Journal of Thoracic Oncology

BRAF V600E mutation is not always present as expected!

--Manuscript Draft--

Manuscript Number:	JTO-D-16-01017R1
Full Title:	BRAF V600E mutation is not always present as expected!
Article Type:	Case Report
Keywords:	collision tumor; BRAF V600E; lung adenocarcinoma; thyroid papillary carcinoma; immunohistochemistry; molecular biology; tumor genotyping
Manuscript Region of Origin:	FRANCE
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BRAF V600E mutation is not always present as expected! A case report of lung and thyroid carcinomas.

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The authors have no conflict of interest to disclose.

ABSTRACT

We report herein the case of a metastatic cervical lymph node comprising both a *BRAF* V600E lung adenocarcinoma and a *BRAF* wild-type thyroid papillary carcinoma in a 71-year-old patient. This case is of double interest because *BRAF* V600E mutation is very frequent in thyroid papillary carcinoma as opposed to lung adenocarcinoma, and because of the collision of the two distinct tumors in a lymph node. In addition, this case report illustrates the usefulness of specific immunostaining for BRAF V600E in the pathological management of

carcinomas. This case also illustrates the potential benefit of genotyping lung adenocarcinomas, even in a context of heavy smoking.

Key words: collision tumor; BRAF V600E; lung adenocarcinoma; thyroid papillary carcinoma; immunohistochemistry; molecular biology; tumor genotyping

INTRODUCTION

We report herein the case of a metastatic cervical lymph node comprising both a *BRAF* V600E lung adenocarcinoma and a *BRAF* wild-type thyroid papillary carcinoma in a 71-year-old patient. This patient dramatically benefited from an anti-BRAF V600E targeted therapy. To our knowledge, this observation is unique and illustrates the importance of tumor genotyping and the utility of immunohistochemistry to detect an abnormal protein reflecting a gene alteration such as *BRAF*.

CASE REPORT

This 71-year-old man was a former smoker (30 Pack-Years) and had been professionally exposed to asbestos. He had a medical history of sleep apnea, diabetes mellitus and hypertensive heart disease.

He presented with mediastinal enlarged lymph nodes of stations 4R, 4L, 5, 6 and 7 discovered on systematic CT-scan (Figure 1-a), along with a 18-mm thyroid lesion. No nodule was detected in the lungs but pleural plaques were observed.

Trans-bronchial fine needle aspiration of mediastinal nodes revealed clusters of tumor cells positive for TTF1 and negative for Thyroglobulin, favoring diagnosis of a lung adenocarcinoma. Molecular analysis (SNaPshot®, fragment analysis and pyrosequencing) of *EGFR*, *KRAS*, *BRAF* and *HER2* retrieved a c.1799T>A (p.Val600Glu) mutation of the *BRAF*

gene, the so-called *BRAF* V600E mutation. ALK immunohistochemistry (D5F3) was negative. Because no conspicuous lung lesion was visualized on scan, given the thyroid lesion and this *BRAF* V600E mutation, a total thyroidectomy was performed with bilateral mediastino-recurrential lymph node dissection (1R, 1L, 2R and 2L stations).

Microscopically, the 18-mm lesion was a vesicular adenoma but it was observed two territories of papillary thyroid carcinoma measuring respectively 3 mm in the right lobe and 2 mm in the left lobe. One node was infiltrated by both the thyroid papillary carcinoma and similar cells to those observed on trans-bronchial fine needle aspiration (Figure 2-a). In accordance with genotyping, BRAF V600E protein expression was detected by immunohistochemistry in the latter but not in the papillary carcinoma cells (Figure 2-b).

The patient was initially treated by CARBOPLATIN-TAXOL, with ill-defined progression of the tumor in the right lower lobe associated with carcinomatous pleural effusion (Figure 1-b). Given the *BRAF* V600E mutation, it was decided to switch to an anti-BRAF V600E treatment (Dabrafenib), leading to dramatic regression of the tumor (Figure 1-c). Given the worse prognosis of this metastatic lung adenocarcinoma compared to that of this rather localized thyroid carcinoma, no adjuvant treatment was given for the latter.

