Dear Editor,

Patients with undifferentiated (anaplastic) thyroid carcinoma (ATC) are refractory to surgical treatment, chemotherapy, and/or radiotherapy, and have a dismal prognosis (Smallridge et al. 2012). Distant metastasis occurs at various sites and is poorly sensitive to chemotherapy or radiotherapy. As a result of aggressive tumor behavior and ineffective therapeutic efficacy, patients do poorly (Besic & Gazic 2013). Positron emission tomography (PET)/computed tomography (CT) is the primary imaging modality for ATC at initial staging and has utility in the early evaluation of treatment response and follow-up (Poisson et al. 2010). Distinct metastases, extensive local infiltration, and regional lymph node metastases have been found at autopsy in 91, 76, and 51% of cases respectively. Regional and distant metastases are shown to be present in 51% of cases, with 40% of cases showing distant metastases alone. The most common site of metastasis is the lung (78%), followed by intrathoracic lymph nodes (58%), neck lymph nodes (51%), pleura (29%), adrenal glands (24%), liver (20%), brain (18%), and heart (18%) (Besic & Gazic 2013). Lung metastases are present in 34 of 38 (89%) cases, with distant metastases found at autopsy, and are known to be present in 27 of these patients while alive. In most patients, lung metastases are detected by chest X-ray (Besic & Gazic 2013). Several genetic alterations are reported in the pathogenesis of ATC, including RAS, BRAF, TP53, CTNNBI (β-catenin), VEGFR1, VEGFR2, KIT, MET, PIK3C, FOXA1, etc. (Liu et al. 2008, Nucera et al. 2009, Smallridge et al. 2012, Antonello & Nucera 2013). The BRAFV600E mutation is the most prevalent genetic alteration in PTC (frequency of about 60%) and may be associated with tumor progression of PTC to ATC (frequency: about 15–44%) (Ricarte-Filho et al. 2009, Cancer Genome Atlas Research Network 2014, Shi et al. 2015). Importantly, although pre-clinical (Nehs et al. 2010, 2012) and clinical (Rosove et al. 2013) trials in ATC patients harboring BRAFV600E suggested that BRAFV600E inhibitors may be promising as anti-cancer therapy, more in depth studies are needed to clarify the genetics and activated aberrant signaling pathways in this rare thyroid carcinoma.

Here, we have performed targeted next-generation sequencing (NGS; Zheng et al. 2014) in six ATCs (high grade malignant pleomorphic cell neoplasms). We found for the first time that one out of six (16.6%) ATC showed overall frequency of the co-occurrence of PTEN p.Cys105LeufsTer2 (c.309_310insT) and TP53 p.Pro153AlafsTer28 (c.455insC) frameshift insertions (identified from 39 genes analyzed, including analysis of 1799T O A B BRAFV600E mutation and translocations of RET, ALK, and ROSI) (Table 1 and Fig. 1). These insertions were present in both primary ATC and in metastatic brain foci which showed increased angiogenesis. Targeted NGS is based on innovative high-throughput screening which is fundamental to identify genomic alterations potentially causative of thyroid cancer progression and drug-resistance. The resulting genomic variants were filtered (Supplementary Materials and Methods, see section on supplementary data given at the end of this article and Table 1) in order to assess functional variants that may be associated with ATC. As example of these concurrent insertions, we describe one case of ATC which genetically harbors PTEN and TP53 insertions. The ATC patient presented with symptoms related to brain metastasis with concurrent PTEN and TP53 frameshift insertions, aberrations later confirmed to be present in the primary ATC as well. The patient was a 68-year-old woman who sought medical attention for two episodes of visual disturbance and expressive aphasia that prompted an emergency department visit in May of 2014. Head CT revealed a 1.9 cm left parieto-occipital mass with vasogenic edema. Subsequent brain magnetic resonance imaging (MRI) confirmed a
### Table 1  Targeted NGS analysis performed in human anaplastic (undifferentiated) carcinomas and matching metastatic foci

