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A Misleading Case of *NTRK*-Rearranged Papillary Thyroid Carcinoma

Marco Maria Germani[1,2], Chiara Boccaccio[1,2], Antonio Matrone[3], Eleonora Molinaro[3], Greta Alì[4], Mirella Giordano[4], Rossella Elisei[3], Gabriella Fontanini[4], Chiara Cremolini[*,1,2]

[1]Unit of Medical Oncology 2, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy
[2]Department of Translational Research and New Technology in Medicine and Surgery, University of Pisa, Pisa, Italy
[3]Unit of Endocrinology, Department of Clinical and Experimental Medicine, Pisa University Hospital, Pisa, Italy
[4]Department of Surgical, Medical and Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy

[*]Corresponding author: Chiara Cremolini, MD, PhD, Unit of Medical Oncology 2, Azienda Ospedaliero-Universitaria Pisana and Department of Translational Research and New Technology in Medicine and Surgery, University of Pisa, Pisa, Italy. Email: chiaracremolini@gmail.com

Abstract

Herein, we present a misleading case of advanced papillary thyroid carcinoma with lung, node, and pleural metastases, initially diagnosed as metastatic lung adenocarcinoma with papillary features, based on the histological and immunohistochemical analysis of a pleural biopsy. Between August 2019 and August 2020, the patient received 2 ineffective lines of systemic therapy, including a first line of chemotherapy with cisplatin and pemetrexed, and a second line of immunotherapy with atezolizumab. Comprehensive genomic profiling by next-generation sequencing on the archival pleural biopsy revealed an *NTRK1-TMP3* fusion and comutation of the *TERT* promoter, commonly found in papillary thyroid carcinoma. After palliative partial thyroidectomy that confirmed the diagnosis of papillary thyroid carcinoma, in February 2021, the patient was enrolled in the STARTRK-2 GO40782 basket trial and received entrectinib, an oral pan-TRK inhibitor specifically targeting *NTRK*-rearranged tumors. After initially experiencing drug-related grade 2 anorexia, dysgeusia, and neurotoxicity and grade 3 asthenia, the dose was reduced, and an excellent and durable objective response was observed.

Key words: thyroid carcinoma; next-generation sequencing; *NTRK1-TMP3* fusion; *TERT* promoter.

Key Points

- *NTRK*-rearranged papillary thyroid carcinoma can have an aggressive behavior.
- Comprehensive genotyping by next-generation sequencing can both individualize treatment and refine the diagnostic workout.
- Oral-targeted therapy demands early and careful monitoring of adverse events to achieve compliance to the treatment.

Patient story

In August 2019, a 57-year-old woman presented to her general practitioner with dysphonia, dyspnea, and fatigue. A contrast-enhanced total-body CT scan was performed, showing bilateral lung and subpleural nodules, a large left pleural effusion, and suspicious mediastinal lymph nodes. The patient's previous medical history was unremarkable, except for the presence of multinodular goiter from several years. Fine needle aspiration cytology (FNAC) performed on the main nodule of the left lobe, several years before, was benign. Then, an advanced lung cancer was suspected, and the patient was referred to the Oncology Department of our hospital.

At the time of the first visit, our patient reported no smoking history and was in good clinical condition (ECOG-PS 0). At physical examination, decreased bilateral respiratory sounds and absence of respiratory sounds in the lower left hemithorax were reported, and 2 palpable left thyroid nodules were detected.

A pleural biopsy was performed, and a lung adenocarcinoma with diffuse papillary features (transcriptional thyroid factor 1 (TTF-1)+, epithelial cell adhesion molecule/EPCAM+, carcino embryonic antigen+, epithelial membrane antigen+, Pan-cytokeratin+) was diagnosed.

Since the dyspnea and the presence of a multinodular goiter, a neck ultrasound was performed, and a FNAC was planned, but due to the respiratory symptoms and the disease stage, initiating systemic therapy was considered a priority. No alterations in *EGFR*, *KRAS BRAF*, *ALK*, and *ROS* genes were detected. Programmed death-ligand 1 (PD-L1) expression at immunohistochemistry (IHC) was 10%.

