We herein report on a challenging variant of nuclear in testis carcinoma (NUTC) exclusively featuring spindle cells as documented in biopsy and resection samples by means of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). NUTC is a deadly variant of cancer caused by t(15;19) translocation, according to which the NUT midline carcinoma family 1 gene [NUT] (expression of which in adults is confined to the seminiferous epithelium and oocytes) is pushed to translocate to bromodomain-containing protein family partners or, less frequently, to other NUT variants, which results in nuclear speckled decoration of tumor cells when a specific IHC assay is used.

Traditionally ascribed to the median line and mediastinum, NUTC is now increasingly being described in the lung, yet it remains a challenging tumor even in this anatomical site. One reason for this could be that the histologic appearance of NUTC is so deceptive morphologically and immunophenotypically as to virtually simulate many unrelated malignancies, such as squamous cell carcinoma, basaloid carcinoma, adenosquamous carcinoma, undifferentiated or poorly differentiated carcinoma, small round cell tumor, high-grade hematologic tumors, amelanotic melanoma, sarcoma, mesothelioma, thymic carcinoma, and extragonadal germ cell tumors. The only way to confidently identify NUTC is to witness accumulation of NUT midline carcinoma family 1 protein (NUT) upon IHC, which is 100% specific when a speckling nuclear pattern is seen to decorate the vast majority of tumor cells. FISH analysis helps to corroborate the final diagnosis of NUTC, but it is not strictly indispensable per se in daily practice, nor is it necessary to uncover the specific partners for the clinical handling of patients.

Malignant tumors made of spindle cells in the lung usually comprise pleomorphic or spindle cell carcinoma, some intermediate- to high-grade neuroendocrine tumors, and (less frequently) sarcomas (either primary or metastatic), amelanotic melanoma, mesothelioma, or thymus-like tumors, whereas the existence of spindle cell–looking NUTC in the lung was unprecedented. Briefly, a 30-year-old white male nonsmoker was admitted to the hospital for sudden thoracic pain owing to a 6.5-cm tumor involving the left lower lobe (Fig. 1A) with minimal homolateral pleural effusion and mediastinal lymph node involvement (Fig. 1B). Bronchial biopsy samples were initially diagnosed as squamous cell carcinoma despite the fact that the patient was young and a nonsmoker. Subsequent lobectomy with extended lymph node excision revealed a solid tumor with pushing edges of growth, spotty necrosis, and vascular invasion that was composed exclusively of spindle cells.
arranged in variably intertwining fascicles with up to 15 mitoses per 2 mm² but no signs of keratinization (Fig. 2A–D).

An extensive IHC characterization revealed diffuse positivity for DeltaNp63/p40, CD34 and vimentin, variable decoration for cytokeratin pool AE1 to AE3, cytokeratin 5 and 6, and CD99, as well as lack of the following: synaptophysin; chromogranin A; CD56; Fli-1; TLE family member 1, transcriptional corepressor; thyroid transcription factor 1; and paired box 8. This profile was not indicative of any type of common lung cancer; instead, it was reminiscent of recurrently described markers in NUTC of the lung and mediastinum.2,3 Accordingly, an IHC assay for NUT protein expression was carried out with the clone C52B1 (Cell Signaling Technology, Danvers, MA) in a 1:50 dilution for 60 minutes after pretreatment with ethylenediaminetetraacetic acid buffer pH9 for 88 minutes and use of the OptiView DAB IHC Detection Kit on the Ventana BenchMark Ultra (Hoffmann-La Roche AG, Basel, Switzerland), which showed widespread nuclear decoration in virtually all tumor cells (Fig. 2E) with a speckling appearance of the staining (see Fig. 2E [inset]). A diagnosis of NUTC was definitively rendered by also reviewing bronchial biopsy samples; it was staged as pT3N2 (IIIB, according to the TNM/American Joint Committee on Cancer criteria [eighth edition]) because of three N1 metastatic lymph nodes and one N2 metastatic lymph node. Owing to this unusual morphology, a FISH confirmation of the NUTC diagnosis was requested to Dr. French’s laboratory, who also revealed NUT-bromodomain containing 4 gene (BRD4) translocation, but the analysis was subsequently repeated in our laboratory (by G.P., M.C., and E.B.) successfully by using a commercially available dual-color break-apart probe kit (ZytoLight SPEC NUTM1 CE IVD, ZytoVision, Bremerhaven, Germany) (Fig. 2F).

The patient underwent four cycles of adjuvant chemotherapy and is currently alive and well with no signs of recurrent disease as of the 12-month follow-up. If this unusual morphologic appearance is likely to underpin an unpredictably longer survival, it will be clarified by further follow-up observation. However, few long-term survivors are on record, even though no relationship has been observed between the type of NUT translocation and clinical course.6 As our case harbored the most frequent NUT-BRD4 translocation in spite of a unique morphologic appearance, it was tempting to speculate that epigenetic and/or microenvironmental factors were likely to account for the challenging shaping and arrangement of tumor cells we have herein documented.

The number of spindle cell NUTCs of the lung is likely destined to increase in the near future thanks to heightened diagnostic awareness, and this report expands its morphologic spectrum. However, NUT protein IHC must enter the pathologist’s daily armamentarium to either avoid missing morphologically demanding lung tumors or strategically manage small biopsy samples without wasting clinical time and resources on inconclusive immunoreactions.

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This study is dedicated to the memory of Carlotta, an extraordinarily lively girl whose untimely death from cancer occurred in the prime of her life. The patient has kindly provided informed consent to the processing of his personal data for this article.
Figure 2. (A-F) Tumor mass presented with pushing margins and spotty necrosis (A [asterisk]), focal vascular invasion (B), and diffuse spindle cell patterning (C). Tumor cells showing no signs of keratinization and up to 15 mitoses per 2 mm² could be enumerated (D). Immunohistochemistry for NUT midline carcinoma family 1 protein revealed diffuse decoration in all tumor cells (E), with the characteristic speckled staining pattern confined to the nuclear area (E [inset]). Fluorescence in situ hybridization analysis by means of a commercially available dual color break-apart kit showed splitting green and red signals, along with fusion yellow signals, indicative of translocation involving the relevant NUT midline carcinoma family 1 gene (NUT) (F).

References