Case Report

Response to BRAF/MEK Inhibition in A598_T599insV BRAF Mutated Melanoma

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Keywords

BRAF A598_T599insV · Targeted therapy · Melanoma · Circulating cell-free tumor DNA

Abstract

Approximately 50% of patients with metastatic melanoma harbor an activating BRAF mutation. Tumors with activating mutation *BRAF* gene proliferate excessively and can be treated with targeted BRAF-inhibitors in combination with MEK inhibitors. The most common BRAF mutation occurs at amino acid position 600. Other *BRAF* mutations are rare and their predictive value for treatment response to BRAF/MEK inhibition is low. Here, we report on a patient with a *BRAF* A598_T599insV mutated melanoma, a mutation that has only been described in one previous melanoma patient in which the treatment response to BRAF/MEK inhibition was transient. Our patient had a large ulcerated metastasis that showed a durable complete response

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implying that BRAF/MEK inhibition should be considered a treatment option for this mutation. We analyzed circulating cell-free tumor DNA (ctDNA) carrying the BRAF A598_T599insV mutation throughout treatment. The allele frequency of *BRAF* A598_T599insV decreased during regression of the tumors, indicating that this method has potential to monitor treatment response. Our case demonstrates durable response to BRAF/MEK inhibition in a melanoma patient carrying a *BRAF* A598_T599insV mutation. In addition, we show that allele frequency analysis of A598_T599insV mutation in blood using ultrasensitive sequencing can be used to monitor treatment response.

Introduction

Activating mutations in the *BRAF* gene occur in approximately 50% of patients with metastatic melanoma [1]. *BRAF* activation increases downstream signaling through the mitogen-activated protein kinase proliferation pathway. As a consequence, melanomas with activated *BRAF* proliferate excessively. Activating *BRAF* mutations usually occur at amino acid position 600 where the most common mutations are substitution of lysine to either glutamic acid (V600E) or valine (V600K). Targeted therapy with BRAF/MEK inhibitors improves survival in patients with V600E/K mutations. The response is fast and symptoms often improve within days or weeks. However, resistance to BRAF/MEK inhibition usually develops within 6–12 months.

BRAF mutations in amino acid positions other than 600 may also cause *BRAF* activation [2] but their predictive value for treatment response to BRAF/MEK inhibition is in most cases unknown. To increase treatment options in melanoma, response rates in patients with unusual *BRAF* mutations need to be evaluated and reported.

The unusual *BRAF* A598_T599insV mutation has only been described in two prior cases, one thyroid cancer and one metastatic melanoma. The melanoma patient was treated with BRAF/MEK inhibition and showed a brief response before progression [3]. In this paper, we used BRAF/MEK inhibition to treat a patient with *BRAF* A598_T599ins mutated melanoma. In contrast to the previous report, our patient experienced long lasting response to BRAF/MEK inhibition followed by immunotherapy. Repeated analysis of ctDNA was evaluated as a tool for early detection of tumor response.

Case Report

In February 2017, a 74-year old woman was referred to the melanoma unit at the Department of oncology, Sahlgrenska University Hospital, Sweden. This is a case of long patient´s delay where the patient 10 years earlier noticed a tumor in her left ankle, most likely the primary melanoma, and shortly after, a lump started growing in her left groin. She did not seek medical help and healthcare personnel first noticed the tumors in January 2017, when she was admitted to hospital due to a bleeding gastric ulcer.

At the first visit to our clinic, the advanced primary tumor was 3 × 4 × 0.5 cm and the groin metastasis was 9 × 11 × 8 cm and ulcerated. Computed Tomography (CT) scan revealed pathological pelvic and retroperitoneal lymph nodes, indicating stage IV M1a disease. During the last months, she had lost weight and experienced swelling of her left leg. Additional negative prognostic signs included poor performance status (ECOG 3), elevated lactate dehydrogenase (LDH) and low serum albumin. Mutation analysis of baseline biopsies from both the primary

tumor and then groin metastasis showed an unusual exon 15 *BRAF* mutation, A598_T599insV. After discussion at a multidisciplinary conference, it was decided to start BRAF/MEK inhibition (dabrafenib and trametinib).

