CASE REPORT

A case of metastatic NUT carcinoma with prolonged response on gemcitabine and nab-paclitaxel

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Abstract

Background: NUT carcinoma is an aggressive malignancy characterized by translocations in the NUTM1 gene. There are currently no consensus treatment recommendations for NUT carcinomas.

Methods: Here, we describe the case of a previously healthy male diagnosed with NUT carcinoma after presenting with sinus pressure, found to have a sinonasal mass and distant metastatic disease in the lungs. While pathologic evaluation and immunohistochemistry were consistent with NUT carcinoma, initial genomic profiling did not demonstrate a NUTM1 translocation.

Results: Whole transcriptomic RNA sequencing of the tumor revealed a YAP1-NUTM1 fusion. Based on an in vitro drug sensitivity screen, the patient was treated with gemcitabine and nab-paclitaxel, achieving a partial response that persisted for 9 months.

Conclusions: Unbiased transcriptomic sequencing may identify previously uncharacterized NUTM1 fusion partners. Gemcitabine and nab-paclitaxel is a well-tolerated combination chemotherapy regimen and could offer a novel treatment approach for NUT carcinoma.

KEYWORDS

chemotherapy, next-generation sequencing, NUT carcinoma, NUTM1 fusion

1 | CASE PRESENTATION

The patient is a 39-year-old, previously healthy white male who initially presented with sinus and ear pressure. He was treated with three courses of antibiotics for presumed sinusitis, with only minimal relief. Ultimately, he saw an otolaryngologist and a computed tomography (CT) scan was performed showing only sinusitis, per report. He underwent endoscopic sinus surgery and turbinate reduction, resulting in some relief, but his symptoms of congestion continued. The patient sought a second opinion from another otolaryngologist and had a repeat CT scan. By report, it again showed opacification of the sinus cavity, but no discrete mass. The second surgeon took him back to the operating room, and a mass was identified during surgical exploration. The biopsy was interpreted as a poorly differentiated non-small cell carcinoma with features consistent with squamous cell carcinoma. Positron emission tomography (PET) imaging showed
a hypermetabolic nasopharyngeal mass, bilateral cervical lymphadenopathy, and pulmonary nodules. He was referred to our institution for a medical oncology consultation.

As part of his workup, pathology materials were requested for in-house review, which demonstrated a poorly differentiated non-small cell carcinoma with indistinct cell borders, enlarged, hyperchromatic, vesicular nuclei with prominent nucleoli, and frequent mitoses and apoptotic debris (Figure 1A). Immunohistochemistry (IHC) showed positive staining with cytokeratins AE1/AE3 and CK5, and focal P40. There was negative staining with TTF-1, Napsin-A, synaptophysin, chromogranin-A, smooth muscle actin, p16, S100, and EBER (EBV-ISH). PD-L1 22C3 was expressed with a combined positive score (CPS) of 25. Based on the pathologic features, absence of risk factors for nasopharyngeal cancer, and the midline nature of the tumor, an additional immunostain for NUT (nuclear protein in testis) was requested. The NUT stain revealed strong, diffusely positive nuclear expression in approximately 95% of tumor nuclei, establishing the diagnosis of NUT carcinoma (Figure 1B).

A transbronchial fine-needle aspiration (TBNA) of the right upper lung mass also demonstrated poorly differentiated non-small cell carcinoma with identical morphologic features on hematoxylin and eosin (H&E) stain, confirming metastatic disease (Figure 1C).

A targeted DNA- and RNA-based next-generation sequencing (NGS) panel (STRATA oncology) demonstrated a CDKN2A deletion, but did not identify a NUT rearrangement. Subsequently, whole transcriptomic RNA sequencing (Tempus xT Panel) demonstrated a \textit{YAP1-NUTM1} rearrangement (Figure 2). Additional genomic variants identified include \textit{BAP1} p.Q665* (variant allele frequency (vAF) 52.3%), \textit{NOTCH1} p.L2203fs (vAF 11.4%), \textit{CDKN2A/B} copy number loss, and \textit{MTAP} copy number loss. Tumor mutational burden was 4.7 mutations per megabase. The patient has a family history of non-melanoma skin cancer and was referred to medical genetics after the identification of the \textit{BAP1} variant.

Treatment options were discussed. Given his young age, the patient very open to the risks or side effects of aggressive trials. Given his tumor had a high PD-L1 score, and the possibility of more durable treatment response with immune checkpoint blockade, he was consented to a clinical trial combining a PD-1 checkpoint inhibitor and a Toll-like receptor 7 (TLR7) agonist (Figure 3A, summary of treatment course). Unfortunately, his cancer grew during treatment. Symptomatically, the patient noted increased headaches in the temples, obstructed nasal passages, and intermittent blurry and double vision. MRI brain showed interval progression of the nasal, sinus, and nasopharyngeal mass, with new intracranial extension through the right cribriform plate and likely involvement of the clivus, as well as increasing extension through the right lamina papyracea with mass effect on the medial rectus muscle posteriorly (Figure 3B). Chest imaging demonstrated interval progression of diffuse bilateral pulmonary metastases with pleural nodules, left pleural effusion, and bilateral hilar lymph node enlargement (Figure 3C). For palliation of his symptoms, he was treated with 45 Gy in 15 fractions of radiation therapy to the nasopharynx, resulting in significant symptomatic benefit and partial response.

