Rediagnosis of Lung Cancer as NUT Midline Carcinoma Based on Clues From Tumor Genomic Profiling

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Abstract

Tumor DNA sequencing can identify rare driver genomic alterations that suggest targets for cancer therapy, even when these drivers cannot be suspected on clinical grounds. In some cases, genomic alterations identified in the tumor can lead to a change in diagnosis with implications for prognosis and therapy. This report describes a case in which evaluation of tumor sequencing results by a molecular tumor board (MTB) led to rediagnosis of a non–small cell lung cancer as highly aggressive NUT midline carcinoma, with implications for targeted therapy using an investigational bromodomain and extraterminal (BET) inhibitor. We discuss the molecular biology and diagnosis of this rare tumor, and suggest how improved annotation of tumor sequencing reports and multidisciplinary expertise of MTBs can facilitate timely diagnosis of rare tumors and application of potential targeted therapies.

J Natl Compr Canc Netw 2018;16(5):467–472

Case Report

A 39-year-old man who never smoked and had no significant past medical history presented to an urgent care clinic with a productive cough. A chest radiograph described an ill-defined density at the left hilum. He was treated with azithromycin and advised to follow up within 10 days to reevaluate the abnormal radiographic findings. A repeat chest radiograph showed the same findings suspicious for malignancy, and he was referred for chest CT, which demonstrated a left lower lobe mass concerning for lung cancer or lymphoma (Figure 1A). PET/CT subsequently showed a left lower lobe lung mass measuring 12 x 6 x 7 cm with intense metabolic activity invading the hilum, bulky mediastinal adenopathy, and another left lower lobe cavitary mass. Multiple lytic bone lesions were identified in the spine, pelvis, and femur (Figure 1B). A brain MRI was negative for metastases. Laboratory tests were notable only for minimally elevated aspartate aminotransferase (39 U/L) and alanine aminotransferase (45 U/L). Fiberoptic bronchoscopy was performed with fine-needle aspiration (FNA) of a station 7 lymph node, and the pathology was read as poorly differentiated squamous cell carcinoma (SCC) (Figure 2). Immunostains were positive for p63 and negative for TTF-1, napsin A, and synaptophysin. Tumor cells were negative for PD-L1, and tumor-associated immune cells showed 5% staining (an antibody SP142). Tissue was sent for sequencing of a 315 cancer-related gene panel (FoundationOne, Foundation Medicine). By the time the patient saw an oncologist, he had lost 10 to 15 pounds. He was referred for consultation at Johns Hopkins University, where repeat PD-L1

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Submitted October 12, 2017; accepted for publication December 21, 2017.

Dr. Hann has disclosed that she has received research funding from GlaxoSmithKline, AbbVie, Bristol-Myers Squibb, and Merrimack Pharmaceuticals; and is a consultant for AbbVie, Bristol-Myers Squibb, and Genentech. Dr. Illei has disclosed that he is on an advisory board or is a consultant for AstraZeneca, Bristol Myers Squibb, Roche, and Abbvie. Dr. Naidoo has disclosed that she has received research funding from Merck, AstraZeneca/Medimmune, and Kyowa Kirin; is a consultant for Bristol Myers Squibb, AstraZeneca/Medimmune, and Takeda; and has received honoraria from Bristol Myers Squibb and AstraZeneca/Medimmune. Dr. Lauring has disclosed that he is a consultant for the Jackson Laboratory. The remaining authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

This work was supported in part by NIH P30 CA006973.

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staining with the 22C3 antibody demonstrated a tumor proportion score of 1% to 2% (low). Several immunotherapy clinical trials were discussed, as well as standard platinum-doublet chemotherapy.

The patient’s tumor sequencing test and clinical history were also referred to the multidisciplinary GAITWAY molecular tumor board (MTB) at Johns Hopkins University to review potentially actionable genomic findings. Only 2 alterations were described in the report: a bromodomain-containing 4 (BRD4) rearrangement in exon 11 and a MutL homolog 1 (MLH1) R325Q mutation. The report stated that the test’s sensitivity was limited by sample quality. Microsatellite stability status was reported as unknown and tumor mutation burden was not calculated, also presumably because of inadequate sample quality or DNA quantity. The MLH1 R325Q mutation has been reported previously in the germline in a few Lynch syndrome families, but it has not been associated with microsatellite instability, and in vitro functional assays show that it is mismatch repair–proficient. This variant has been classified as likely benign.

The patient’s family history was not suggestive of Lynch syndrome, with a paternal grandfather with lung cancer (heavy smoker) and a paternal grandmother with a gynecologic cancer (age unknown). The MTB did not recommend germline testing or further workup.