Targeted Therapy: February–June 2017

The patient started BRAF/MEK inhibition, dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) and responded immediately. After 5 months of treatment, only a small fraction of the groin metastasis remained (Fig. 1) and the abdominal lymph nodes also regressed. However, the advanced primary tumor only showed marginal response. We evaluated ctDNA analyzed by SiMSen-Seq as a tool to monitor treatment response during BRAF-MEK inhibition [4]. The variant allele frequency of *BRAF* A598_T599insV rapidly decreased, from 23% before treatment to 0% after one month, in agreement with the dramatic clinical benefit. Thus, ctDNA analysis constitutes a potentially valuable molecular marker to monitor treatment response.

Pembrolizumab; June–October 2017

After 5 months of BRAF/MEK-inhibition, the patient was switched to PD-1 inhibition with pembrolizumab (2 mg/kg every three weeks) before developing resistance to BRAF/MEK-inhibition. Pembrolizumab showed an excellent effect on the remaining groin metastasis (Fig. 1) and intra-abdominal lymph nodes but the advanced primary tumor progressed. After 5 months, pembrolizumab treatment was permanently stopped due to grade III colitis. The colitis slowly subsided with tapering doses of prednisone.

From October 2017 to April 2018, no active treatment was given and the patient was monitored with clinical controls and CT scans every three months. During this period, the primary tumor progressed, whereas the groin and intra-abdominal lymph node metastases regressed.

Electrochemotherapy of Ankle Tumor; April 2018

In April 2018, the growing primary tumor was treated with electrochemotherapy, a locoregional treatment combining chemotherapy with electroporation [5]. After treatment the tumor regressed and showed no signs of recurrence after one year (Fig. 2A).

Immediately before electrochemotherapy, a new biopsy was obtained from the primary tumor. The biopsy, harbored the same *BRAF* A598_T599insV mutation as the baseline biopsies of both the primary tumor and the groin metastasis. Interestingly, PD-L1 expression was high in the groin metastasis but very low in the primary tumor (Fig. 2B). Low PD-L1 expression in the primary tumor may explain the poor response to PD-1 inhibition. Another possible explanation is differences in mutation patterns between the primary tumor and metastasis. In our patient, the non-responding primary tumor was treated with electrochemotherapy which had an excellent and durable effect.

Last Follow-Up Visit; April 2019

The patient is in excellent health, she has gained 10 kg in weight and the serum albumin level is normalized. Currently, she has no active treatment but will continue follow-up with clinical visits and CT scans every three months.

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Conclusion

In conclusion, our case demonstrates durable response to BRAF/MEK inhibition in a melanoma patient carrying a *BRAF* A598_T599insV mutation. In addition, we show that allele frequency analysis of A598_T599insV mutation in blood using ultrasensitive sequencing can be used to monitor treatment response.

Methods

Histopathology

Hematoxylin and eosin (H&E) staining was performed by standard methods. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tumor resection specimens. In brief, antigen retrieval was performed in Dako PT-Link using EnVision™ FLEX Target Retrieval Solution. Immunohistochemical staining was performed in a Dako Autostainer Link using the EnVision™ FLEX detection system according to the manufacturer's instructions. Primary antibodies were anti-SOX10 (mouse monoclonal clone BC34, Abcam, Cambridge, UK) and anti-PD-L1 (mouse monoclonal Dako Omnis clone 22C3, Agilent, Santa Clara, CA, USA).

Molecular Analysis

DNA was isolated from FFPE sections using QIAamp DNA FFPE tissue kit (Qiagen Gmbh, Hilden, Germany). 10 ng of DNA was used to prepare barcoded libraries using the Ion AmpliSeq Colon and Lung Cancer Research Panel v2 (Thermo Fisher Scientific, Waltham, MA, USA). Template preparation and enrichment were performed with the Ion Chef system and sequencing performed on an Ion S5 XL system (Thermo Fisher Scientific). After alignment to the hg19 human reference genome and variant calling by the Torrent Suite Software v4.2.1.0 variant were visually inspected with the Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, MA, USA).