DISCUSSION

BRAF V600E mutation is retrieved in slightly less than 50% of papillary thyroid carcinoma (1) but in only 2% of lung adenocarcinoma (2). Two features of this observation are noteworthy: the co-existence of two different carcinomas, one of the thyroid gland and one of the lung, with a so-called collision tumor in a lymph node; the fact that, contrary to what might have been expected according to epidemiological data, the lung lesion harbored a *BRAF* V600E mutation while the thyroid carcinoma did not. Perhaps more importantly, this

case report illustrates the importance of genotyping lung adenocarcinoma, even when patients are current or former smokers.

ACKNOWLEDGMENTS

The authors are indebted to Nikki Sabourin-Gibbs (Rouen University Hospital) for assistance with language editing.

REFERENCES

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FIGURE LEGENDS

Figure 1: Computerized tomography scans. i: mediastinal window. ii: parenchymal window.

a- initially (day 0)

b-after treatment by CARBOPLATIN-TAXOL (day 170). Mediastinal lymph nodes have increased in size (i) and a ill-defined lesion has appeared in the right lower lobe with right pleural effusion (ii).

c-after second-line treatment by Dabrafenib (day 483). Mediastinal lymph nodes and the right lung lesion have dramatically regressed and only a mild right pleural effusion remains.

Figure 2: Photomicrographs of formalin-fixed paraffin-embedded tissue section of a cervical lymph node after staining by Hematoxylin Eosin and Safran (2-a) and immunostaining for BRAF V600E (2-b). This lymph node is infiltrated by both a papillary thyroid carcinoma (star), negative for BRAF V600E staining and a BRAF V600E positive adenocarcinoma (arrow). Bar scale 50 µm.