The tumor sequencing report stated that alterations removing the C terminus of BRD4 are predicted to disrupt its transcriptional and chromatin regula-
Molecular Insights in Patient Care

Genetic Finding Changes Cancer Diagnosis

More recent studies have identified the pathognomonic rearrangements in patients ranging in age from 0.1 to 81 years, with a median age of 29 years.8 NMC is a very aggressive cancer with a median survival of only 6.7 months in the largest case series reported.9 Survival for adult patients at 1 year is <20%. There is no accepted standard of care, but the best reported outcomes have been in patients with primary head and neck tumor sites treated with intensive local therapy, including gross total resection and/or radiation or chemoradiation.3,9 Chemotherapy regimens for SCC, sarcomas, and germ cell tumors have been used and frequently include platinum drugs, anthracyclines, and alkylating agents, but no regimen has shown clear superiority. Initial responses to chemotherapy have been reported, but the tumor typically recurs quickly as a refractory, rapidly progressive cancer.9,10 Only a few long-term survivors have been reported, all of whom received intensive combination chemotherapy and radiation.10–12 Several of these patients were treated according to the Scandinavian Sarcoma Group IX protocol using alternating courses of vincristine, doxorubicin, and ifosfamide with cisplatin, doxorubicin, and ifosfamide.11,12

Preclinical functional studies have demonstrated that the BRD4-NUT fusion results in extensive recruitment of NUT to large “megadomains” of DNA ranging from hundreds of kilobases to megabases, through tethering by the acetyl-histone–binding BET (bromodomain and extraterminal) domain of BRD4. Increased histone lysine acetylation leads to feed-forward recruitment of more BRD4 to these loci, causing widespread epigenetic transcriptional activation, including key loci such as MYC.11 Recently, several pharmaceutical companies have developed inhibitors of BET domain–containing proteins, including BRD4. These drugs are being tested in ongoing clinical trials, some of which have cohorts for NMC. A recent case series described dramatic, rapid responses of several cases of NMC to one of these investigational BET inhibitors, OTX015/MK-8628.14 Of 10 evaluable patients with NMC, 2 responses and 4 cases of stable disease were reported in a phase I/II trial of the BET inhibitor GSK525762.15 There is also a case report of a response to the histone deacetylase inhibitor vorinostat.16

The MTB observed that the patient was relatively young at 39 years of age and was a never-smoker, despite having been diagnosed with SCC of the lung. Given the histology and the BRD4 rearrangement, the MTB was suspicious that his disease was in fact NMC, and suggested performing immunohistochemistry for the NUT protein, which is highly sensitive and specific for NMC.17 After some delay in obtaining remaining tissue from the patient’s FNA, the specimen tested positive for NMC with strong, diffuse NUT staining (Figure 2).

In the interim, the patient experienced worsening bone pain, dyspnea, and fever, and was hospitalized locally. CT imaging demonstrated an increase in the primary lung mass and mediastinal adenopathy, with encasement of the left airways and postobstructive changes, new liver lesions, and T5 vertebral compression fracture. Hepatic transaminases were both >1.5 times the upper limit of normal. Given the constraints of his worsening performance status, he was treated with radiation to the mediastinum and lung mass with concurrent weekly dosing of carboplatin and paclitaxel (subsequently changed to nab-paclitaxel). He was discharged and received further radiotherapy to the right hip and sacrum, kyphoplasty, and denosumab. His symptoms improved and he was seen again at Johns Hopkins University nearly 3 months after his tumor sequencing report was issued to discuss participation in a clinical trial of the BET inhibitor GSK525762 with a cohort for NMC (ClinicalTrials.gov identifier: NCT01587703). Unfortunately, he
had abnormal hepatic transaminase levels that precluded immediate trial participation, and several days later was readmitted to the hospital with epidural spinal cord compression at T2. He underwent surgical decompression and then initiated treatment with doxorubicin and cyclophosphamide. However, he had continued progression of bone metastases and died before the second cycle of chemotherapy.

Analysis of the raw sequencing data from the patient’s tumor sequencing test performed after his death revealed a complex set of chromosomal rearrangements involving the \textit{BRD4} locus on chromosome 19p13.1, with multiple breakpoints in which a portion of chromosome 6 is inserted into the intronic sequence downstream of exon 11 in \textit{BRD4}, with respect to the transcriptional orientation (Figure 3). This is then followed by an apparent inversion of a short subsequent chromosome 19 segment, which then harbors a breakpoint to chromosome 15 upstream of the \textit{NUTM1} locus. There is also evidence of a breakpoint downstream to these events, with respect to the \textit{BRD4} locus, involving chromosome 8. There was no definitive evidence of a rearrangement locating the upstream \textit{BRD4} exons (1–11) into the \textit{NUTM1} locus in the same transcriptional orientation. Given the targeted nature of this sequencing assay, which does not directly have sequencing baits/probes for \textit{NUTM1}, we were unable to definitively evaluate whether additional breakpoints within this region might be present that would support a \textit{BRD4-NUTM1} fusion. However, the combination of the various genomic alterations in the \textit{BRD4} locus (including a breakpoint to just upstream of the \textit{NUTM1} locus) and the strong, diffuse NUT immunohistochemical staining is suggestive of a cryptic \textit{BRD4-NUT} fusion or some form of transcriptional upregulation of NUT as a consequence of the rearrangement.

