

REVIEW

Sustained response to brigatinib in a patient with refractory metastatic pheochromocytoma harboring R1192P anaplastic lymphoma kinase mutation: a case report from the Austrian Group Medical Tumor Therapy next-generation sequencing registry and discussion of the literature

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Metastatic pheochromocytoma and paraganglioma (PPGL) are rare diseases with dismal prognosis and standard therapies are lacking. We herein report the first case of a germline anaplastic lymphoma kinase (ALK) mutation in a patient with chemorefractory metastatic pheochromocytoma in the absence of mutations of known PPGL-associated predisposing genes. Therapy with the ALK inhibitor (ALKi) brigatinib led to dramatic and durable disease remission, despite previous disease progression on the ALKi alectinib. This case underscores the potential clinical use of molecular profiling in rare diseases with limited treatment options and suggests that the ALK-R1192P point mutation might predict sensitivity to brigatinib.

Key words: pheochromocytoma, ALK, brigatinib, NGS

INTRODUCTION

Pheochromocytoma and paraganglioma (PPGL) are rare catecholamine-secreting neoplasms arising from chromaffin cells located in the adrenal medulla or neuronal crest. Although completely resected PPGL have an excellent prognosis after surgical removal, >10% of patients with PPGL develop distant disease. Metastatic pheochromocytomas have a rather poor 5-year overall survival of ~60% and prognosis has not significantly improved over the past two decades, especially for those cases where treatment options such as radionuclide therapy [e.g. ¹³¹I-meta-iodobenzylguanidine (¹³¹I-MIBG)] or peptide receptor radioligand therapy (e.g. ¹⁷⁷Lu-DOTATATE) are not feasible.¹

Novel treatment approaches for metastatic PPGL, such as targeted molecular therapies with the multikinase inhibitor sunitinib or the mechanistic target of rapamycin inhibitor

everolimus, could only achieve modest improvements in the recent past.²⁻⁴ This case report deals with an adult patient suffering from distant recurrences of a therapy-refractory pheochromocytoma harboring R1192P anaplastic lymphoma kinase (ALK) mutation (NM_004304.3: c.3575G>C; NP_004295.2: p.Arg1192Pro), a mutation that has not been described previously in PPGL.

CASE PRESENTATION

In November 2017, a 48-year-old male patient presented with abdominalgia and arterial hypertension. Computed tomographic imaging detected a large mass measuring 14 × 14 × 13 cm³ on top of the right kidney close to a pathologically enlarged lymph node measuring 1.7 × 1.1 cm². Histological analysis obtained by computed tomography (CT)-guided biopsy suspected a paraganglioma with positive immunohistochemical staining for synaptophysin and chromogranin A. Endocrinologic examination revealed increased normetanephrine levels in the 24-hour urine and elevated serum parathyroid hormone concentration. Alpha-blockade with doxazosin 1 mg twice a day was established to treat hypertension and to avoid perioperative complications. The patient underwent radical nephrectomy with ipsilateral adrenalectomy. Histopathological assessment of

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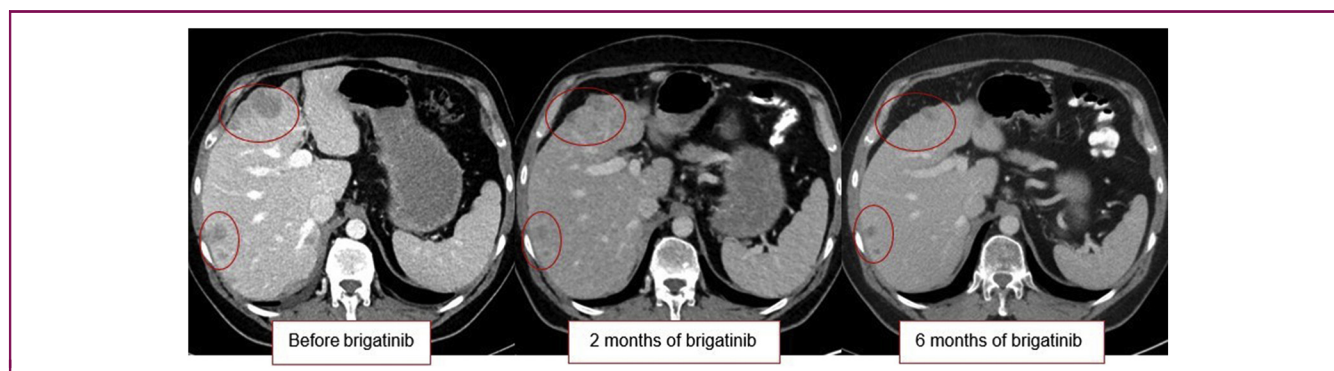


