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Excellent Response to MEK Inhibition in an *AGK-BRAF* Gene Fusion Driven Carcinoma: Case Report and Literature Review

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Abstract. *Background: Soft tissue myoepithelial carcinomas (STMC) are a rare, malignant subgroup of myoepithelial tumors that arise typically in glandular or ductal tissues, but also in the bone and soft and cutaneous tissues. Due to its rarity, there is no consensus regarding the treatment of STMC, including chemotherapy or other systemic agents for metastatic STMC. Case Report: A chemotherapy- and regorafenib-refractory STMC, harboring an AGK-BRAF fusion, was successfully treated using MEK-inhibition with cobimetinib in monotherapy. MEK-inhibition with cobimetinib effectively silenced paradoxical MAP kinase/ERK-signaling pathway activation after regorafenib monotherapy, and resulted in a significant and durable clinical response. Conclusion: This effect of MEK-inhibition in STMC harboring an AGK-BRAF fusion has not been previously reported and contributes to the existing, yet limited, knowledge on the treatment of BRAF fusion-driven tumors. Also, our case highlights the importance of next generation sequencing in driving further rational therapeutic choices to provide disease control and palliation.*

Soft tissue myoepithelial carcinomas (STMC) are a rare, malignant subgroup of myoepithelial tumors with an age-adjusted incidence of only 0.0018 per 100,000 persons per year (1). Myoepithelial tumors usually occur at sites where normal myoepithelial cells are found in relation to glandular or ductal structures, such as salivary gland and breast tissue. However, myoepithelial tumors are increasingly reported in tissues that normally lack myoepithelial cells, such as the bone and soft tissue (2, 3). STMC are typically found in the extremities affecting women and men equally before the 4th decade of life, although they can occur at any age, with approximately 20% of cases in children (2-4). In fact, children and adolescents have a higher incidence of STMC, whereas adults more commonly develop oral cavity/pharynx myoepithelial carcinomas (1).

Characteristically, STMC demonstrate heterogeneous morphologic and immunophenotypic features. They are subclassified into mixed tumor/chondroid syringoma, myoepithelioma, and myoepithelial carcinoma (2, 5). The mixed tumor and myoepithelioma types generally do not metastasize and only reoccur in up to 20% of cases, usually following incomplete excision (5). In contrast, myoepithelial carcinomas behave more aggressively, with locoregional recurrence and metastasis in up to 40-50% of cases (5).

Histologically, STMC are characterized by moderate-to-severe atypia with vesicular nuclei and prominent nucleoli next to high mitotic rates and necrosis (5). STMC variably co-express epithelial antigens [broad-spectrum cytokeratin (pan-keratin, AE1/AE3, Cam5.2) and/or epithelial membrane antigen (EMA)] and muscle/myoepithelial markers [smooth muscle actin (SMA), HHF-35, p63, glial fibrillary acidic protein (GFAP), S-100 protein, CD10, Sox-10, desmin and/or calponin] (2, 4-7). Translocations in the *ESWR1* gene, present on chromosome 22q, and deletions of *SMARCB1* gene are often found in 40-50% and 30% of cases, respectively (2, 4). A small subset of STMC harboring an *ESWR1* gene translocation have alternate Fused in sarcoma (*FUS*) rearrangement with documented fusion partners *POU5F1* (6p21), *PBX1* (1q23), *ZNF444* (19q23), *ATF1* (12q13), *PBX3* (9q33), and *KLF17* (1p34) (4). However, the biologic significance of these fusion gene products remains unknown (4).

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Based on the degree of cytologic atypia and the additional presence of necrosis and mitoses, STMC are graded as low, intermediate, and high (5). The presence of cytological atypia, high mitotic count, and high tumor necrosis is associated with a more aggressive clinical course. These tumors commonly metastasize to the lung, bone, lymph nodes, and soft tissue (2, 5). The location and size of the primary lesion also seem to impact the clinical course. A series of 29 STMC in children reported that large, deep-seated tumors often rapidly progressed, whereas frequently, small lesions in superficial soft tissues initially behaved more indolently, but ultimately metastasized in a high percentage (36%) of cases (8).

Localized STMC are generally treated after multidisciplinary discussion in an expert sarcoma center with surgical resection in conjunction with (neo-)adjuvant radiation depending on the surgical margins (2). However, due to its rarity, there is no consensus regarding the treatment of STMC, and there is a paucity of evidence for chemotherapy or other systemic agents in the presence of metastatic disease (2, 8).