Additional treatment options were considered. In searching for treatment options for our patient, the clinical literature (reviewed below) did not suggest a clear therapeutic choice. We therefore chose gemcitabine (1500mg/m$^2$ day 1, 15 of 28-day cycle) and nab-paclitaxel (175mg/m$^2$ day 1, 15 of 28-day cycle) based on an \textit{in vitro} drug screening study performed in a panel of NC cell lines, which demonstrated increased cytotoxicity with microtubule poisons and topoisomerase inhibitors.\textsuperscript{1} The combination of gemcitabine and nab-paclitaxel resulted in response at both his primary site and in the lung lesions (representative images, Figure 3D,E). Treatment was briefly interrupted to allow recovery after the patient was hit by a car while biking, and was complicated by a single episode of culture-negative febrile neutropenia, grade 1 neuropathy, and a soft-tissue infection. The patient remained on gemcitabine and nab-paclitaxel for 9 months prior to progression of lung nodules. He was subsequently treated on a clinical trial of a BET inhibitor, with progressive disease.

\textbf{FIGURE 1} A. H&E slide of nasal biopsy demonstrating a non-small cell carcinoma. B. IHC for NUT protein demonstrating positive staining in tumor nuclei (brown). C. H&E slide of lung biopsy demonstrates morphological features similar to nasal biopsy.
as best response but remains alive currently, approximately 21 months after initially developing symptoms.

2 | DISCUSSION

NUT carcinoma (NC) is a rare, highly aggressive malignancy defined by the presence of rearrangements in the \textit{NUTM1} (aka \textit{NUT}) gene. Previously referred to as NUT midline carcinoma because it often arises in midline structures of the head/neck and thorax, subsequent reports have noted that NC can arise from a number of anatomic sites.\textsuperscript{2} Histologically, NUT carcinomas have a monomorphic, poorly differentiated appearance with focal squamous differentiation seen in a subset of cases. As these histopathologic features can overlap with other poorly differentiated carcinomas, the diagnosis is dependent on staining or molecular testing for NUT. In normal human tissues, the NUT protein is expressed only in post-meiotic spermatids. Strong diffuse NUT immunostaining is highly specific and diagnostic of NUT carcinoma in the appropriate setting. Currently, the WHO guidelines state that only strong and diffuse (greater than 50\%) nuclear staining should be considered a positive result.\textsuperscript{3} Pathologists need to be aware; however, that variable staining has also been reported in some germ cell tumors.\textsuperscript{4}

2.1 | Molecular testing

Early cytogenetic characterization indicated the majority of NCs (70\%) harbor a clonal, reciprocal translocation between the \textit{NUTM1} and \textit{BRD4} genes t(15;19), generating an in-frame fusion gene encoding \textit{BRD4-NUTM1}. Subsequently, multiple additional translocation partners for \textit{NUTM1} have been identified, including \textit{BRD3}\textsuperscript{5} and \textit{NSD3}.\textsuperscript{6} Recent DNA- and RNA-based next-generation sequencing (NGS) has demonstrated additional fusion partners in NC\textsuperscript{7} and revealed \textit{NUTM1} translocations in other solid malignancies (sarcomas, poromas, and CNS tumors) and leukemia.\textsuperscript{8} While identifying the specific translocation is not necessary for diagnosis, analysis of clinical outcomes suggests there may be differences in prognosis based on the binding partner.\textsuperscript{9} Moreover, identification of the translocation partner may also have implications for treatment as discussed below.

\begin{figure}
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\includegraphics[width=\textwidth]{figure2}
\caption{\textit{YAP1-NUTM1} rearrangement. The 5\' breakpoint in \textit{YAP1} is in intron 3 and the 3\' breakpoint in \textit{NUTM1} is in intron 2.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{A. Timeline of diagnosis and treatment course. B. Baseline MRI Brain with and without contrast demonstrating sphenoid sinus mass. C. Baseline chest CT demonstrating pulmonary nodule. D. MRI Brain with and without contrast after 2 cycles of gemcitabine and nab-paclitaxel. E. Chest CT after 2 cycles of treatment.}
\end{figure}
Several techniques are available for molecular characterization of NUTM1 rearrangements, each with unique test characteristics. While cytogenetics was used to initially describe the classical t(15;19) rearrangement, this assay is difficult to perform in practice due to the need for fresh tissue and would not identify novel NUTM1 fusions. Fluorescence in situ hybridization (FISH) using break apart probes for NUTM1 is another sensitive method to identify rearrangements, but cannot identify the translocation partner and is not a widely available test. With the broader use of NGS in oncology, multiple commercially available multigene panels now include NUTM1. However, the ability of a targeted DNA-based sequencing panel to identify fusions and particularly novel partners can be limited as we observed in our patient. While NUTM1 was included on the initial NGS assay performed in our patient, that assay was designed to detect fusions specifically with BRD3, BRD4, CIC, and WHSC1L1, thus a translocation involving a novel partner was not identified. In contrast, “partner agnostic” RNA-based sequencing using assays such as the Archer FusionPlex or whole transcriptome RNA sequencing can identify novel fusions.