Figure 1. Computed tomography images of the abdomen showing response of liver metastases to brigatinib. Red circles indicate locations of metastases.

the surgical specimen revealed a malignant pheochromocytoma pT3, pN1 (1/11), L1, V1, R1, UICC stage III. Functional imaging with ^{68}Ga -DOTATOC positron emission tomography (PET)/CT showed no evidence of metastatic disease, whereas a choline-PET/CT revealed an adenoma of the parathyroid causing primary hyperparathyroidism. The family history revealed a 16-year-old daughter who was diagnosed with neuroblastoma at the age of 2 months. The initial genetic testing for pheochromocytoma-associated syndromes according to the secretory phenotype encompassed the *VHL* gene, the *SDHx* genes including *SDHB*, *SDHC*, and *SDHD*, as well as *MAX*. In none of these genes, a pathogenic variant was detected by gene panel sequencing of germline DNA.

One year after incomplete resection of the malignant pheochromocytoma, the normetanephrine levels began to rise and follow-up ^{68}Ga -DOTANOC-PET/CT showed local recurrence in the right retroperitoneum as well as bone and lymph node metastases. Radionuclide therapy with ^{131}I -MIBG or peptide receptor radioligand therapy such as ^{177}Lu -DOTATATE was not applicable because of low uptake of MIBG and gallium-68-labeled somatostatin analogs. Systemic chemotherapy was not yet indicated regarding the low tumor burden. Thus, the patient received palliative treatment with the somatostatin analog octreotide long-acting release 30 mg every 4 weeks with adequate symptom control.

Considering the patient's young age, fitness, and advanced disease stage combined with limited treatment options, molecular profiling was performed shortly after the detection of metastases within the next-generation sequencing (NGS) Registry of the Austrian Group Medical Tumor Therapy. NGS of the tumor tissue (as described in 'Methods' section) identified a gain-of-function mutation in the *ALK* gene (R1192P) with an allelic frequency of 86% in the absence of an *ALK* rearrangement. In addition, one variant of unknown significance, CTCF P3155, was detected. The tumor was microsatellite stable with low tumor mutational burden (3 mutations/Mb). A positive family history of neuroblastoma in the daughter prompted genetic counseling, which revealed the *ALK* R1192P mutation as being a germline mutation in the patient as well as his daughter.

Three months after initiation of octreotide monotherapy the disease progressed on PET/CT scan. Therefore, the

second-generation *ALK* inhibitor (*ALKi*) alectinib [600 mg twice a day in analogy to non-small-cell lung cancer (NSCLC)] was added on an individual basis, resulting in disease stabilization at radiologic assessment after 1 month.^{5,6} Combination treatment was continued for 5 months until hepatic metastases were noticed.

Given the high tumor burden, treatment was switched to chemotherapy consisting of cyclophosphamide (750 mg/m²), vincristine (1.4 mg/m²), and dacarbazine (600 mg/m²) every 3 weeks.^{7,8} Although two courses of this regimen were administered, normetanephrine levels rose constantly and imaging confirmed nonresponsiveness. Given the known *ALK* mutation, we initiated individual treatment with brigatinib (90 mg for 7 days followed by 180 mg a day), another second-generation *ALKi*.^{9,10} Radiological re-evaluation after 2 months of therapy revealed a partial response, as shown in Figure 1.

The patient demonstrates a sustained partial response 10 months on therapy, with minor side-effects mainly being moderately elevated liver enzymes and mild myalgias. Figure 2 summarizes the treatment course from time of metachronous metastasis and urinary concentration of normetanephrine during therapy.

METHODS

Germline panel sequencing

Genomic DNA was extracted from peripheral blood leukocytes with the QIASymphony (Qiagen). Library preparation was performed using the TruSight Rapid Capture Kit and TruSight Cancer Sequencing Panel (Illumina). The library was paired-end sequenced on a MiSeq (Illumina) using the MiSeq Reagent Kit v2 for 300 (2 × 150) cycles (Illumina). Reads were aligned to the human reference sequence GRCh37 (hg19) and target regions [*VHL* (NM_000551.3), *SDHB* (NM_003000.2), *SDHC* (NM_003001.3), *SDHD* (NM_003002.3) and *MAX* (NM_002382.4)] were analyzed with the SEQNEXT Software (JSI). Variants were classified according to the consensus recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.¹¹ Benign and likely benign variants were not reported.

