Detection of multiple activating NRAS variants under BRAF/MEK-inhibitor therapy in BRAF positive malignant melanoma using liquid biopsy

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INTRODUCTION

Malignant melanoma is one of the most common skin cancers worldwide.[1] The most frequent driver mutation is BRAF V600E, observed in about 50% of patients and a target for kinase inhibitor therapy.[2] In addition, activating mutations in NRAS are found in up to 20% of melanomas,[2] showing a more aggressive presentation and a poorer prognosis.[3] Monitoring of therapy response by radiography or clinical parameters is poorly correlated with patient survival.[4] In recent years, liquid biopsy (LB) is increasingly used to monitor therapy responses in patients with cancer.[5] LB is a minimal invasive technique based on release of fragments of genomic DNA (circulating tumor DNA [ctDNA]) from dying tumor cells in body fluids as blood and detection and quantification of ctDNA by next-generation sequencing or other technique. It has been shown to be a reliable tool for disease monitoring and minimal residual disease detection. It has also become a prognostic factor for progression free and overall survival.[6] In addition, it allows a better capture of tumor heterogeneity due to subclonal events compared with genomic DNA from biopsies,[4] which is especially important when therapy resistance emerges. However, most previous LB studies of melanomas focused on BRAF detection[7] and little attention has been directed to the development of new variants and their impact on disease.

Abbreviations used:

ctDNA:	circulating tumor DNA
LB:	liquid biopsy
VAF:	variant allele frequency

Here we illustrate a patient's course with a BRAF positive melanoma who developed 4 different activating NRAS variants under targeted therapy.

CASE PRESENTATION

A 28-year-old man patient presented with melanoma stage pT3b on the lower abdomen and a positive sentinel lymph node (clinical stage IIIC). After complete resection, the tissue was analyzed using targeted next-generation sequencing and a BRAF exon 15 hot spot mutation (V600E) was detected (variant allele frequency [VAF] 6%). Due to advanced tumor stage, he was treated with adjuvant pembrolizumab therapy. Three months later, computed tomography scan revealed a focus in the lung, indicating metastatic progress (Fig 1, *A*). Therapy was switched to nivolumab/ipilimumab and therapy monitoring with LB, which was negative at this stage, was initiated (Fig 1, *C*; LB1, VAF 0%). However, the patient experienced severe immunotherapy-associated side effects, necessitating termination of therapy. After a brief period of clinical stability and negative LBs (Fig 1, *C*; LB1 until LB2), in LB3 the known BRAF mutation (VAF 13%) was

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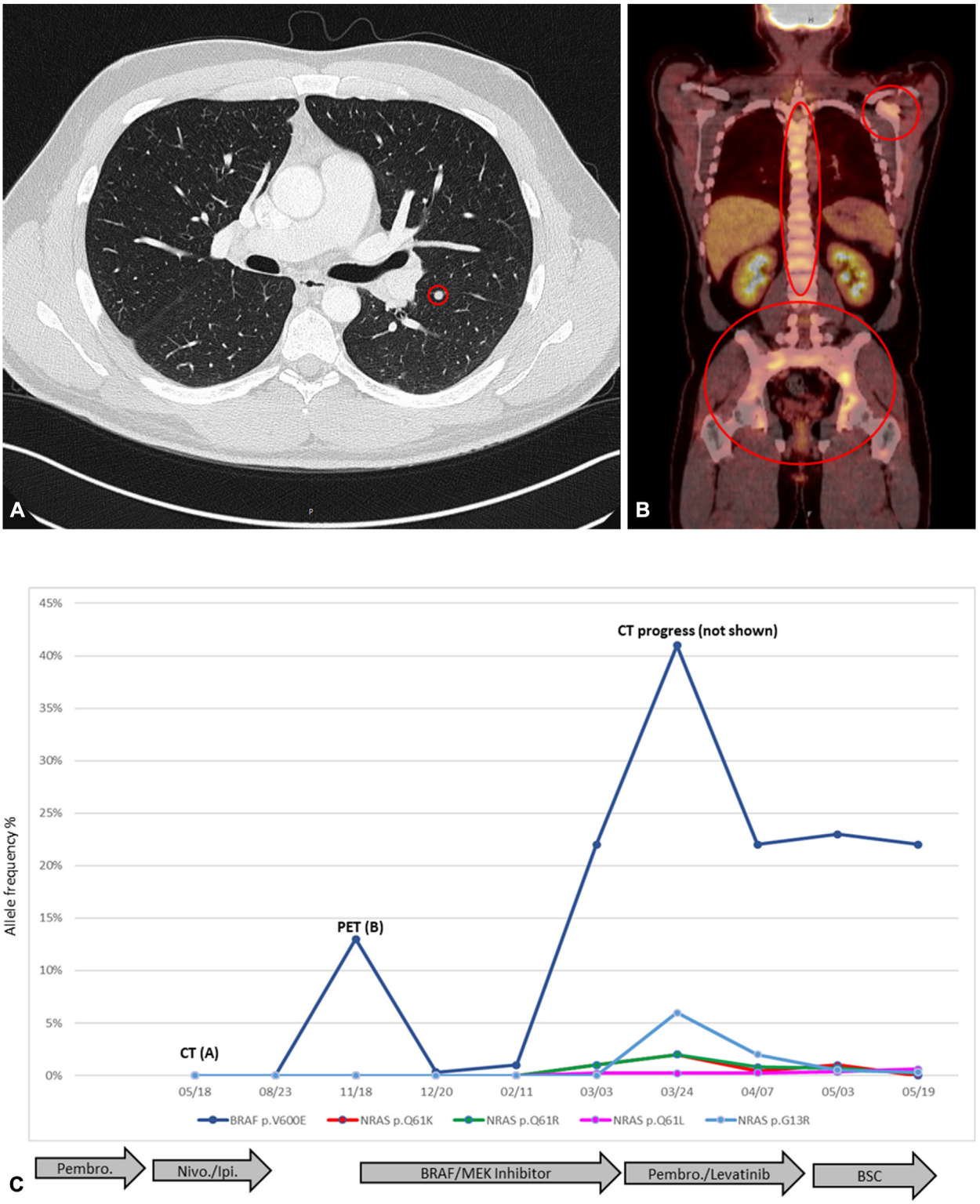