First-line chemotherapy with cisplatin and pemetrexed was administered (at that time immune checkpoint inhibitors were not reimbursed in Italy when PD-L1 expression was <50%).

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In January 2020, after 5 cycles of Cisplatin and Pemetrexed, disease progression at pleural, lung, and nodal sites was detected, while thyroid nodules were stable and second-line immunotherapy with atezolizumab was started.

Given the potential for interaction of immunotherapy on thyroid function,[1] the patient was referred to the Endocrine Unit of our hospital. Hyperthyroidism was diagnosed. Thyroid scintigraphy confirmed the presence of cold thyroid nodules and FNAC of the nodule of the left lobe (3 cm) was suggestive for papillary thyroid carcinoma (TIR 5 according to Italian Consensus).[2]

By August 2020, imaging showed lung, nodal, and pleural progression, with newly enlarged abdominal nodes. Overall, her general condition and dyspnea worsened (ECOG PS 1). Screening in the STARTRK-2 GO40782 basket study was offered to identify potentially actionable mutations by means of FoundationOne CDx panel.[3,4]

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The archived formalin-fixed, paraffin-embedded tissue sample of the pleural biopsy performed before the first-line chemotherapy was analyzed. Two remarkable findings were reported: an *NTRK1-tropomyosin 3* (*TPM3*) fusion and a C228T mutation of the telomerase reverse transcriptase (*TERT*) promoter.

NTRK1/2/3 (Neurotrophic Tyrosine Kinase Receptor 1/2/3) encode for the tropomyosin receptor kinases A/B/C (TRK A, B, C), which play an important role in the embryogenesis of the nervous system.[5] Aberrant fusions of *NTRK* genes result in the translation of aberrant chimeric TRK receptors that constitutively activate downstream signaling pathways.[5] Most notably, *NTRK* rearrangements are targets of the oral pan-TRK inhibitors larotrectinib and entrectinib, that showed relevant efficacy across *NTRK* fusion-positive solid tumors. Indeed, 2 pooled analyses of single arm phase I and II trials reported, in adults, objective response rates (ORR), median progression-free survival (mPFS), and overall survival (mOS) of 67% and 61%, 26 and 14 months, and not reached and 34 months, with larotrectinib and entrectinib, respectively.[6,7]

TERT promoter mutations induce cellular proliferation through the activation of the telomerase reverse transcriptase and are detected in several solid tumors, with a frequency of 2%-5% in NSCLC, and 11% and 30%-50% in papillary and poorly differentiated carcinomas, respectively.[8] The presence of a *TERT* promoter mutation usually confers a greater aggressiveness to thyroid cancers, especially when combined with other somatic mutation as happened in the present case.[9]

These molecular findings, together with the previous pathological report of pleural metastasis of lung adenocarcinoma with papillary features, prompted us to reconsider the initial diagnosis of advanced lung cancer, and the diagnosis was revised to papillary thyroid carcinoma with lung and pleural metastases. This was corroborated by the consideration that *NTRK* rearrangements are detected in around 9% of patients with papillary thyroid carcinoma, and that *TPM3*, the fusion partner gene found in our patient, is the second most frequent 5′ in thyroid cancers harboring *NTRK* rearrangements (18%).[5,10]

Patient Update

Based on the NGS molecular findings and on the FNAC results, a total thyroidectomy was planned in January 2021. Histology showed the presence of poorly differentiated thyroid carcinoma and 3 nodal metastases (pathological staging: pT3bN1a R2; immunophenotype: TTF-1+, PAX-8+; thyroglobulin+). Additional analyses on the archival pleural biopsy showed positivity for PAX-8 and thyroglobulin, confirming the histologic diagnosis of metastatic thyroid cancer (Fig. 1). TTF-1 immunostaining alone would have not been sufficient