Figure 1

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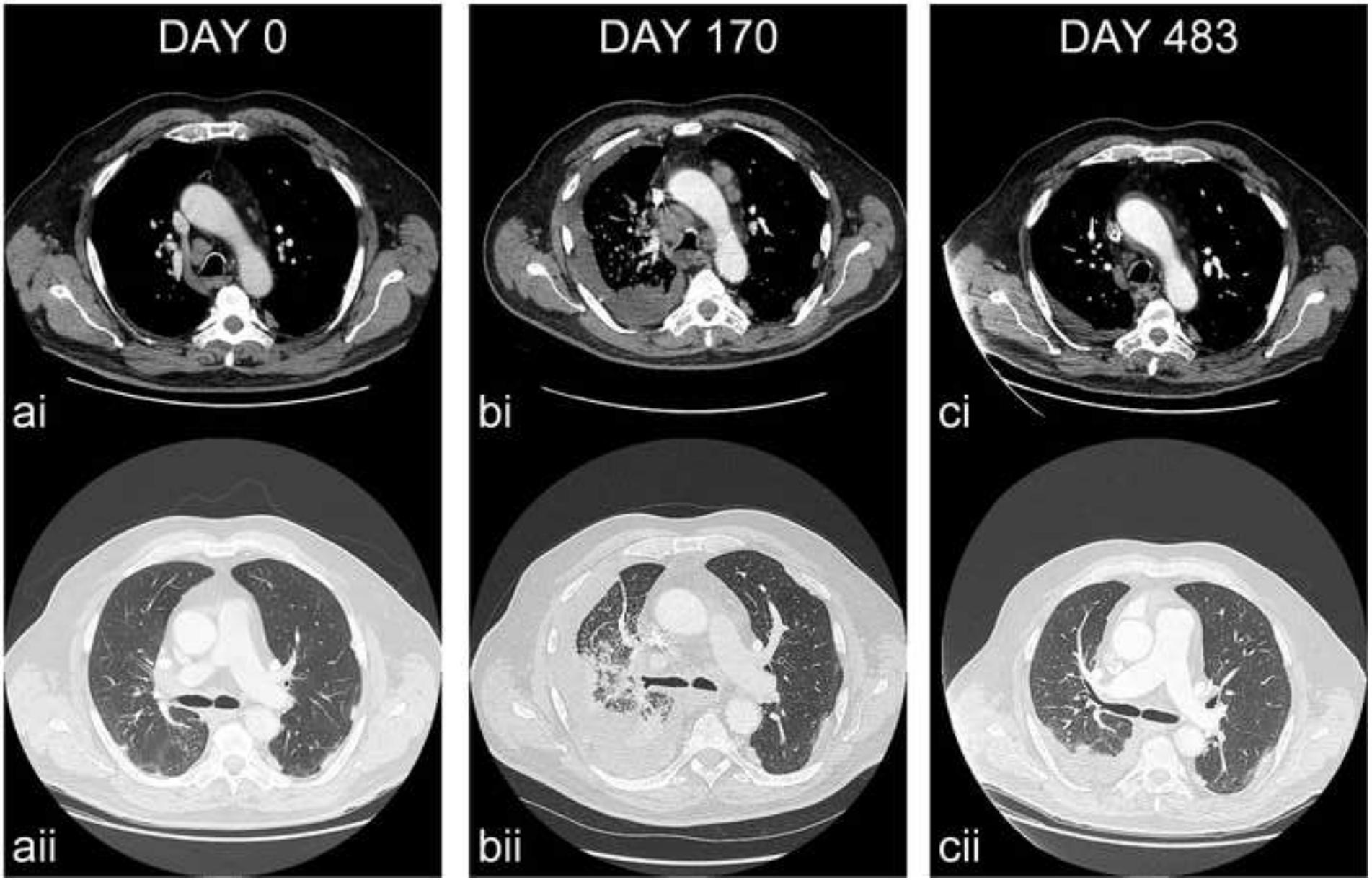
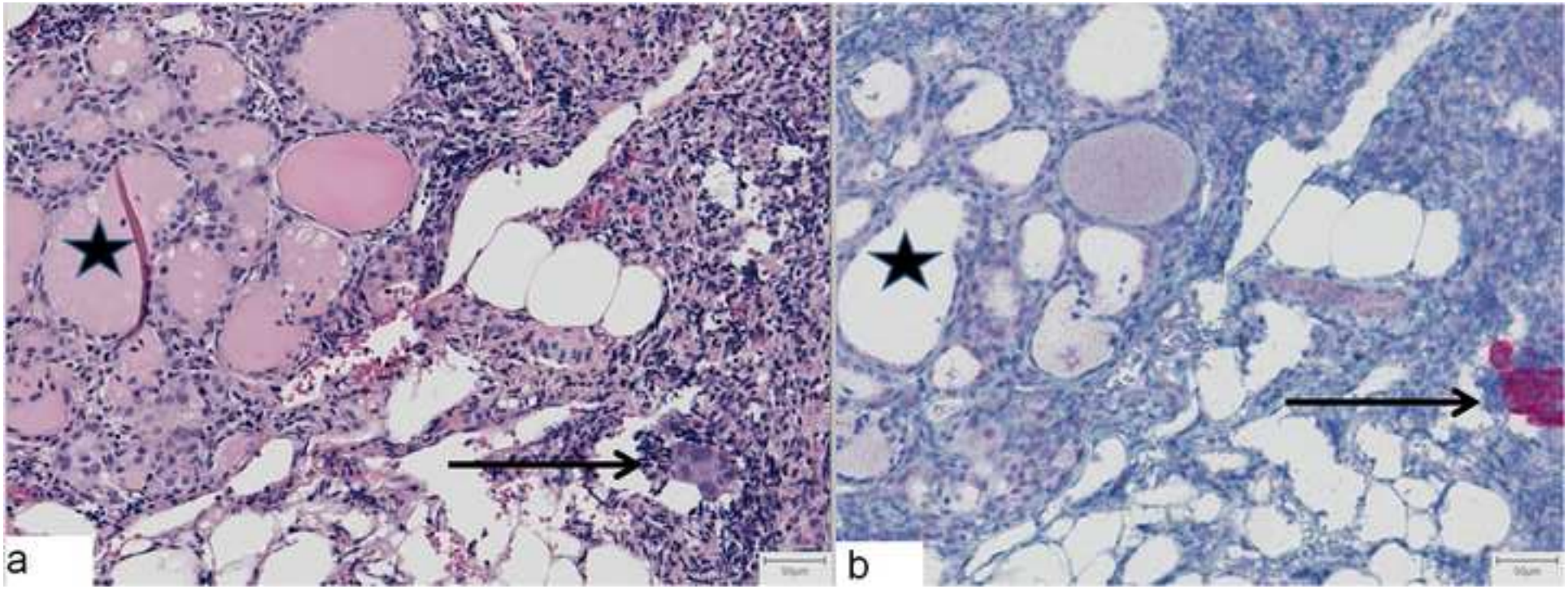


