Non-small-cell lung cancer infiltrated with chronic myelomonocytic leukaemia

Koopman, Bart; Hiddinga, Birgitta I.; Platteel, Inge; Kluiver, Joost L.; Timens, Wim; Mulder, André B.; van Doesum, Jaap A.; Schuuring, Ed; Diepstra, Arjan; Kempen, Léon C. van

Published in:
Histopathology

DOI:
10.1111/his.14326

Publication date:
2021

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Histopathology 2021 DOI: 10.1111/his.14326

Lesson Of The Month

Non-small-cell lung cancer infiltrated with chronic myelomonocytic leukaemia: a molecular diagnostic challenge to recognise mixed cancers in a single biopsy

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Introduction

Molecular profiling techniques such as targeted next-generation sequencing (NGS) have become increasingly important in routine cancer diagnostics. Genomic alterations that are characteristic in certain malignancies are sometimes also detected in other cancers. Detection of rare variants may challenge the initial diagnosis or uncover a co-existing malignancy.[1,2] We report on a non-small-cell lung cancer (NSCLC) case with an oncogenic mutation in *PIK3CA* and unusual mutations in both *MET* and *IDH2*, the last of which was shown to originate from tumour-infiltrating chronic myelomonocytic leukaemia (CMML).

Case presentation

An 80-year-old male presented with a mass in the right upper lobe with accompanying brain and bone lesions, suspicious of a stage IV (cT4N0M1c) primary tumour of the lung. A histological needle biopsy of the pulmonary mass showed a solid and trabecular growing (non-small-cell) carcinoma in a minority of Alcian blue-positive intracytoplasmic vacuoles. Immunohistochemistry showed a diffuse strong nuclear staining for thyroid transcription factor 1 (TTF1), diffuse strong cytoplasmic staining for napsin-A and lack of staining for p40, leading to a conclusion of poorly differentiated adenocarcinoma. NGS revealed the presence of an oncogenic mutation in *PIK3CA* (NM_006218): c.3140A>G p.(His1047Arg) with 34% variant allele frequency (VAF). Additionally, two unexpected mutations were detected: an unknown frameshift mutation in *MET* exon 14 (NM_000245): c.2913_2914delinsT p.(Asp972Metfs*13) with 48% VAF and a mutation in *IDH2* (NM_002168): c.419G>A p.(Arg140Gln) with an almost fourfold lower VAF (13%). NanoString-targeted transcript analysis demonstrated *MET* exon 14 skipping transcripts. This mutational profile was intriguing, because of the unknown mutation in *MET* resulting in exon 14 skipping and the *IDH2* mutation which is only rarely found in NSCLC and almost exclusively limited to haematological malignancies.[3] The patient provided written informed consent for publication.

Review of the patient's health record revealed a history of CMML with an *IDH2* p.(Arg140Gln) mutation. The CMML had been regularly followed-up every 8–12 weeks prior to the diagnosis of NSCLC. Complete blood counts and white blood cell differentials had repeatedly demonstrated stable disease without indication of transformation to acute myeloid leukaemia. Flow cytometry-based analysis on peripheral blood performed after the lung cancer diagnosis demonstrated 1% myeloblasts and 8% CD64 strong-positive/CD300e-negative monoblasts/promonocytes. Considering the patient's age and lack of anaemia, thrombocytopaenia or transformation to acute myeloid leukaemia, there was no indication for treatment. Re-analysis by NGS of the bone marrow tissue previously used to diagnose CMML confirmed the presence of *IDH2* p.(Arg140Gln) and demonstrated wild-type *PIK3CA* and *MET*. Together, this suggested that *IDH2* mutation-positive leukaemic cells were present in the lung adenocarcinoma biopsy.

Re-analysis of the histology did not clearly identify the presence of leukaemia; however, leukaemic cells in CMML can be virtually indistinguishable from normal monocytes by histology alone. Microscopic analysis showed a modest presence of inflammatory-like cells, with an interstitial distribution pattern in the stroma and in association with the tumour cells, closely resembling reactive inflammation as often seen in the context of NSCLC. Immunohistochemical staining revealed that these cells were consistently CD163-positive, most probably reflecting CMML cells (Figure 1). There were no sheets of CMML cells and there were no blastic plasmacytoid dendritic cells or plasmacytoid dendritic cell aggregates – which can sometimes be present in peripheral tissues involved with CMML – recognisable by histology and flow cytometry.