Case Report

A 57-year-old woman presented in 2014 with an indolent swelling in the right groin. In 2012, she underwent right femoral artery catheterization for angiography and coiling of a ruptured brain aneurysm, which resulted in a slowly enlarging groin swelling. This was shown in 2013 by angiography to be a pseudo-aneurysm of the right arterial artery. The patient underwent an uncomplicated resection of a pseudo-aneurysm of the right arterial artery in 2014. However, during the procedure, a large pathologic lymph node was visualized and resected for pathological review. The initial diagnosis was a poorly differentiated non-small-cell adenocarcinoma, composed of papillary-like structures lined by a broad layer of pleomorphic cells with vacuolization and eosinophilic cytoplasm. There was central tumoral necrosis and diffuse mitotic figures. Prominent immunostaining for S100 and neurospecific enolase was observed, with varying staining for pan-cytokeratin. The myoepithelial marker p63 showed sporadic positivity. Other immunohistochemical markers were negative, including keratin 7, 8, 19, and 20, renal cell carcinoma, thyroid transcription factor 1, estrogen receptor, CD23, alpha smooth muscle actin, chromogranin, synaptophysin, epithelial membrane antigen, human melanoma black – 45, melan A, and desmin. No translocation in the *EWSR1* gene (cytogenetic location: 22q12.2) was identified, nor was there an amplification of the *MDM2* gene, suggesting the tumor was not an Ewings sarcoma or liposarcoma. After review by a panel of expert pathologists, the ultimate diagnosis was a poorly differentiated STMC, with negative resection margins.

Staging with positron emission tomography (PET) at the time of diagnosis did not identify a primary tumor site, nor any other disease sites. As the resected STMC was thought to be a metastasis of unknown primary site, the multidisciplinary tumor board decided to administer adjuvant chemotherapy with four cycles of cisplatin and gemcitabine, according to the ESMO clinical practice guidelines for cancers of unknown primary site (9), preceding consolidation radiotherapy (60 Gy in 30 fractions) of the right groin.

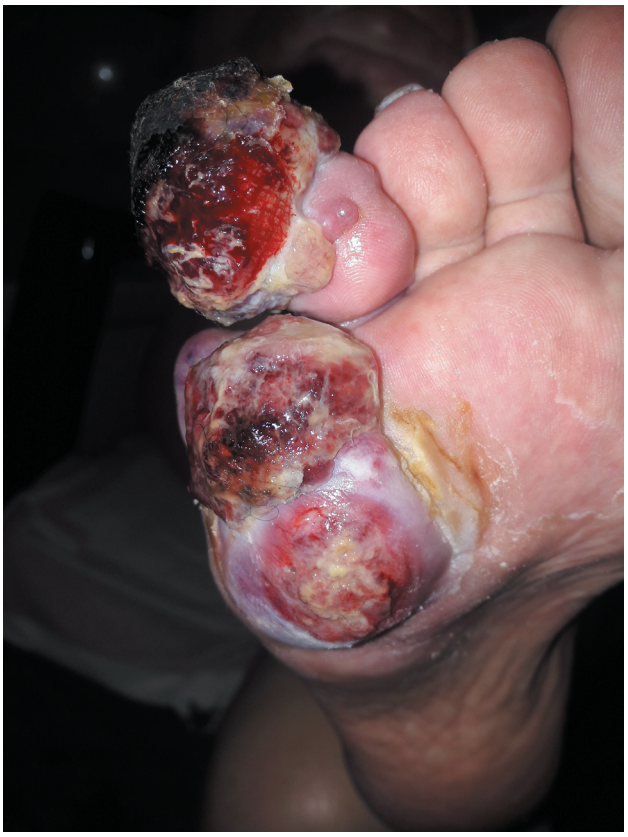

Figure 1. *Image from March 2018 of the chronically inflamed, malignant wound of the right fourth and fifth toe.*

One year after radiotherapy, in May 2015, the patient presented with two palpable subcutaneous nodules on the right upper leg and in the popliteal fossa of the left leg. Both lesions were resected and pathology revealed malignant, poorly differentiated lesions, with comparable histology to the previous metastatic myoepithelial carcinoma of the lymph node of the right groin. PET demonstrated again no further metastatic disease nor a primary tumor site. Due to borderline resection of the popliteal metastatic lesion, adjuvant consolidating radiotherapy (39 Gy in 13 fractions) was administered.