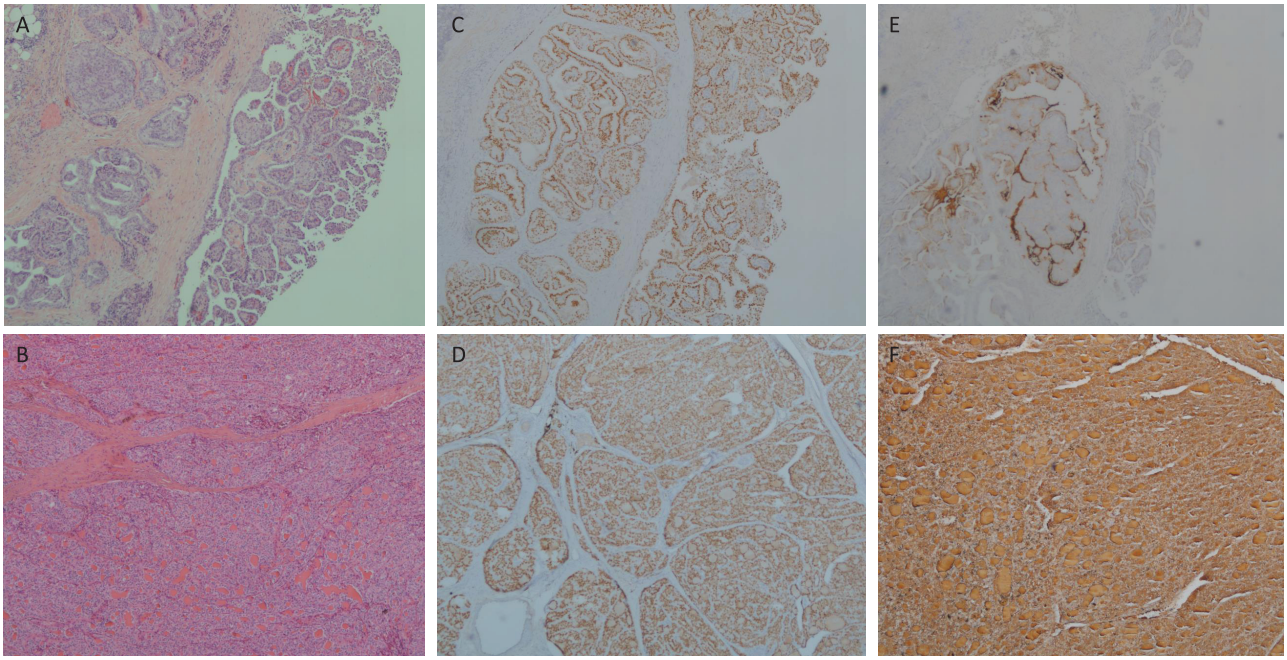

Figure 1. Representative images showing Haematoxylin-Eosin staining of pleural metastases (**A**) and primary papillary thyroid carcinoma (**B**); TTF-1 immunohistochemical staining of pleural metastases (**C**) and primary papillary thyroid carcinoma (**D**); Tireoglobuline immunohistochemical staining of pleural metastases (**E**) and primary papillary thyroid carcinoma (**F**). Magnification ×10. Abbreviations: TTF-1: transcriptional Thyroid Factor 1.

to differentiate between lung and thyroid origin, because TTF-1 is commonly expressed both in lung and thyroid carcinomas (Table 1).[11-15] Furthermore, the same genomic alterations were checked in the thyroid tumor tissue, and the same *NTRK* rearrangement and *TERT* promoter mutation were confirmed.

The next therapeutic choice considered several factors: first, the poorly differentiated histology could mitigate clinical benefit from radioiodine therapy[16]; second, the tyrosine kinase inhibitor (TKI) lenvatinib may have been as active as NTRK inhibitors (ORR: 65%; mPFS: 18 months), although more toxic (G3-4 adverse event rate with lenvatinib and NTRK inhibitors: 76% vs. 12%)[6,7,17]; third, targeting of *NTRK* could elicit earlier relief of the patient's symptoms. From these considerations, in February 2021, the patient was enrolled in the treatment phase of the STARTRK-2 GO40782 trial, and entrectinib was started at a dosage of 600 mg daily.[3] After only 11 days the dose was reduced to 400 mg daily due to drug-related G3 asthenia, G2 neurotoxicity, dysgeusia, and anorexia. Following dose reduction, good tolerance and full compliance with treatment were achieved. After only 2 months of treatment, total-body CT scan showed partial response with 70% dimensional reduction of target lesions according to RECIST. After 4 months, maximum shrinkage was achieved (–92%; Fig. 2). At the same time, the patient's clinical condition improved (ECOG-PS 0), and dysphonia and dyspnea regressed. After 2 years and 3 months, treatment with entrectinib at 400 mg daily is ongoing, with no evidence of radiological disease progression. G1 dysgeusia, neurotoxicity, and osteomuscular pain remain the only persistent side effects from entrectinib.