Cell-Free DNA Extraction

Whole blood was collected in cfDNA preservative tubes (Norgen) and processed within 30 days to separate plasma according to manufacturer's instructions. Plasma was kept at –80°C. Before DNA extraction plasma was centrifuged at 4,300 g and 4°C for 20 min with an Eppendorf 5804 R centrifuge to remove cellular debris. Subsequently, cfDNA was extracted using the QIAamp Circulating Nucleic Acids Kit and eluted in 20–150 µL AVE buffer (both Qiagen). CfDNA was stored at –20°C. If necessary cfDNA was concentrated 15–30× using Vivacon 500, 30,000 MWCO reverse spin columns (Sartorius) according to the manufacturer's instructions.

SiMSen-Seq

SiMSen-Seq libraries were generated as described [4]. All PCRs were performed in a T100 thermal cycler (Bio-Rad). Briefly, the first barcoding PCR contained 1× Phusion HF buffer (Thermo-Fisher-Scientific) 40 nM of each PAGE-purified barcode primer (Intregrate DNA Technologies), 0.5 M L-Carnitine (Sigma-Aldrich), 0.1 U Phusion polymerase (Thermo-Fisher-Scientific), 200 µM dNTPs (Sigma-Aldrich) and up to 4 µL of cfDNA in a total reaction volume of 15 µL. The following thermal profile was used: 98°C for 30 s; 3 cycles of barcoding: 98°C for 10 s, 62°C for 6 min, and 72°C for 30 s; 65°C for 15 min; 95°C for 15 min and final hold at 4°C. At the 65°C incubation step 30 µL of TE buffer pH 8.0 (Ambion) containing 30 ng/µL

Streptomyces griseus protease (Sigma-Aldrich) was added to each reaction. The second adapter PCR contained 15 µL diluted barcoded PCR products were amplified using 1× Q5 high-fidelity ready mix (NewEngland BioLabs) and 400 nM Illumina adapter primers (Sigma-Aldrich, desalted) in a total volume of 60 µL. The following thermal profile was used: 98°C for 3 min; 30 cycles of amplification: 98°C for 10 s, 80°C for 1 s, 72°C for 30 s, 76°C for 30 s (All with ramping at 0.2°C/s) and hold at 4°C. All concentrations are shown as final reaction concentrations. Adapter PCR products were purified using the Agencourt AMPure XP system (Beckman Coulter) according to the manufacturer's instructions. The applied volume ratio between beads and PCR products was 1:1 and the purified product was eluted in 30 µL TE buffer pH 8.0 (Ambion). Prior to sequencing, SiMSen-Seq libraries were assessed on a Fragment Analyzer using the NGS HS kit (both Advanced Analytic Technology) to ensure correct library size and purity. Libraries were pooled and quantified using qPCR using a modified version of the NEBNext Library Quant Kit for Illumina (New England BioLabs). Clustering was performed at 1.3 pM on a MiniSeq instrument in 1 × 150 bp mode, supplemented with 20% Phix Control v3 using a 150 bp High Output Reagent Cartridge (all Illumina).

Data Analysis

Raw FASTQ were analyzed using a modified version of Debarcer (https://github.com/oicr-gsi/debarcer/releases/tag/v0.3.1) on a CentOS 6.9 cluster. Briefly, valid reads within each amplicon were identified as those which contained a barcode sequence in the correct position relative to the hairpin stem. Reads were then grouped into families by amplicon and barcode. For reads within each family a consensus sequence was determined for each base. Non-reference sequences were reported in consensus sequences if they composed 100% of the reads in families with 3–20 reads, or at least 90% of reads in families with >20 reads.

Acknowledgements

Statement of Ethics

The patient was given written and oral information about ctDNA being analyzed for research purposes. She signed informed consent and the study is approved by the ethical committee at the University of Gothenburg (D-151–16). The patient also provided a separate written informed consent to this report being published as a case.

Disclosure Statement

Max Levin has received lecturing fees from Bristol-Myers Squibb, Novartis and Roche. Anders Ståhlberg is co-inventor of SiMSen-Seq that is patent protected.

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Author Contributions

All authors have contributed to study design and acquisition of data. ML and SB drafted the manuscript, all co-authors contributed to revising the draft and approved of the submitted manuscript.