In August 2016, a new solitary subcutaneous lesion of the left upper thigh was visualized on PET. The lesion was completely resected, demonstrating a third cutaneous

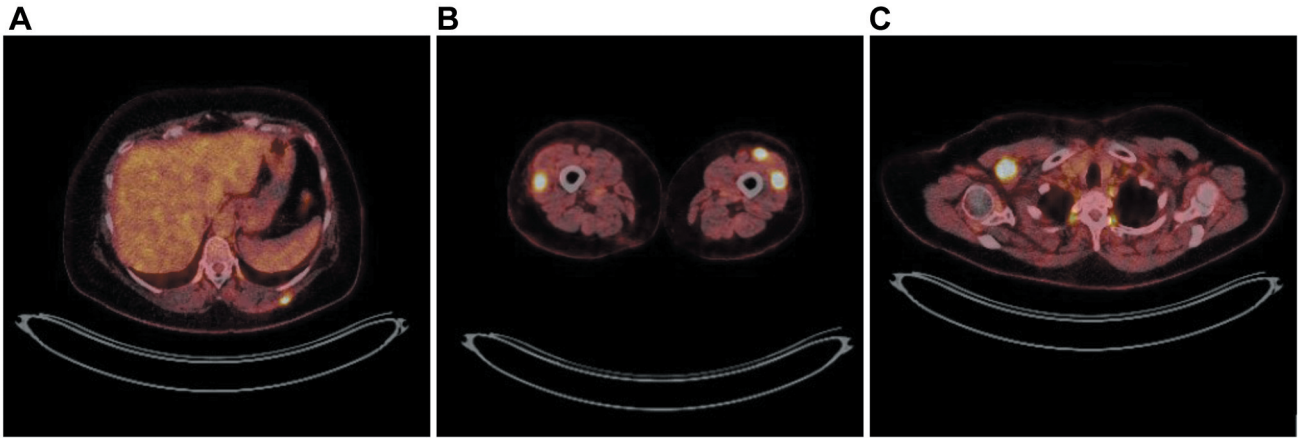

Figure 2. *Positron emission tomography images from March 2018 showing subcutaneous lesions in (A) the left torso (B) both upper legs and, (C) right supraclavicular lymph node.*

metastasis, followed by adjuvant consolidating radiotherapy (39 Gy in 13 fractions).

In March 2017, a slowly progressive solitary lung lesion of the left lower lobe was identified during follow-up with computed tomography (CT) scan, for which the patient underwent a wedge resection of the left lower lobe. Histopathological diagnosis confirmed a lung metastasis of the myoepithelial carcinoma.

Six months later, in March 2018, the patient presented with a chronically inflamed, malignant wound of the right fourth and fifth toe (Figure 1). Biopsy confirmed the presence of the myoepithelial malignancy, and additional PET identified multiple subcutaneous lesions at the torso and both legs, and a suspicious right supraclavicular lymph node (Figure 2).

Following amputation of the right fifth toe for hygiene reasons, palliative chemotherapy that consisted of carboplatin and gemcitabine, was re-initiated in April 2018. Unfortunately, at first response evaluation after 6 cycles, progression of the multiple subcutaneous lesions was observed. The patient was referred to a clinical trial in the Clinical Research Unit of the Department of Oncology at the University Hospital Antwerp (UZA) in Belgium.

Tumor biopsies were analyzed by mRNA in situ hybridization (ISH) to investigate amplification of the fibroblast growth factor receptor (*FGFR*) 1, 2, 3 and 4 mRNA, involved in modulating downstream MAP kinase/ERK and PI3K/AKT signaling pathways. The tumor showed amplification of *FGFR 1* and subsequently treatment was initiated in December 2018 on a phase I trial with a combination of the phosphoinositide 3-kinase (PI3K) inhibitor copanlisib and the pan-FGFR 1, 2, 3 inhibitor, rogaratinib. After an initial therapeutic break due to severe fatigue, the combination treatment was terminated because of disease progression in March 2019.