Discussion

Comprehensive genome profiling with a NGS approach yielded a striking impact in 2 points of this clinical case. First, it allowed identification of a key driver molecular alteration, *NTRK* rearrangement, that could be successfully targeted with entrectinib; second, it provided an additional molecular finding, *TERT* promoter mutation, which prompted us to revise the histopathological diagnosis and subsequent treatment considerations.

While *NTRK* rearrangements are described both in NSCLC and thyroid cancers, the translocation partner *TPM3* is not highly typical of thyroid cancer being found also in NSCLC, but their prevalence is higher in thyroid tumors (1%-6% vs. <1%),[10,18-20]. Additional arguments for revising the patient's diagnosis were the detection of a mutation in the *TERT* promoter, which is much more frequent in thyroid carcinoma compared to NSCLC, and the presence of thyroid nodules.

Notably, the *NTRK* rearrangement was found by DNA-NGS in our case. This is the preferred approach in tumors with relatively low frequency of *NTRK* fusions whenever a NGS platform is available, because it allows *NTKR* diagnostics to take advantage also of simultaneous comprehensive genome profiling.[21,22] However, since the detection of a *NTRK* fusion product at NGS does not imply its translation, confirmatory IHC may be advantageous to validate protein expression.[21,22] If NGS cannot be offered, or a comprehensive genomic profiling is not recommended for that specific histology, the reverse approach with IHC followed by confirmative NGS in IHC-positive cases is currently recommended by ESMO especially for tumors at low prevalence of NTRK rearrangements and without other known drivers.[21,22] Upfront fluorescence in situ hybridization (FISH) followed by confirmatory RNA-NGS should be offered in tumors with expected high frequency of *NTRK* fusions (ie, secretory carcinoma of the salivary gland and breast secretory carcinoma).[21,22]

In our case, it is also worthy of consideration that if *NTRK* rearrangement had been diagnosed with IHC, the C228T *TERT* promoter mutation would have remained undetected, and the clinical relevance of thyroid nodules would have been underestimated.[23] Even though it may be argued that the added value of NGS in detecting the cancer origin was likely mitigated in this case by the tissue-agnostic approval of *NTRK* inhibitors, resulting from their well-known efficacy across different malignancies,[6,7] the correct identification of the primary tumor of origin remains of paramount

Table 1. Immunohistochemical and molecular biomarkers of lung and thyroid carcinoma.

	NSCLC		Non-anaplastic thyroid carcinoma (%)
	Adenocarcinoma (%)	Squamous cell carcinoma (%)	
IHC biomarkers			
TTF-1	80-85	1	90%-100%
p63	33	94	70[a]
p40	16	94	≈0
CK7	95	≈0	97
CK5/6	2	90	≈0
Napsin-A	74	1	9-30[a]
Molecular biomarkers			
NTRK rearrangement	<1	≈0	2-6
TERT promoter mutation	3	<2	11[b]

[a]Papillary thyroid carcinoma, virtually absent in follicular and medullary carcinoma.
[b]Papillary thyroid carcinoma; 30%-50% in poorly differentiated histology.
Abbreviations: CK: cytokeratin; IHC: immunohistochemistry; NSCLC: non-small cell lung cancer; NTRK: Neurotrophic Tyrosine Kinase Receptor; TERT: Telomerase Reverse Transcriptase; TTF-1: Transcriptional Thyroid Factor 1.