Figure 2

Click here to download Figure Figure 2-a 2-b.bmp



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~~performed and~~ retrieved a c.1799T>A (p.Val600Glu) mutation of the *BRAF* gene, the so-called *BRAF* V600E mutation. ALK immunohistochemistry (D5F3) was negative. Because no conspicuous lung lesion was visualized on scan, given the thyroid lesion and this *BRAF* V600E mutation, a total thyroidectomy was performed with bilateral mediastino-recurrential lymph node dissection (1R, 1L, 2R and 2L stations).

~~Macroscopically, the thyroid gland was modified by a nodular goiter.~~ Microscopically, the 18-mm lesion was a vesicular adenoma but it was observed ~~examination confirmed goiter and revealed a vesicular adenoma in the left lobe and~~ two territories of papillary thyroid carcinoma measuring respectively 3 mm in the right lobe and 2 mm in the left lobe. One node was infiltrated by both the thyroid papillary carcinoma and similar cells to those observed on trans-bronchial fine needle aspiration (Figure 2-a). In accordance with genotyping, BRAF V600E protein expression was detected by immunohistochemistry in the latter but not in the papillary carcinoma cells (Figure 2-b).

The patient was initially treated by CARBOPLATIN-TAXOL, with ill-defined progression of the tumor in the ~~left~~ right lower lobe associated with carcinomatous pleural effusion (Figure 1-b). Given the *BRAF* V600E mutation, it was decided to switch to an anti-BRAF V600E treatment (Dabrafenib), leading to ~~-~~dramatic regression of the tumor (Figure 1-c). Given the worse prognosis of this metastatic lung adenocarcinoma compared to that of this rather localized thyroid carcinoma, no adjuvant treatment was given for the latter.

DISCUSSION

~~This patient presented with a *BRAF* wild-type thyroid papillary carcinoma and a *BRAF* V600E lung adenocarcinoma, with collision of these lesions in a lymph node.~~

BRAF V600E mutation is retrieved in slightly less than 50% of papillary thyroid carcinoma (1) but in only 2% of lung adenocarcinoma (2). Two features of this observation are

noteworthy: the co-existence of two different carcinomas, one of the thyroid gland and one of the lung, with a so-called collision tumor in a lymph node; the fact that, contrary to what might have been expected according to epidemiological data, the lung lesion harbored a *BRAF* V600E mutation while the thyroid carcinoma did not. Perhaps more importantly, this case report illustrates the importance of genotyping lung adenocarcinoma, even when patients are current or former smokers.