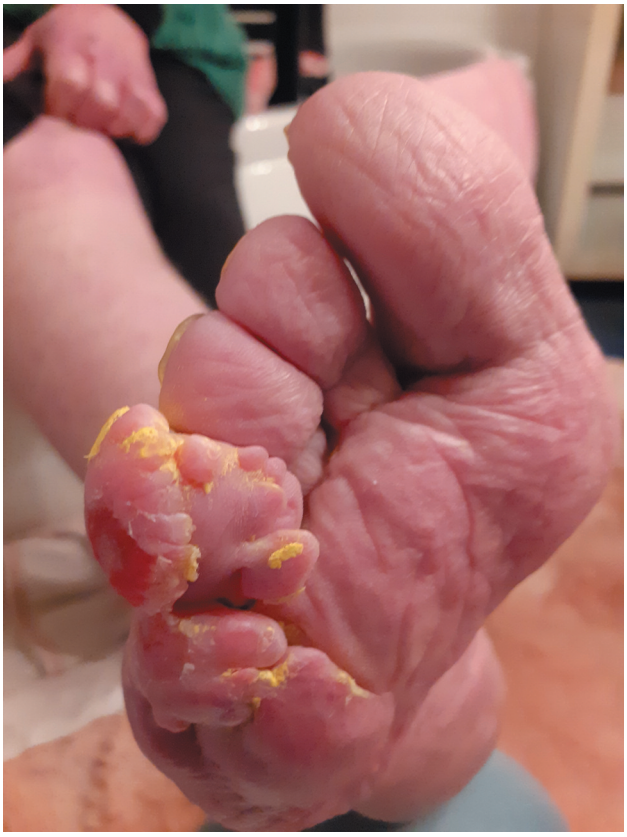

Figure 3. *Image of the malignant wound of the right fourth and fifth toe after one year of treatment with MEK-inhibitor cobimetinib.*

Concurrently with the mRNA ISH, molecular testing of the tumor DNA, involving a hybrid capture-based next generation sequencing (NGS) platform (FoundationOne™), was

performed. NGS-data showed an *AGK-BRAF* gene fusion, a *CDKN2A/B* loss, and a *TERT* promoter-146C>T mutation.

We hypothesized that the *AGK-BRAF* gene fusion would activate the MAP kinase/ERK-signaling pathway, stimulating cell proliferation and rendering resistance to therapy, hence the patient commenced treatment with the multikinase inhibitor regorafenib. However, after one month, clinical disease progression of some cutaneous lesions was observed, with the remaining lesions clinically stable.

Ultimately, following recent evidence of superior sensitivity of cancers with *BRAF* mutations to mitogen-activated protein kinase (MEK)-inhibitors, treatment with cobimetinib as a single agent was initiated in June 2019 at a dose of 60 mg a day (cycles of 3 weeks of treatment followed by 1 week of rest). This oral agent resulted in a significant and durable clinical response, with tumor reduction of multiple subcutaneous lesions and malignant wound of the right fourth and fifth toe (Figure 3), and an overall persisting stabilization of the disease according to Response Evaluation Criteria in Solid Tumors (RECIST), which continues as of August 2021. Treatment with cobimetinib was tolerated excellently with no clinical or biochemical toxicity. This case report was approved by the ethics committee of the Antwerp University Hospital (UZA). The patient provided written informed consent for the publication of this case report.

Discussion

BRAF gene mutations and fusions. *BRAF* encodes for the serine/threonine protein kinase B-Raf downstream of RAS, activating the MAP kinase/ERK-signaling pathway (10). Under normal physiological conditions, cell proliferation and survival is promoted upon activation of RAS by extracellular factors (11). In case of activating point mutations or fusions in *BRAF*, mutated B-Raf or a Raf kinase fusion protein signals as a monomer independent of upstream growth stimuli (10), leading to constitutive activation of the MAP kinase/ERK-signaling pathway. Ultimately, this results in excessive cell proliferation and survival (11-13), driving cancer growth.

BRAF is mutated in approximately 8% of all cancers, frequently in thyroid cancer (59%) and melanoma (51%) and less commonly in colon (10%) and lung cancer (7%) (13). The predominant *BRAF* gene mutation involves a thymidine to adenosine transversion at nucleotide 1,799 (14), resulting in a $BRAF^{V600E}$ mutation, which encodes the constitutively active $BRAF^{V600E}$ oncoprotein, and accounts for 92% of the observed mutations in *BRAF* (15).