<table>
<thead>
<tr>
<th>Histology</th>
<th>Gender (F/M)</th>
<th>Size (cm)</th>
<th>Age (years)</th>
<th>Metastasis</th>
<th>TNM</th>
<th>NGS in the primary ATC</th>
<th>NGS in the metastatic foci</th>
<th>TTF-1 expression in ATC</th>
<th>PAX8 expression in ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATC #2 + PDTC and Hurthle cell carcinoma</td>
<td>F</td>
<td>9</td>
<td>47</td>
<td>Neck cervical lymph nodes, neck skin</td>
<td>pT4aN1bMx</td>
<td>Point mutations: PK3R1 p.Thr675Ile (c.2024C&gt;T)</td>
<td>Material not available</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ATC #3</td>
<td>F</td>
<td>8.5</td>
<td>85</td>
<td>Bilateral lung</td>
<td>pT4aN1bM1</td>
<td>Point mutation: TP53 p.Pro151Ser (c.451C&gt;T)</td>
<td>Material not available</td>
<td>Negative</td>
<td>Not available</td>
</tr>
<tr>
<td>ATC #4</td>
<td>M</td>
<td>6</td>
<td>68</td>
<td>Lung, neck cervical lymph nodes, neck skin</td>
<td>pT4aN1bM1</td>
<td>Point mutation: BRAF* 1799T &gt; A (Val600Glu)</td>
<td>Lymph node: material not available</td>
<td>Wak/negative</td>
<td>Positive</td>
</tr>
<tr>
<td>ATC #5</td>
<td>M</td>
<td>5</td>
<td>50</td>
<td>Bilateral lung</td>
<td>pT4aN1bM1</td>
<td>No variants were detected</td>
<td>Lymph node: no variants were detected</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>ATC #6</td>
<td>M</td>
<td>5</td>
<td>57</td>
<td>Lung, pretracheal, paratracheal and prelaryngeal/ Delphian lymph nodes</td>
<td>pT4bN1bM1</td>
<td>No variants were detected</td>
<td>Lymph node metastasis: no variants were detected</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Tumor sequencing was performed on formalin-fixed paraffin-embedded (FFPE) tissues using anchored multiplex PCR (AMP), a multiplex PCR assay developed to detect single nucleotide variants (SNVs) and insertion/deletion (indel) in genomic DNA, using targeted next-generation sequencing (NGS). The genomic DNA was sheared with the Covaris M220 instrument, followed by end repair, adenylation, and ligation with an adapter. A sequencing library targeting hotspots and exons in 39 genes (including analysis of 1799T > A BRAF[V600E] mutation and translocations of RET, ALK, and ROS1) was generated using two hemi-nested PCRs. Illumina MiSeq 2 × 151 bp-end sequencing results were aligned to the hg19 human genome reference using BWA-MEM. MuTect and a laboratory-developed insertion/deletion analysis algorithm were used for SNV and indel variant detection respectively. Oncotator was used for mutation annotation. Minimal thresholds of 100 × coverage and 5% allelic fraction were applied. The filters include a panel of normal DNAs. The resulting variants were filtered against SNP databases (including dbSNP 1000 Genomes Project) and annotated using the Bioconductor Variant Annotation package. Overall coverage was calculated using a 21-bp window for hotspot point mutation targets (± 10 bp) and 5-bp intronic flanks for whole exon targets.

*Genotyping for this sample was performed with a more focused panel using SNaPshot technology from Applied Biosystems, as previously described. SNaPshot analysis was performed according to Dias-Santagata et al. (2010).
1.6 cm enhancing mass in the posterior left parietal lobe of the brain with surrounding edema but no midline shift. Following admission at a local hospital, she experienced two seizures with subsequent craniotomy and resection of a left occipital mass. Histologic review confirmed a metastatic high-grade carcinoma with multifocal necrosis (Fig. 1). Immunohistochemistry demonstrates tumor cells to express CK7, AE1/3, TTF-1 (diffusely positive), and nuclear clearing, and prominent nucleoli (arrowhead); 1000× magnification. (I) Thyroglobulin (Tg) is demonstrated in the normal macrofollicular thyroid tissue (lower half) and entirely absent in tumor cells (above); 200× magnification. (J) PTEN immunohistochemical evaluation of the primary tumor with normal thyroid macrofollicles showing nuclear positivity for PTEN and tumor cells below (blue nuclei) display loss of PTEN protein, indicative of PTEN genomic insertion; 200× magnification. (K) PAX8, a thyroid developmental transcription factor is prominently staining the nuclei of normal thyrocytes (left) and multifocally positive in malignant cells (right); 200× magnification. (L) TTF-1, an additional thyroid developmental transcription factor is strongly expressed in normal thyrocyte nuclei (left) and focally in tumor cells (right), with loss of TTF-1 protein (but not absence) a characteristic of undifferentiated thyroid carcinomas; 200× magnification. (M) TP53 immunohistochemical evaluation of the primary tumor shows an absence of p53 in both normal thyroid follicles (lower 1/4 of panel) and tumor cells, indicated either lack of expression or a mutated protein. A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-15-0198.