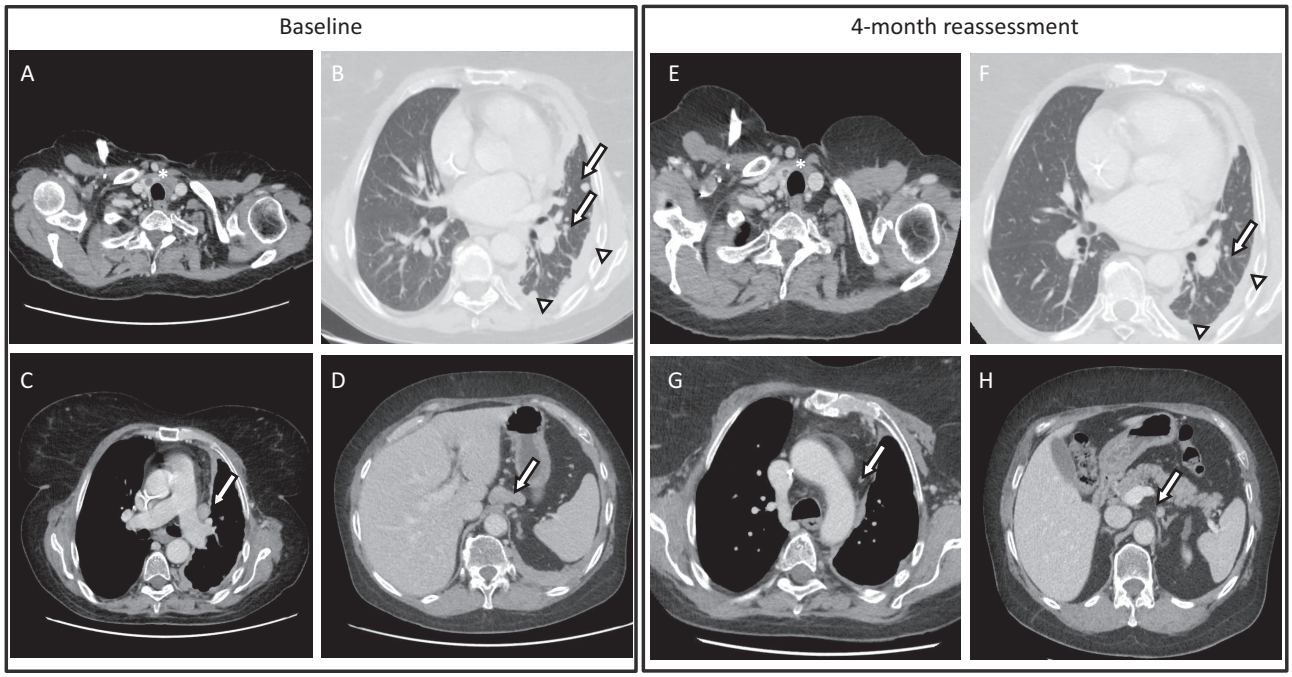

Figure 2. CT-scan with contrast performed before (**A**-**B**-**C**-**D**) and after 4 months of entrectinib (**E**-**F**-**G**-**H**). In (A) and (E), residual thyroid tissue is highlighted with an asterisk; in (B) and (F), arrows and arrowheads point to lung and subpleural nodules and thickenings, respectively; in (C) and (G), an arrow points to a mediastinal node; in (D) and (H), an arrow points to an abdominal lymph node cluster.

importance for prognostic assessment and possible subsequent therapies (ie, TKIs).[24]

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Conflict of Interest

Rossella Elisei reported consulting or advisory roles with EISAI, Sanofi-Genzyme, EXELIXIS, Lilly, LOXO, and IPSEN. Chiara Cremolini reported honoraria from Amgen, Bayer, Merck, Roche, and Servier; consulting or advisory roles with Amgen, Bayer, MSD, and Roche; speakers' bureau with Servier; research funding from Bayer, Merck, and Servier; and travel, accommodations, and expenses from Roche and Servier. The other authors indicated no financial relationships.

Author Contributions

Conception/Design: M.M.G., C.B., R.E., C.C. Provision of study material or patients: All authors. Collection and/or assembly of data: M.M.G., C.B., A.M., G.A., M.G., R.E., C.C. Data analysis and interpretation: M.M.G., C.B., A.M., G.A., R.E., G.F., C.C. Manuscript writing: M.M.G., C.B., A.M., R.E., C.C. Final approval of manuscript: All authors.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

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