BRAF gene fusion represents a rare event [55 *BRAF* fusions detected in an analysis of 20, 573 tumors (0.53%) (16)]. Different mechanisms of BRAF activation have been described in several solid tumor types and interestingly, they are enriched in Spitzoid melanomas (75%), low-grade pediatric astrocytomas (70%), acinar pancreatic cancers (67%), pilocytic gliomas (30%), and pediatric and overall papillary thyroid cancers [19% (17) *versus* 3%] (13, 16). The breakpoint of *BRAF* gene fusions can occur in introns 7, 8, 9, or 10 within *BRAF,* and is able to fuse with more than 110 different fusion partner genes (18), including acylglycerol kinase (*AGK*). This, ultimately results in a RAF kinase fusion protein (13), which constitutively activates the MAP kinase/ERK-signaling pathway (12). Interestingly, *BRAF* fusions have also recently been held accountable for resistance in multiple tumors types including *EGFR* mutant lung cancers (19, 20), gastric cancer (21) and $BRAF^{V600E}$ mutant melanomas (22) treated with tyrosine kinase inhibitors, FGFR inhibitors, and vemurafenib, respectively.

Data regarding the prevalence of *BRAF* mutations and fusions in STMC are lacking. However, a comprehensive genomic profiling of metastatic and relapsed salivary gland carcinomas found *BRAF* genomic alterations in 5% of myoepithelial salivary gland carcinomas. These consisted of $BRAF^{V600E}$ mutations (46%), activating non-$BRAF^{V600E}$ base substitutions (33%), and fusions (12%), along with other limited alterations in the PI3K/MTOR pathway, the sonic hedgehog pathway (PTCH1), and rare kinase growth factor GA (PDGFRB) (23).

Treatment of BRAF gene fusions. Information on the drug sensitivity of tumors with B-Raf fusion kinases is limited due to the relatively low frequency of *BRAF* fusions and scarcity of cell lines carrying these alterations (16, 18). Also, *BRAF* fusions display significant differences in their phenotypes and degrees of response to MEK-inhibition such as trametinib (24). As such, clinical case studies of *BRAF* fusion-driven malignancies treated with multikinase inhibitors report partially conflicting results (18).

The multikinase inhibitor sorafenib showed significant clinical response in a melanoma harboring an *AGK-BRAF* fusion (12, 25) and in a soft tissue sarcoma harboring a *KIAA1549-BRAF* fusion (26). However, the primary tumor response in the latter case study could also be attributable to concurrently administered temsirolimus (an mTOR inhibitor) and the antiangiogenic agent bevacizumab (26). Nonetheless, *in vitro,* an increased sensitivity to sorafenib was observed in the patient-derived melanoma cell line harboring an *AGK-BRAF* gene fusion compared to melanoma cell lines harboring an $BRAF^{V600E}$ mutation; this is likely due to the binding properties of sorafenib. As the kinase domain of AGK-BRAF does not contain any mutation in contrast to $BRAF^{V600E}$, sorafenib may be more effective to prevent the activation of the wild-type conformation in *AGK-BRAF* expressing cell lines compared to melanoma cell lines with $BRAF^{V600E}$ mutation (12). Additionally, Palanisamy *et al*. demonstrated in a prostate cancer cell line that sorafenib and MEK inhibitors are active against *BRAF* fusions (27).

However, sorafenib produced unexpected and unprecedented acceleration of tumor growth in children with low-grade astrocytoma irrespective of the tumor *BRAF* status (*KIAA1549-BRAF* fusion, *BRAF* duplication, and *BRAF* wild-type), most likely due to paradoxical activation of the MAP kinase/ERK-signaling pathway (28). Indeed, classical RAF inhibitors are known to paradoxically activate the MAP kinase/ERK-signaling pathway by the physical binding of the RAF inhibitor to the BRAF or CRAF protomer and promoting dimerization with an uninhibited CRAF protomer through conformational changes in the drug-bound RAF protomer (18, 29, 30). This results in therapeutic resistance and/or tumor growth (18, 28). In the case of *BRAF* fusions in melanoma, Botton *et al*. provided evidence that paradoxical activation of the MAP kinase/ERK-signaling pathway in response to first generation RAF inhibitors, such as sorafenib and regorafenib, was due to the presence of the dimerization domain encoded by 5' fusion partner gene (18).