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c-after second-line treatment by Dabrafenib (day 483). Mediastinal lymph nodes and the right lung lesion have dramatically regressed and only a mild right pleural effusion remains.~~Photomicrographs of a cytoblock after staining by Hematoxylin Eosin and Safran (1-a) and immunostaining for TTF1 (1-b), showing clusters of basophilic tumor cells with a quite abundant cytoplasm and an irregular nucleus with conspicuous nucleolus (1-a). These cells display nuclear staining for TTF1 (1-b). Bar scale 50 µm.~~

Figure 2: Photomicrographs of formalin-fixed paraffin-embedded tissue section of a cervical lymph node after staining by Hematoxylin Eosin and Safran (2-a) and immunostaining for BRAF V600E (2-b). This lymph node is infiltrated by both a papillary thyroid carcinoma (star), negative for BRAF V600E staining and a BRAF V600E positive adenocarcinoma (arrow). Bar scale 50 µm.

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To the Associate Editor of *the Journal of Thoracic Oncology*

Ref: JTO-D-16-01017

October 10th 2016

Dear Professor Novello,

Further to your proposal, we are pleased to submit our revised manuscript entitled:
" ***BRAF* V600E mutation is not always present as expected! A case report of lung and thyroid carcinomas**" for consideration for publication in your journal.

Following comments made by the reviewers, we included more information in the manuscript and added CT-scan images.

Please find a point-by-point response to the comments of the two reviewers on the next page.

We trust you will find both the changes in this revised manuscript and the answers to the reviewers to be convincing and sincerely hope that our work is now suitable for publication in *The Journal of Thoracic Oncology*.

Let us know should you require any further information.

Yours sincerely,

Doctor Nicolas Piton

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Responses to reviewer comments:

Reviewer #1: *Comments to the Authors,*

This manuscript reported the co-existence of two different carcinomas with a so-called collision tu-mor in a lymph node. What's more, the BRAF V600E mutation status of the two carcinomas was con-trary to epidemiological date. This case report would give great help for the study of BRAF V600E mutation in lung adenocarcinoma. However, I have several concerns to make the manuscript more solid.

1, In the part of CASE REPORT, the author state that mediastinal enlarged lymph nodes were pre-sented in the 71-year-old male patient and then trans-bronchial fine needle aspiration of mediastinal nodes and bilateral mediasino-recurrential lymph node dissection was performed. It is preferable to describe more details about the lymph nodes and surgery, such as the station of each enlarged lymph node, the extent of lymph node dissection in the surgery.

As requested, we indicated the stations of the enlarged lymph nodes (line 44) and the lymph node dissection during surgery (line 54).

2.Although the author has mentioned the chemotherapy and anti-BRAF V600E treatment after the surgery, it is important to provide more details and objective data to describe the progression and re-gression of the tumor, especially about the progression of left lower lobe.

Thanks for your comment and please apologise our mistake regarding the side of this lung lesion (right lung). We included CT-scans illustrations (Figure 1) and described more precisely the progression of the tumor in the right lower lobe (lines 61 and 93 to 95).

3, In the current manuscript, thyroid papillary carcinoma and lymph node metastasis of it was men-tioned, so it is recommended to state the treatment and prognosis of the thyroid carcinoma too.

Thanks for your remark, we modified the manuscript to state which therapeutic attitude was decided regarding thyroid carcinoma (lines 64 to 66).

In summary, I hope the authors could give more details and more explicit description to this special case.

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Reviewer #2: *This case (71-year-old man) is interesting for us. He had a metastatic cervical lymph node comprising both a BRAF V600E lung adenocarcinoma and a BRAF wild-type thyroid papillary carcinoma, but on the diagnosis, no nodule could be detected in the lungs.*
Even though he was initially treated by CARBOPLATIN-TAXOL, he had the tumor progression in the left lower lobe associated with carcinomatous pleural effusion.
Therefore, this tumor developing later in the left lower lobe is considered to be a primary lesion of the lung. The authors should show us the information of the primary lesion in detail (Let us show any CT images and so on).

As requested, we detailed the description of the primary lesion as much as possible regarding the word limit of 500 (lines 61 and 93 to 95) and included CT images (Figure 1) to illustrate the disease at diagnosis (Day 0), the disease after conventional chemotherapy (day 170) and after targeted therapy (day 483).