Figure 1

Histopathology and immunohistochemical evaluation of the primary anaplastic (undifferentiated) carcinoma (ATC) and the brain metastasis. (A) ATC (left, T) infiltrating brain parenchyma (right, B); 40× magnification. The tumor infiltrates with insular nests and the tumor is surrounded by hemorrhage. (B) Tumor cells are present in insular nests with scattered apoptotic figures (arrows) and multinucleated forms (arrowheads); 400× magnification. (C) Also noted is abundant tumor necrosis (lower third, asterisks); 400× magnification. (D) Oil immersion view of the tumor shows bizarre mitotic figures (arrows); 1000× magnification. (E) Normal macrofollicular thyroid tissue at the bottom left (N) with adjacent fibrotic thyroid tissue. The upper half of the figure shows a malignant (M) nodular infiltrate of cells with a no appreciable follicles; 40× magnification. (F) Closer look at the malignant cells shows the nuclei to have nuclear clearing and irregular borders with scattered multinucleated forms (arrowheads) and apoptotic figures (arrows); 200× magnification. (G) Bizarre mitotic figures (similar to those seen in the brain (D) are also noted in the primary tumor (arrows); 400×. (H) Oil immersion view shows multinucleated forms (arrow), nuclear clearing, and prominent
PAX8 (diffusely positive). Tumor cells are negative for PTEN (lost) (Supplementary Fig. 1), Napsin-A (Supplementary Fig. 1), CK20, CD10, HMB45, SOX10, ER, mammaglobin, chromogranin, TP53 (Supplementary Fig. 1), and GFAP. The ki67 tumor proliferative index is up to 80%. Immunohistochemical staining with an antibody directed against CD31 demonstrated intra- and peritumoral blood vessels (increased angiogenesis; Supplementary Fig. 1), and absence of D2-40 staining highlighted a dearth of lymphatic spaces. Positivity for keratins, TTF-1 and PAX8 with negative napsin-A (Supplementary Fig. 1) essentially confirms a thyroid primary and excludes other sites. Post-operative brain MRI revealed minimal ring enhancement along the post-surgical cavity without nodularity along with a second enhancing lesion of the deep right frontal lobe measuring 5 mm. CT of the chest, abdomen and pelvis revealed a hyperdense, 2.8 × 2.5 cm left thyroid nodule with multiple lung nodules as follows: LUL (left upper lobe of the lung; 7 mm), lingula (1.4 cm), right lower lobe of the lung (RLL; 1.9 cm) along with a cirrhotic-appearing liver. 18-fluorodeoxyglucose (18FDG)–PET/CT confirmed FDG avidity of the left thyroid nodule (standardized uptake value (SUV): 5.7) and uptake of the lingular nodule and RLL nodule (SUV: 3.2). There was a 1 cm nodule in the retroperitoneum with a smaller lateral nodule both with FDG uptake. She had a left thyroid lobectomy for purposes of both comparative diagnosis and treatment. The tumor is unusual in that it is of intermediate size (3.3 cm), confined to the thyroid gland with no evidence of direct extrathyroidal extension of tumor, and shows a mixed morphological appearance. The majority of the tumor has nested, clear cell morphology with prominent, multifocal comedo-type necrosis. There are foci with marked pleomorphism, including cells with tri- and tetrapolar and circular mitoses, and some with clearly bizarre chromatin patterns. Multinucleated cells are prominent. These features are most consistent with an ATC (stage: pT4a NX M1). This unifocal and unencapsulated ATC (Fig. 1) arises in association with a poorly differentiated thyroid carcinoma with clear cell and oncocytic features, widely invasive, with extensive vascular invasion, locally intrathyroidal, with negative surgical margins. Ki67 proliferative index was up to 50%. TTF-1 nuclear expression was focal, rare and weak (Fig. 1); whereas, PAX8 nuclear expression was multi-focally positive and keratin seven marked rare ATC cells.