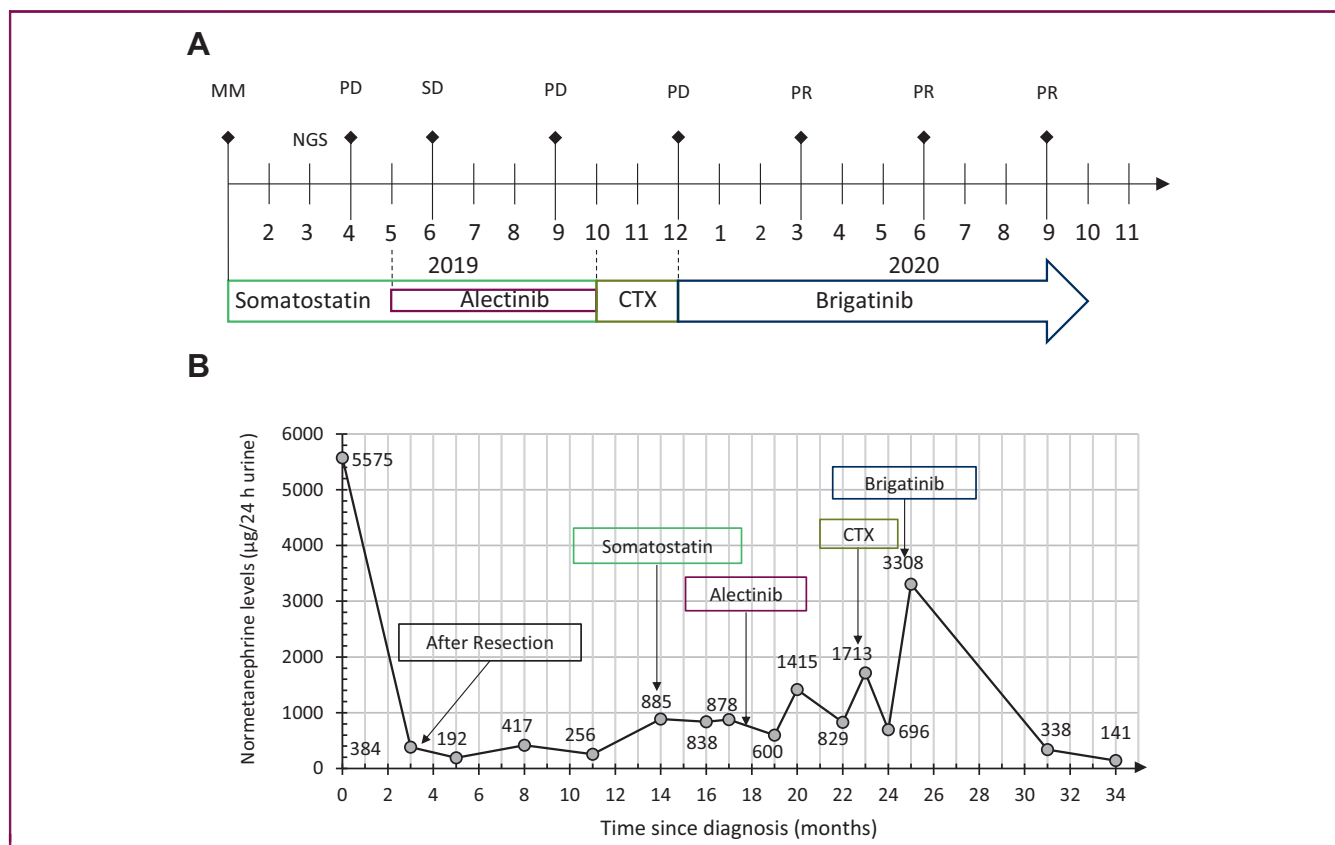


Figure 2. (A) Timeline of patient's diagnosis of distant disease and treatments. (B) Normetanephrine levels during therapy. The arrows point at the start of the respective therapy.

CTX, chemotherapy; MM, metachronous metastasis; NGS, next-generation sequencing; PD, progressive disease; PR, partial response; SD, stable disease.