Fig 1. A, CT scan with pulmonary focus (*red circle*), indicating progressive disease under immunotherapy with pembrolizumab. **B,** PET-CT scan with multiple osseous metastases (*red circles*) indicative of progressive disease under immunotherapy with nivolumab and ipilimumab, after exhausting all treatment options the patient was treated according to the BSC concept. **C,** Molecular monitoring using targeted next-generation sequencing with circulating tumor DNA. Variant allele frequencies of BRAF V600E and 4 different activating NRAS mutations are given on the y-axis, the dates of blood draws are shown on the x-axis in chronological order. *BSC*, Best supportive care; *CT*, computed tomography; *PET*, positron emission tomography.

detected and accompanied by positron emission tomography-computed tomography showing massive bone progression (Fig 1, *B*). At that time point BRAF/MEK directed therapy (encorafenib + binimetinib) was initiated. With LB a molecular response was observed with BRAF VAF falling to 0.3%. Imaging could not verify a response to therapy, but the patient was nevertheless considered to have stable disease with no further progression. Subsequent LBs demonstrated molecular progression of the disease, with BRAF VAFs increasing up to 41% and detection of 4 different activating NRAS variants with progressively increasing VAFs (VAFs up to 2%, 2%, 0.2%, and 6%, for details see Fig 1). Based on imaging and clinical symptoms the progress was detected 5 weeks later than by LB. Therapy was then switched to pembrolizumab with lenvatinib. Again, the patient developed immune-mediated pneumonitis. After exhausting all available treatment options, the patient was finally treated according to best supportive care concept. The patient died with further pulmonary progression 1 year and 9 months after the initial diagnosis.

METHODS

Genomic DNA was extracted from formalin-fixed, paraffin-embedded biopsy and ctDNA from blood samples in STRECK tubes. Anchored multiplex polymerase chain reaction amplicons were generated using Archer LiquidPlex Assay kit (Invitae). Sequencing was performed on a MiSeq sequencer (Illumina) with average molecular coverage >500×. For variant identification Archer analysis software was used. Somatic variants altering protein structure with at least 5 reads and a VAF >0.55% were reported.

The clinical and radiologic data were extracted from the digital patient file (Meona).

DISCUSSION

The presented case of the course of a BRAF positive melanoma under initial BRAF/MEK-inhibitor response with the development of 4 subclonal activating NRAS variants leading to fulminant progression and death illustrates several aspects of LB analysis for therapy monitoring.

First, although concurrent BRAF/NRAS variants have already been described,[8] in this case the particularly unusual development of 4 different activating NRAS variants under BRAF/MEK therapy likely representing 4 different subclones was observed. Detection of these 4 subclones in conventional biopsies is rather unlikely and demonstrates the sampling advantage of LBs.

Second, after an initial response, the detection and increase in VAFs of the 4 subclones with different NRAS variants (up to 8% in sum) was accompanied by a much stronger increase in BRAF mutated ctDNA (up to 41%). VAFs usually reflects tumor burden[9] and would therefore here indicate a stronger expansion of tumor clone that carries only the BRAF mutation compared with the NRAS-carrying subclones. One possible explanation would be that BRAF/MEK therapy induces genetic changes that were not captured with our panel (eg, amplifications), which then lead to no further response to therapy resulting in tumor progression. The NRAS-carrying subclones may also represent a survival advantage, even for tumor cells not carrying this mutation, by alternating cell-cell signaling. Our data do not allow us to analyze this more precisely and further investigations are needed. Third, compared with LB analysis of other solids entities reported in the literature the VAFs are exceptional high (up to 41% VAF). As already described, this confirms the usefulness and simplified accessibility of using LB in patients with melanoma for disease monitoring and treatment evaluation.[7]

Fourth, this case also demonstrates the great utility of LB regarding early relapse or progress detection with a lead time of 5 weeks for molecular relapse detection compared with imaging and clinical parameters. Early detection of tumor recurrence can be crucial for adapting treatment strategies and patient's outcome. As we and others showed, LB could be used as a supplemental tool between clinical symptoms and imaging techniques in patients with melanoma.

CONCLUSION

LB is a useful tool to evaluate therapy response, especially in melanoma, where high VAFs in ctDNA are frequent. The usage of targeted next-generation sequencing with panels as compared digital polymerase chain reaction approaches detecting only the initial driver mutation is recommendable, as detection of resistance variants under targeted therapy may be a more reliable indicator of a molecular progress than the targeted driver mutation. To better understand the function and role of acquired NRAS mutations, a larger cohort with more comprehensive genetic analysis is needed.

Conflicts of interest

None disclosed.

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