal antibody against recombinant NUT protein that had a sensitivity rate of 87%, a specificity rate of 100%, and a positive predictive value of nearly 100%.

Histological findings are not diagnostic for NUT midline carcinoma. The morphological findings generally consist of poorly differentiated carcinoma or squamous cell carcinoma not broadly known to most pathologists.\textsuperscript{2,4} Occasionally, it has been classified as other tumors (eg, thymic carcinoma).\textsuperscript{3} Similarly in our patient, the original differential diagnosis was broad; however, because the diagnosis was initially thought to be a germ cell tumor or lymphoma, the specimen from the biopsy was first assigned to the genitourinary pathologist who did an extensive workup. We also consulted with a thoracic pathologist, who suggested NUT midline carcinoma given the findings on morphology, the patient’s clinical history, and the location of the tumor. Based on these findings, additional immunostains were performed to check for squamous differentiation. NUT IHC showed clear reactivity within the tumor cells. NUT IHC can detect NUT in NUT midline carcinoma, the expression of which in normal, mature adult tissue is restricted to the testis.

Because incidence rates and published data are lacking, no standardized treatment exists for NUT midline carcinoma. It is an aggressive, fatal disease that is unresponsive to aggressive chemoradiotherapy.\textsuperscript{3,14} Its median survival is 9 months, ranging from 28 to 96 weeks depending on the molecular characteristics of the tumor.\textsuperscript{2,11}

In the absence of optimal treatment, platinum-based and lymphoma-type regimens have been used.\textsuperscript{3,15} In a large series by Bauer et al,\textsuperscript{16} no differences were seen in progression-free and overall survival rates by patient age, sex, histology findings, type of translocation, or lymph-node involvement. The 1- and 2-year rates of progression-free survival among 25 adults were both 4%, whereas the overall survival rates were 16% and 5% at 1 and 2 years, respectively.\textsuperscript{16} In this series, large resection and radiotherapy delivered early appeared to be associated with improved progression-free survival and overall survival rates.\textsuperscript{16}

Balla et al\textsuperscript{8} reported on a 10-year-old boy with NUT midline carcinoma. They used a histone deacetylase inhibitor to treat the patient for 5 weeks, who had an objective clinical response before toxicities limited its continued use. The patient’s disease recurred, and he died 11 months after the initial diagnosis.\textsuperscript{5}

Looking toward the future, targeted therapeutic approaches are being developed, such as direct-acting bromodomain inhibitors and histone deacetylase inhibitors.\textsuperscript{10,16} For more information on current trials of NUT midline carcinoma, please visit www.nmcregistry.org/clinictrial.html.

Conclusions
Nuclear protein of the testis midline carcinoma is an aggressive and usually fatal disease that should be considered in the differential diagnosis of midline poorly differentiated carcinoma in a young male. Optimal surgery and radiotherapy regimes are improving the rates of progression-free and overall survival, and advances are taking place in our understanding of the specific translocation in the pathogenesis of the disease. In the future, targeted therapy could lead to better treatment options for this aggressive and rare cancer.

References
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