Germline Sanger sequencing

Genomic DNA was extracted from peripheral blood leukocytes with the QIASymphony (Qiagen). The complete ALK [NM004304.5] exon 23 (c.3516_c.3645) was PCR amplified and Sanger sequenced with the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) on the ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems). The original tumor biopsy sample was submitted as formalin-fixed, paraffin-embedded tumor tissue for hybrid capture-based NGS using FoundationOne CDx, a commercially available platform covering 324 cancer-related genes.¹² The patient was treated with brigatinib within an individual patient treatment program after the collection of the written informed consent. Morphologic response was assessed using CT according to RECIST version 1.1 23.¹³

DISCUSSION

Thirty percent of all PPGLs are associated with inherited cancer syndromes with germline mutations in the *VHL*, *RET*, *NF1*, *SDHx*, *SDHAF2*, *MAX*, and *TMEM127* genes.¹⁴ Point mutations in ALK have been described in anaplastic thyroid tumors, neuroblastoma, and as a resistance mechanism in ALK fusion-positive NSCLC after previous ALKi therapy.¹⁵⁻¹⁹ ALK-R1192P point mutation has been reported as a germline mutation in neuroblastoma, but so far PPGLs have not been linked to ALK mutations at all, based on COSMIC

(Catalogue of Somatic Mutations in Cancer) and literature retrieval from PubMed.

R1192P mutation lies within the protein kinase domain and causes gain of function through ligand-independent activation of the ALK kinase domain and increased downstream signaling via PI3K–PKB/Akt, STAT3, and MAPK/ERK.^{20,21} The exact signaling pathway depends on tumor type and method of ALK activation (e.g. ligand, fusion partner, overexpression, or activating mutation).

Standard treatment options for metastatic PPGL include surgical removal, radiotherapy, targeted radiolabeled carriers (e.g. ¹³¹I-MIBG or ¹⁷⁷Lu-DOTATATE), and chemotherapy with cyclophosphamide, vincristine, and dacarbazine or temozolomide. ALKis are competitive inhibitors of the ATP pocket of the atypical ALK tyrosine kinase leading to insufficient energy supply and downregulation. Alectinib, a second-generation ALKi, was included based on data of patients with ALK-rearranged metastatic NSCLC, in which ALKis are approved for first-line treatment.^{5,6,22} A restaging CT scan, taken 3 months after initiation of alectinib, however, revealed no response. Brigatinib, another second-generation ALKi, was administered for compassionate use after failure of chemotherapy. Our patient has achieved up to now a partial response for >10 months.

Though in vitro studies showed activity of brigatinib and alectinib against R1192P, the patient achieved no response

to alectinib. A possible explanation might lie in the differential potency of the two second-generation ALKis in inhibiting ALK-R1192P mutation compared with the first-generation ALKi crizotinib: alectinib inhibits ALK-addicted neuroblastoma cell line proliferation with 14-fold decrease in half-maximal inhibitory concentrations, whereas brigatinib exhibits 26 times greater potency in kinase assays than crizotinib.^{23,24} Furthermore, a tyrosine kinase inhibitor analysis performed by Amin et al²⁵ found that ALK-R1192P mutation in nucleophosmin-ALK confers resistance to several ALKis except for brigatinib. In line with published preclinical data, this case corroborates that ALK-R1192P mutation might confer sensitivity to brigatinib. Indeed, brigatinib was administered in the absence of repeated molecular characterization and we cannot exclude the occurrence of acquired mutations following alectinib.

CONCLUSIONS

On the one hand, this case demonstrates that using ALKis in ALK-mutated tumors other than NSCLC and neuroblastoma may be a highly effective treatment when all other standard therapy options have failed, and on the other, this is the first report of a germline ALK mutation in a patient with pheochromocytoma in the absence of mutations of known PPGL-associated genes that might predispose to the development of pheochromocytoma. However, further sequencing of the ALK exon 23 in patients with PPGL are needed to firmly establish this link. Without the validity of a clinical trial, this case underscores the possible benefit from molecular evaluation of orphan diseases such as PPGL. With the increasing use of commercially available NGS testing platforms in oncology, we strongly encourage participation in academic registries such as the NGS Registry (NCT03301493) of the Austrian Group Medical Tumor Therapy in order to be able to analyze the impact of NGS testing on treatment choices and patient outcome.

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DISCLOSURE

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DATA SHARING

The authors declare that data supporting the findings of this study are available within the paper. The complete molecular report of the NGS analysis and primer sequences are available upon request from the corresponding author.

ETHICS DECLARATIONS

Written informed consent was obtained from the patient and from the patient's daughter for publication of this case report and any accompanying images.

This analysis was performed within the next-generation sequencing (NGS) Registry of the Austrian Group Medical Tumor Therapy (AGMT) (NCT 03301493). This registry has been approved by the Ethics Committee of the provincial government of Salzburg (Nr.: 415-E/2129).

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