To demonstrate the presence of the *IDH2* mutation in the inflammatory-like component, macrodissection and mutation-specific digital droplet polymerase chain reaction (ddPCR; BioRad, Lunteren, the Netherlands) analysis of adenocarcinoma and adenocarcinoma-free stromal tissue was performed. Both the *PIK3CA* and *IDH2* mutations were detected in the dissected area containing both the TTF1-positive adenocarcinoma and CD163-positive inflammatory-like cells with allelic frequency of 44% and 8%, respectively. The *MET*

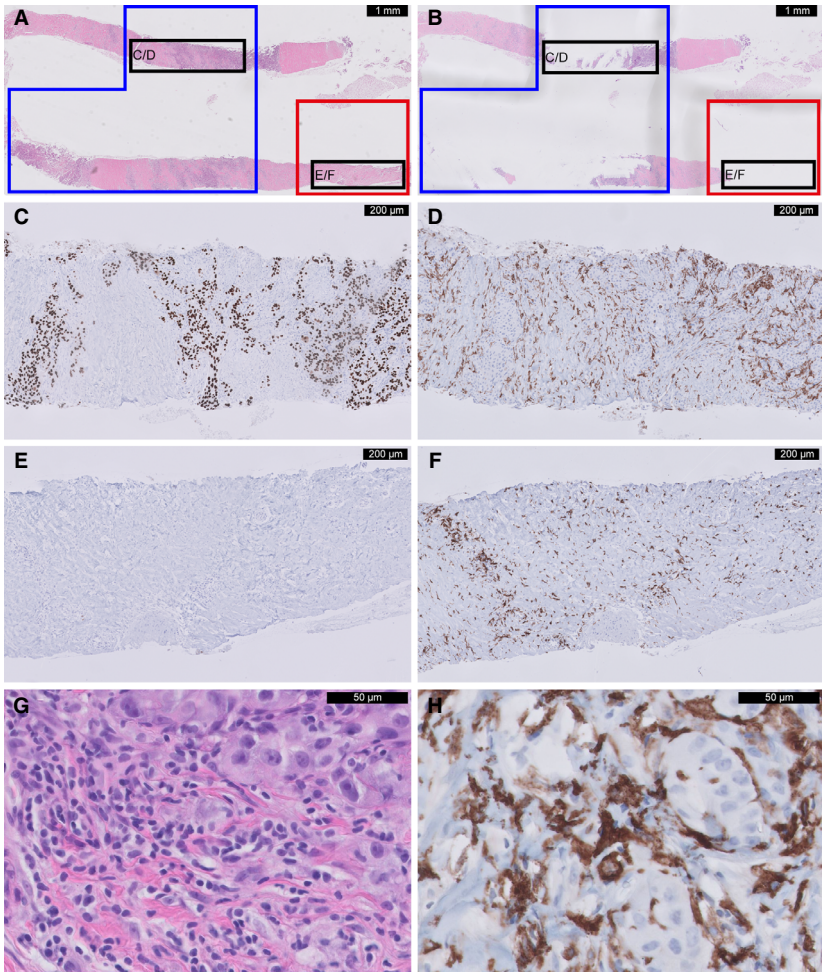

Figure 1. Histological images demonstrating the concurring presence of myelomonocytic leukaemia cells and adenocarcinoma cells. Histological images of the lung biopsy obtained by endobronchial ultrasound-guided fine needle aspiration, with corresponding TTF1 and CD163 immunohistochemistry. **A**,**B**, H&E stains of predissection (**A**) and post-dissection (**B**) tissue slides, with adenocarcinoma-containing, TTF1-positive and chronic myelomonocytic leukaemia (CMML)-containing, CD163-positive areas marked in blue and tumour-free, TTF1-negative and CD163-positive areas marked in red. Part of the etching in the glass slide to mark the area for dissection is visible in **B**. **C**,**D**, Adenocarcinoma-containing, TTF1-positive (**C**) and CMML-containing, CD163-positive (**D**) area, in which both *PIK3CA* p.(His1047Arg) and *IDH2* p.(Arg140Gln) were detected with mutation-specific ddPCR. **E**,**F**, Tumour-free, TTF1-negative (**E**) and CD163-positive (**F**) area, testing positive for *IDH2* p.(R140Q) but negative for *PIK3CA* p.(H1047R) with mutation-specific ddPCR. **G**,**H**, Area containing both adenocarcinoma (large cells, upper right) and suspected CMML cells (small cells, lower left), stained with H&E (**G**) and CD163 (**H**). CD163, cluster of differentiation 163; ddPCR, digital-droplet polymerase chain reaction; H&E, haematoxylin and eosin; IDH2, isocitrate dehydrogenase [NADP(+)] 2; PIK3CA, phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alpha; TTF1, thyroid transcription factor 1.