**Discussion**

This case illustrates a missed opportunity to match a promising targeted therapy with a specific genomic driver alteration in a rare cancer type. Many oncologists may not be aware of NMC as a clinical entity. Although outcomes to date have been poor, making the correct diagnosis can lead to early use of aggressive combined modality therapy, which offers the best chance for disease control. Specific diagnosis

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Complex chromosomal rearrangements within the \textit{BRD4} locus involving chromosomes 6, 8, 15, and 19. (A) The DNA sequence read alignments at the \textit{BRD4} locus are shown, with the chromosomal region and \textit{BRD4} exon structure indicated at the top of the figure. Reads are organized by chromosome of origin of the mate pair. As expected, most reads have both mates mapping to the \textit{BRD4} locus on chromosome 19 (only a portion of these reads are shown in the figure). A smaller number of reads have one mate pair mapping to \textit{BRD4} on chromosome 19 and the other mate pair mapping to chromosome 6, 8, or 15 (red box), indicating a complex pattern of chromosomal rearrangements clustered distal to \textit{BRD4} exon 11. (B) The DNA sequence read alignments near the \textit{NUTM1} locus are shown, in which the chromosome 19 to 15 rearrangement is present upstream of the \textit{NUTM1} locus (red box). All reads are stratified by the chromosome of the mate pair and overlaid by the labeled gene structures.
also allows for enrollment of patients with NMC on clinical trials, which may define the role of novel agents in the management of this disease. Evidence for BET inhibitors in this disease is still preliminary, and we do not know whether this patient would have responded, but this approach represents the kind of specific therapeutic targeting that tumor sequencing is intended to enable. The MTB’s ability to discern the possibility of NMC based on the reported BRD4 rearrangement, together with the clinical and pathology data, illustrates one of the many ways MTBs can add value in interpreting whether tumor molecular profiling results are clinically actionable.

It is likely that other cases of NMC are diagnosed as poorly differentiated SCC of the lung or other sites, although it will be difficult to define how often this occurs until more widespread molecular testing of the relevant genes is reported. One large series of unselected lung carcinomas examined for NUT expression did not identify any positive cases. However, over a 4-year period, a specialty referral center described 8 cases of primary pulmonary NMC, which shared very similar clinical features to this case. Retrospective analysis by this group of 166 consecutive lung cancer biopsies lacking glandular differentiation identified 1 case as NMC (0.6%). Clinicians should consider NMC in the differential diagnosis of poorly differentiated carcinomas occurring in the head and neck or thorax, particularly in nonsmokers.

This tumor had a complex set of chromosomal rearrangements, and the genomic coverage of this test does not conclusively link the upstream exons 1–11 of BRD4 to NUTM1. The cautious language of the report is certainly accurate and appropriate. DNA-based cancer gene panel tests have limitations in their ability to identify gene fusions, and sensitivity is highest when specific baits are designed to capture the exon or intron sequences expected to be involved. Despite the sequencing test’s inability to identify the canonical BRD4-NUT rearrangement in this case, the clinical presentation, histopathology, and NUT immunohistochemistry all support the diagnosis of NMC. It is possible that NUT overexpression is activated in this case by disruption of transcriptional regulatory elements near the NUTM1 locus, rather than by a gene fusion. However, we favor the existence of a cryptic rearrangement, given that the BRD4 locus rearrangement is probably not coincidental. Of note, complex multichromosome rearrangements due to chromoplexy that lead to productive BRD4-NUT fusion transcripts have been identified using whole-exome sequencing in NMC.

Even in the absence of definitive evidence of a typical BRD4-NUT fusion in this case, an annotation that had commented on the existence of BRD4-NUT rearrangements in NMC and the possibility of confirmatory testing in the appropriate clinical context (poorly differentiated SCC) could have directed a clinician toward the appropriate diagnostic workup and clinical trial options for NMC. As noted earlier, NUT immunohistochemistry is a reliable and simple method to identify NMC. Other methods include cytogenetics, fluorescence in situ hybridization, and RNA-based approaches, such as next-generation RNA sequencing, RT-PCR, and the Archer FusionPlex assay (ArcherDX, Inc.). Tumor sequencing providers currently suggest considering additional testing in other situations. For example, annotations for deleterious mutations in genes such as BRCA1 and BRCA2 advise that in some cases these could represent germline cancer-predisposing variants and suggest germline testing if clinically indicated.

Conclusions

This case highlights a disconnect between traditional pathology and commercial tumor gene sequencing, wherein sequencing results are often provided back to oncologists or surgeons and may not be reviewed by the pathologists who originally reviewed the tissue, missing the opportunity to revise a diagnosis accordingly. Rare tumors will always pose clinical challenges, but improvements to tumor sequencing annotation and more accessible and timely review of findings by MTBs with both oncology and molecular pathology expertise could refine clinical diagnoses and match patients, such as the one discussed herein, to appropriate molecularly targeted clinical trials before the therapeutic window closes.

Acknowledgments

The authors would like to thank the entire GAITWAY MTB for their input and assistance with this case.
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