This provides a rationale for combining BRAF-inhibitors with additional downstream inhibition of MEK1/2, to increase MAP kinase/ERK-signaling pathway inhibition and prevent resistance to BRAF-inhibitor monotherapy by paradoxical activation. Indeed, BRAF and MEK-inhibitor combinations are more effective than BRAF-inhibitor monotherapy (31), and are approved for use in various cancers, such as melanoma and NSCLC harboring a $BRAF^{V600E}$ mutation (32, 33). In support of this, Guidry *et al*. reported remission in a patient with a primary cutaneous myoepithelial carcinoma with a $BRAF^{V600E}$ mutation after administration of a combination of the BRAF-inhibitor vemurafenib and the MEK-inhibitor cobimetinib (34).

However, several case studies also report clinical activity of monotherapy with a MEK-inhibitor in melanoma harboring a *BRAF* fusion. In a patient with Spitzoid metastatic melanoma featuring a *ZKSCAN1-BRAF* fusion, the major tumor response was achieved with the MEK-inhibitor trametinib rather than with a RAF kinase inhibitor (16). Trametinib-based treatment of two heavily pretreated patients with metastatic melanoma harboring a *PPFIBP2-BRAF* and a *KIAA1549-BRAF* fusion resulted in radiological response in both extracranial and intracranial sites and slowed disease progression, with symptomatic improvement of both patients (35). MEK-inhibition monotherapy has also been reported to show remarkable clinical response for *RAF1* fusions in metastatic melanoma (36, 37) and an anaplastic pleomorphic xanthoastrocytoma with leptomeningeal dissemination (38). However, their clinical efficacy is likely context dependent, as McEvoy *et al*. reported an inferior response using trametinib-based MEK-inhibition for a *RAF1*-fused pancreatic acinar cell carcinoma (39).

Interestingly, recent evidence demonstrated that a *TERT* promoter mutation determines the therapeutic response of $BRAF^{V600E}$ mutated cancers to BRAF and MEK-inhibitors, and induced robust apoptosis in cancer cells harboring both $BRAF^{V600E}$ and *TERT* promoter mutations, but little or not in cells harboring only $BRAF^{V600E}$ (40). In line with these findings, a dramatic response to combination therapy with BRAF and MEK-inhibition was seen in a patient with a $BRAF^{V600E}$ and *TERT* promoter mutated epithelioid glioblastoma (41). Although no literature is available concerning the response of tumors harboring a *TERT* promoter mutation and a *BRAF* fusion to BRAF and MEK-inhibitors, the presence or absence of a *TERT* promoter mutation could also explain the difference in treatment efficacy of these therapies in tumors harboring a *BRAF* fusion.

Conclusion

This is a case report of a successful treatment of a patient with a metastasized chemotherapy- and regorafenib-refractory STMC harboring an *AGK-BRAF* fusion using MEK-inhibition with cobimetinib as monotherapy. We postulate that the MEK inhibition effectively silenced paradoxical MAP kinase/ERK-signaling pathway activation after regorafenib monotherapy, resulting in a significant and sustained clinical response. This effect of MEK-inhibition in STMC harboring an *AGK-BRAF* fusion has not yet previously been reported and contributes to the existing, yet limited, knowledge of the treatment of *BRAF* fusion-driven tumors. Moreover, however highly speculative, the additional *TERT* promoter-146C>T mutation could provide an additional genetic explanation for the observed effect.

In recent years, next generation sequencing (NGS) has revolutionized our understanding of tumorigenesis. Through the identification of "driving mutations" in key molecular pathways, NGS has become vital in exploring new treatment options. *BRAF* fusions, however, are not detected with standard whole-exome sequencing, due to the location of the fusion junctions in the introns. High-depth sequencing of selected "hotspot" introns can sensitively detect rearrangements at the level of the introns and identify involved partner genes by analyzing the junction sequence, allowing novel *BRAF* rearrangements, including fusions, to be detected (22). For patients with rare cancers, the use of NGS is an important option to drive further rational therapeutic choices to provide disease control and palliation.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

A.D., C.V.P., M.R., E.S., M.P. and H.P. wrote and drafted the manuscript. K.Z., S.L. and P.P. performed the pathological, immunohistochemical and molecular analyses. All Authors have reviewed the manuscript, and read and agreed to the published version of the manuscript.

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