mutation was not analysed due to the challenging design of the ddPCR probe and primers. In contrast, only the *IDH2* p.(Arg140Gln) mutation with an allelic frequency of 39% was detected in the tumour-free component. These observations indicate that only the CD163-positive inflammatory-like cells carried the *IDH2* mutation.

The case was discussed by the Molecular Tumor Board (MTB) at the University Medical Center Groningen.[4] There was no indication to treat the CMML due to lack of clinical manifestations such as anaemia or thrombopenia, nor suspicion of transformation to acute myeloid leukaemia. Targeted MET inhibition was recommended for treating the NSCLC based on the detected *MET* exon 14 skipping. The MTB acknowledged the unknown effect of the *PIK3CA* mutation on MET inhibition but did not recommend dual inhibition, as the treatment efficacy of PI3K inhibitors in NSCLC is unknown. Crizotinib in compassionate use (250 mg twice daily) was initiated

and resulted in a 39% radiological volume reduction (7.6–4.6 cm) of the primary tumour within 12 weeks (Figure 2). Despite clinical and radiological improvement, the patient developed an occlusion of the superior mesenteric artery with intestinal ischaemia and succumbed to subsequent abdominal sepsis. The patient's family did not consent to post-mortem examination and the cause of the obstruction therefore remained unknown.

Discussion

The two unexpected mutations found in this patient's biopsy each highlight unique diagnostic challenges derived from genomic profiling. The *IDH2* p.(Arg140Gln) mutation most probably originated from tumour-infiltrating CMML. Although uncovering a second primary malignancy based on genomic profiling is not uncommon,[1,2] detection of two different types of cancer in a single biopsy is rare. Here the histological and molecular work-up of the case identified CMML-infiltrating lung adenocarcinoma.

In addition, we identified a novel uncharacterised *MET* exon 14 frameshift mutation p.(Asp972Metfs*13) which was shown to induce *MET* exon 14 skipping. Mutations in *MET* that involve the splice site acceptor or donor site of exon 14 result in the exclusion of exon 14 in the *MET* transcript with subsequent loss of the ability to down-regulate the signalling activity.[5] Evidence from CRISPR-Cas9-altered cell lines indicates that frameshift mutations can also induce splicing events that result in exclusion of the affected exon from the transcript.[6] Although the exact mechanism remains elusive, expression of a *MET* exon 14 skipping RNA transcript was verified. The observed 39% volume reduction of the primary tumour after 12 weeks of targeted MET inhibition treatment demonstrated that this novel *MET* frameshift variant is indeed actionable.

This case illustrates that seemingly unexpected oncogenic mutations can be derived from a tumour-