In our patient, a YAP1-NUTM1 fusion was identified by whole transcriptome RNA sequencing. YAP1 is a transcriptional co-activator, functioning downstream of the evolutionarily conserved HIPPO signaling pathway. To our knowledge, YAP1-NUTM1 fusions have not been seen in NUT carcinoma but have recently been described in poromas and certain porocarcinomas. Our case did not appear morphologically consistent with a porocarcinoma. Sekine and colleagues demonstrated YAP1-NUTM1 fusions activate TEAD-dependent transcription, suggesting a mechanism for their oncogenic function.

2.2 | Therapeutic options

Given the rarity of the disease, there are no prospective studies examining treatment options for NUT carcinoma. A registry has been established (NMCregistry.org) to collect pathologic and outcomes data. Clinical series have demonstrated NC patients treated with multi-modality therapy including surgery, radiation, and chemotherapy have better outcomes but prognosis is still very limited. For patients with metastatic disease, an optimal chemotherapy regimen has not been established. Case series have described success with multi-agent pediatric sarcoma treatment regimens; however, these can have significant toxicity.

BRD4 is a bromodomain (BRD) and extra-terminal domain (BET) family member that plays an important role in gene transcription by binding to acetylated histones. BET family proteins have been shown to contribute to carcinogenesis and treatment resistance in multiple malignancies. Specifically, in NUT carcinoma, preclinical studies with BET inhibitors lead to growth arrest and differentiation with the MYC oncoprotein being an important target of BRD4-NUTM1. Interestingly, BRD4 appears to play an important role in YAP mediated transcription and BET inhibitors have preclinical activity in YAP-dependent malignancies. This suggests BET inhibitors may be a therapeutic option for our patient despite the presence of a YAP1-NUTM1 translocation. Two recent early phase clinical trials have examined the activity of BET inhibitors in NC patients. Birabresib (MK-8628/OTX015) is a BRD2, BRD3, and BRD4 inhibitor. In the phase Ib study of the nine evaluable patients with NC three partial responses was observed with an additional three patients having stable disease. Duration of response ranged from 1.4 to 8.4 months. The results of a phase I/II study of molibresib (GSK525762) were recently reported. In the NC cohort, the overall response rate was 11% (2/19 confirmed partial responses). Overall 4 patients remained on study drug for longer than 6 months. Interestingly, these patients all had non-thoracic primaries and the NUTM1 fusion partner was BRD3, which may suggest differential activity against BRD family proteins. While these studies demonstrate proof of concept for BET inhibition in NC, responses were not durable. Moreover, there may be differences in the activity of BET inhibitors based on the specific breakpoints of the translocation. Combination strategies targeting the unique transcriptional dependencies of this aggressive malignancy are also being studied, with hope for improving outcomes for this challenging disease.

Given the limited response observed with BET inhibitors, we sought alternate therapeutic approaches. Stirnweiss et al had previously reported genomic profiling and high-throughput drug screening of a series of NUT carcinoma cell lines. Their analysis demonstrated recurrent RECQL5 alterations and deficiency in DNA repair. Screening for drug sensitivity demonstrated increased sensitivity to anthracyclines (daunorubicin), topoisomerase inhibitors (topotecan, gemcitabine), and microtubule inhibitors (docetaxel, vincristine), with activity in the low nanomolar range. This observation prompted our decision to treat with gemcitabine and nab-paclitaxel, given this is a well-established combination regimen and would provide the patient exposure to 2 agents that were active in the in vitro drug screen. Interestingly, this report also demonstrated that BET inhibitors were only active in a subset of the cell lines, specifically those harboring BRD4-NUTM1 fusions, suggesting that more detailed understanding of the fusion partner may be important for treatment selection. In addition to this cell line drug screen, a previous case report described a complete response in a patient with NUT carcinoma treated sequentially with cisplatin/docetaxel followed by gemcitabine monotherapy. The tolerability and encouraging treatment response seen in our patient suggest the combination of gemcitabine and taxanes is worth further study in NUT carcinoma. More detailed genomic analysis of the nature of the NUTM1 translocation, fusion partner, and...
co-occurring alterations may also play an important role in treatment selection.

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CONFLICTS OF INTEREST
The authors declare no relevant conflicts of interest. No funding for this report has been provided.

AUTHOR CONTRIBUTIONS
SAP: Contributed to conception and design, collected data, and wrote the manuscript. BS: Contributed to data collection and writing the manuscript. CS: Contributed to data collection and writing the manuscript. AMZ: Contributed to data collection and writing the manuscript. WGY: Contributed to data collection and writing the manuscript. JW: Contributed to conception and design, collected data, and wrote the manuscript.

ETHICS STATEMENT
Written informed consent from the patient was obtained for publication of this case report.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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