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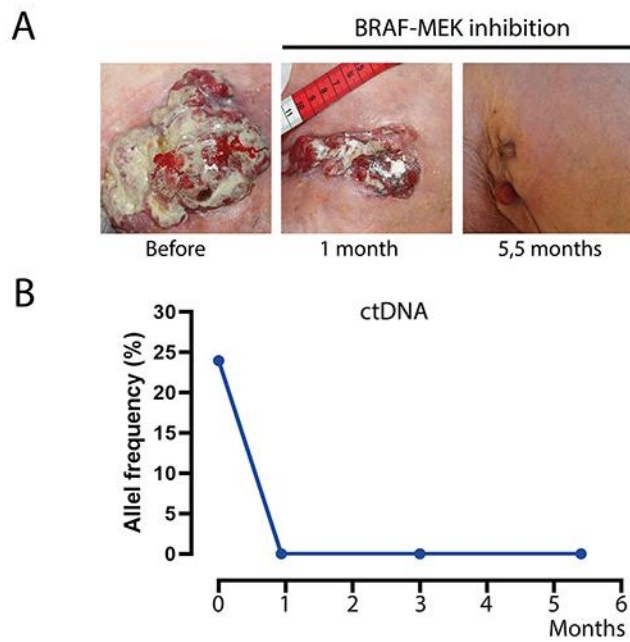
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Fig. 1. Clinical response of groin tumor and CtDNA levels during treatment. **A**. Clinical response of groin metastasis to BRAF/MEK inhibition. **B**. The allele frequency of *BRAF* A598_T599insV analyzed by SiMSen-Seq at four time points; immediately before treatment and at 1, 3 and 5.5 months after starting treatment with BRA/MEK inhibitors (dabrafenib and trametinib). Note rapid regression of groin metastases and concomitant decline in *BRAF* A598_T599insV ctDNA.
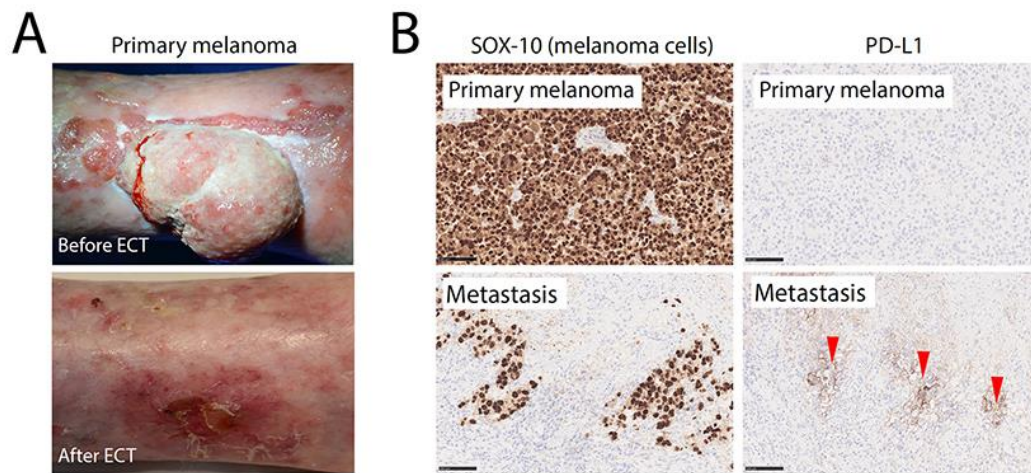
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Fig. 2. PD-1 inhibitor resistant ankle tumor. **A**. The top picture shows the large ankle tumor after progression on PD1-treatment. The bottom picture shows the same tumor one year after electrochemotherapy (ECT) treatment. **B**. Immunohistochemical staining of advanced primary tumor (top row) and groin metastasis (bottom row). SOX-10 stains for melanoma cells and the biopsies show a visibly larger number of melanoma cells in the primary tumor compared to the groin metastasis. The pictures furthest to the right show staining for PD-L1 expression. Note the absence of PD-L1 in the immunotherapy resistant primary tumor, in contrast to the finding in the groin metastasis where PD-L1 is expressed. Scale bar 100 µm.