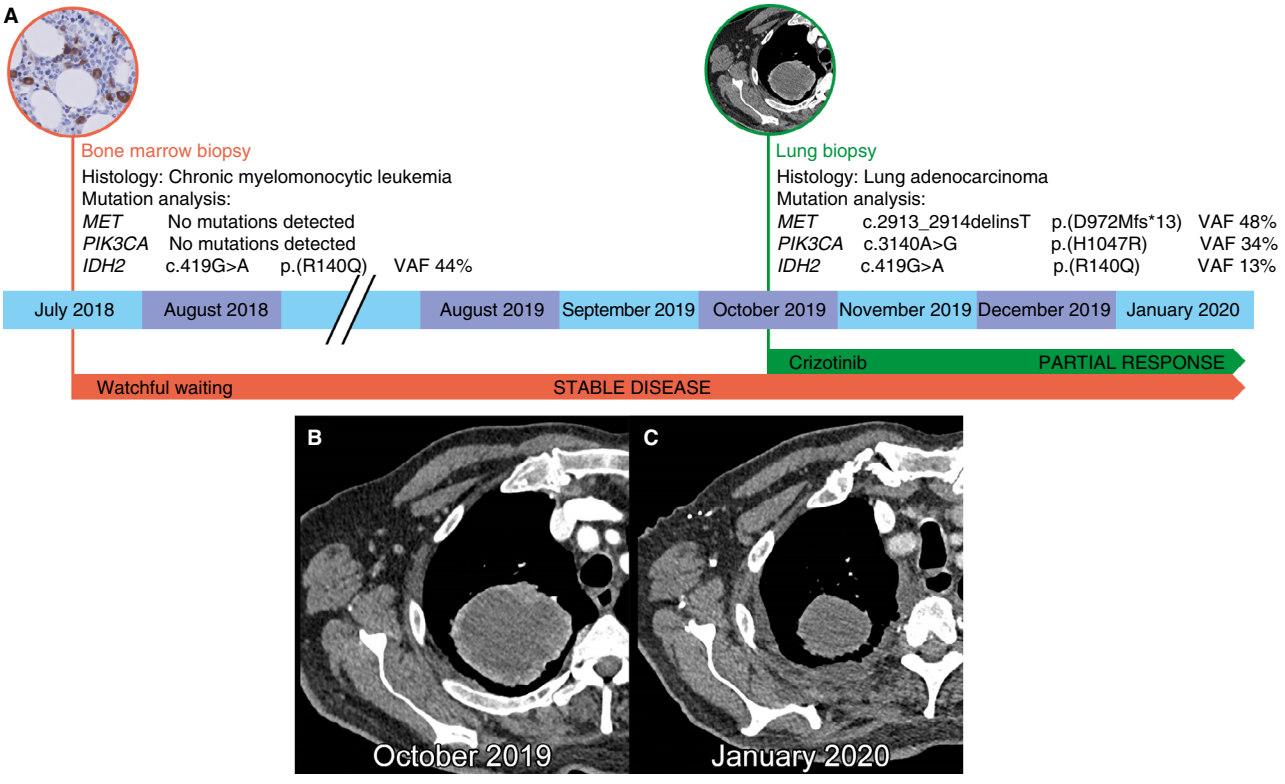

Figure 2. Patient clinical history and radiological imaging. **A**, Time-line of the patient's clinical history, with respective NGS results marked for separate bone marrow and lung biopsies. **B**, Computed tomography imaging of primary lesion (maximum diameter 7.6 cm) in the right upper lobe of the lung prior to initiating treatment with crizotinib. **C**, Computed tomography imaging after 3 months of treatment with crizotinib showing a partial response, with a volume reduction of 39% (maximum diameter 4.6 cm). IDH2, isocitrate dehydrogenase [NADP(+)] 2; MET, MET proto-oncogene, receptor tyrosine kinase; NGS, Next-generation sequencing; PIK3CA, Phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alpha.

infiltrating second malignancy that may be unrecognisable by histomorphology alone. This patient's NSCLC biopsy harboured an unexpected *IDH2* mutation which was shown to originate from tumour-infiltrating chronic myelomonocytic leukaemia. We conclude that the presence of a second malignancy should be considered when an unexpected genetic variant is detected in the molecular analysis of solid malignancies.

Acknowledgements

This work was supported by ZonMW (the Netherlands Organisation for Health Research) within the Personalised Medicine Program (PATH project; grant number 846001001). We thank the members of the University Medical Center Groningen Molecular Tumor Board for their contribution to this report.

Conflict of interest

The authors declare no conflicts of interest related to this study.

Bart Koopman[1]
Birgitta I Hiddinga[2]
Inge Platteel[1]
Joost L Kluiver[1]
Wim Timens[1]
André B Mulder[3]
Jaap A van Doesum[4]
Ed Schuuring[1]
Arjan Diepstra[1]
Léon C van Kempen[1]

[1]*Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen,* [2]*Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen,* [3]*Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, and* [4]*Department of Hematology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*

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