**Figure 2**
Genomic variants detected by targeted next-generation sequencing (NGS). (A) PTEN p.Cys105LeufsTer2 (c.309_310insT) (ENST00000371953). The sequencing results showed a frameshift T insertion in exon 5 of PTEN, changing the cysteine at amino acid position 105 to leucine and introducing a new stop codon adjacent to this residue. (B) TP53 p.Pro153AlafsTer28 (c.455insC) (ENST00000420246). The sequencing results showed a frameshift C insertion in exon 4 of TP53, changing the proline at amino acid position 153 to alanine and introducing a new stop codon 27 residues downstream. In both panels, the genomic position, the top and bottom genomic strands, and all the reading frames are depicted at the top. A subset of sequence reads is shown with pink horizontal lines representing forward reads and blue horizontal lines representing reverse reads. Mismatches are highlighted in red, blue, green, and gold. The insertions are centered and denoted with purple vertical bars. A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-15-0198.
The ATC was negative for thyroglobulin (retained in surrounding normal thyroid as control) (Fig. 1), TP53 (Fig. 1), and β-catenin (normal membranous staining). PTEN expression was lost in tumor cells (retained in surrounding normal thyroid as control) (Fig. 1). TTF-1 is often lost in ATC or may be focal. PAX8, another thyroid transcription factor, is often more consistently retained in ATC, even though often reduced in overall expression. Therefore, the two immunostains are usually run in tandem, as one stain may show better expression than the other. These tumors often display variable tumor morphology, with a combination of a better differentiated tumor and an undifferentiated tumor. It is likely that the variable expression of these early differentiation-inducing transcription factors contributes to the diverse morphologies of ATC, with distinct heterogeneity even with a single tumor. Additionally, these changes might be noted by varying expression levels of any individual antigen in primary vs metastatic tumor, depending upon which clonal population of the tumor has spread. Importantly, our genomic analysis revealed PTEN (c.309_310insT) and TP53 (c.455insC) frameshift insertions (Fig. 2) as genetic aberrations in the primary ATC specimen. The patient died of disease 10.5 months following her initial brain surgery and subsequent thyroid lobectomy with no evidence of residual disease in the thyroid bed (March 2015). However, she did have residual brain disease and was subsequently involved in a clinical trial. The clinical presentation is atypical in many ways, primarily as she presented with distant symptoms rather than primary symptoms. The fact that her primary tumor was still resectable at the time of diagnosis of brain metastasis is extraordinary and very atypical for this diagnosis.

In summary, ATC is a biologically aggressive malignancy with a complex tumor microenvironment (Nucera et al. 2011, Duquette et al. 2013) prone to rapid local growth and distance metastasis. Here, we identify, for the first time, concomitant frameshift insertions in PTEN and TP53 associated with brain metastasis. Most patients with ATC die from local symptoms, including loss of airway and hemorrhage. The reason we present this case as unique is specifically because it is such an unusual presentation with brain metastasis rather than local symptoms. And, even more remarkably, the patient had a resectable thyroid tumor. This indicates that the tumor biology in this case allowed for early metastasis to a site in the brain that facilitated early clinical attention rather than to a place that would allow for more insidious advancement of the tumor prior to clinical presentation. The genetic alterations seen in this tumor are in genes with known association with aggressive thyroid cancers, PTEN in differentiating thyroid cancers, albeit rare. In human carcinomas, monoallelic mutation of PTEN without loss or mutation of the second allele is prevalent at presentation, whereas complete loss is observed at low frequencies with the exception of advanced carcinomas (Salmena et al. 2008). Similarly, TP53 is more often mutated in ATC (Kunstman et al. 2015). How this combination of PTEN and TP53 insertions assert this tumor’s behavior biologically is unclear.

Although ATC is known to be biologically aggressive with a complex tumor microenvironment, this particular clinical presentation and concomitant insertions may provide new insights for ATC treatment; specifically, because it is such an unusual manifestation with symptoms arising from the brain metastasis rather than its usual presentation with locally aggressive behavior. ATC harboring BRAFV600E present targeted adjuvant options; however, genetic alterations in PTEN and TP53 genes may represent a therapeutic challenge in patients with ATC harboring those aberrations, warranting the need for pre-clinical models to better elucidate the biological cooperation between PTEN and TP53 insertions and their underlying molecular mechanisms in ATC.

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Supplementary data
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Declaration of interest
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