

Ameloblastic fibrosarcoma: clinicopathological and molecular analysis of seven cases highlighting frequent *BRAF* and occasional *NRAS* mutations

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Aims: Ameloblastic fibrosarcoma (AFS) is an aggressive odontogenic neoplasm featuring malignant mesenchymal stroma in addition to an ameloblastic epithelial component, and is hence considered to be the malignant counterpart of ameloblastic fibroma (AF). AFS is exceedingly rare, with <110 cases having been reported so far. Although *BRAF* mutations are recognised driver mutations in ameloblastoma, the molecular pathogenesis of AFS remains elusive.

Methods and results: We herein describe seven AFSs that were analysed, for the first time, for mutations in the *BRAF*–*NRAS* pathway. The patients were four females and three males aged 23–57 years (median, 26 years). Three tumours developed after one or multiple recurrences of AF (4–20 years after initial diagnosis), two showed transition from AF-like bland areas, and two developed *de novo*. All patients were treated with surgery; adjuvant chemotherapy was

given to one patient. At the last follow-up, five patients were alive and well (19–344 months). The remainder were lost to follow-up. Histological examination showed variable sarcomatous overgrowth with varying degrees of atypia and increased mitotic activity. The epithelial component varied greatly according to the degree of sarcomatous overgrowth. Molecular testing revealed *BRAF* V600E mutations in five cases and *NRAS* p.Gln61Lys mutation in one case. One tumour was wild-type.

Conclusion: To our knowledge, this is the first study on *BRAF*/*NRAS* mutations in AFS. Given the activity of RAF and MEK inhibitors across different cancers harbouring V600E mutations, our data strongly suggest that all AFS cases should be genetically tested, and that targeted treatment approaches for this extremely rare sarcoma subtype should be clinically investigated.

Keywords: ameloblastic fibroma, ameloblastic fibrosarcoma, *BRAF*, molecular pathogenesis, *NRAS*, targeted therapy

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Introduction

Ameloblastic fibrosarcoma (AFS) is an aggressive neoplasm of odontogenic origin that features malignant mesenchymal spindle cell stroma in addition to an ameloblastic (ameloblastoma-like) epithelial component.¹ By definition, the epithelial component is histologically benign, in contrast to the exceedingly rare ameloblastic carcinosarcoma.² AFS is exceedingly rare with <110 cases reported so far.^{1,3} AFS represents 0.3% of all odontogenic tumours and 24% of malignant odontogenic tumours.⁴

AFS is considered to be the malignant counterpart of ameloblastic fibroma (AF). Indeed, sarcomatous overgrowth and a frankly malignant stromal appearance are the only distinguishing features between AF and AFS.¹ Although frequent *BRAF* (and rarely also *NRAS* and *KRAS*) mutations have been recognised as driver mutations in ameloblastoma,^{5–7} the molecular pathogenesis of AFS has remained elusive. In this study, we describe a series of seven previously unreported AFS cases with an emphasis on the mutation status of the RAF–RAS pathway.

Materials and methods

The seven cases were retrieved from the consultation files of the authors (A.A., A.F., A.S., and D.B.) and from the surgical pathology files of the University Dental Hospital, Riyadh, Saudi Arabia. Samples were used in accordance with ethical guidelines for the use of retrospective tissue samples provided by the local ethics committee of the Friedrich-Alexander University Erlangen-Nuremberg (ethics committee statements 24.01.2005 and 18.01.2012). Histological diagnosis was based on criteria defined in the most recent World Health Organization classification of head and neck tumours.¹ Assessment of the proliferation fraction was performed with Ki67 (mouse monoclonal anti-human Ki67 antibody; clone MIB-1, dilution 1:100; Dako, Hamburg, Germany). Only unequivocal nuclear staining was considered to be positive. The proliferation fraction (Ki67 index) was counted separately for the bland-looking component (AF) and the malignant component of the tumour. *BRAF* V600E mutation-specific immunohistochemistry was performed with the mouse monoclonal antibody (clone VE1; Spring Biosciences, Indianapolis, IN, USA), on the Ventana Benchmark XT instrument (VentanaRoche Tissue Diagnostics, Tucson, AZ, USA), used according to the manufacturer's specifications—detailed methods are available in Toon *et al.*⁸

MOLECULAR TESTING

To detect gene mutations involving components of the RAS–RAF signalling pathway that have been implicated in the benign counterparts of AFS, i.e. AF, tumour DNA was isolated after manual microdissection of the two components in tumours with a low risk of contamination and from the stromal component only in those tumours with a limited epithelial component. Amplicon-based massive parallel sequencing was performed with a commercial 15-gene panel, the TruSight Tumor 15 (TST15) panel (Illumina, San Diego, CA, USA), and a MiSeq system according to the manufacturer's instructions (Illumina). The 15-gene panel is focused on the detection of hotspot mutations within the coding regions of 15 genes (*AKT1*, *BRAF*, *EGFR*, *ERBB2*, *FOXL2*, *GNA11*, *GNAQ*, *KIT*, *KRAS*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA*, *RET*, and *TP53*) that are frequently altered by mutations in solid tumours. Raw sequencing data were automatically aligned to the human genome (hg19), and the reported variants were annotated with VARIANT STUDIO 3.0 (Illumina).

Results

CLINICAL FEATURES

All tumours presented as progressively enlarging masses (Table 1). One tumour presented during pregnancy (at 2 months of gestation) and was resected postpartum. Two tumours developed following recurrent AF; one 6 years after initial resection of AF, and the other after two recurrences of AF (47 months). One patient (27 years old) developed AFS after three recurrences of an unspecified ameloblastic tumor since age 7. Four of the six tumours with detailed information were located in the mandible, and two were located in the maxilla. All patients underwent surgical resection with clear margins. One patient received unspecified adjuvant chemotherapy. Follow-up was available for five patients, and ranged from 19 months to 344 months (median, 37 months); all were alive without recurrence or metastases. Two patients were lost to follow-up after surgery.

PATHOLOGICAL FINDINGS

Histological examination showed similar features, albeit with significant variations in the degree of atypia and extent of the sarcomatous stromal features (Figure 1A). Significant cellularity and overgrowth of the stromal component were the most striking and remarkable features at low power (Figure 1B). The

epithelial component showed unequivocal ameloblastic features with peripheral palisades of elongated dark-staining cells with reversed polarity surrounding a predominant component of reticulated pale-staining epithelium (Figure 1C). A variable, but no more than mild, degree of atypia with occasional mitoses was seen in the epithelial component, but overt features of malignancy were lacking (Figure 1C). By contrast, the stromal component was predominant and showed overt features of malignancy, including significant cellularity, at least moderate nuclear atypia, and high mitotic activity, and was in some areas indistinguishable from undifferentiated spindle cell sarcoma (Figures 1D–F and 2A–C). Focally, a more rounded cell morphology and variable myxoid stromal changes were noted (Figure 2C,D). Mitotic activity averaged >5 mitoses in 10 high-power fields in all cases. The Ki67 fraction could be determined in the benign-looking and sarcomatous components in four tumours. It showed a striking difference between the AF-like (range, 1–10%; mean, 4%) and the sarcomatous (range, 45–90%; mean, 67%) areas (Figure 2E,F).

MOLECULAR RESULTS

BRAF mutations were detected in five cases, and all represented the known V600E mutation. One case was analysed separately for stromal and epithelial components, and both components showed V600E mutation. The *BRAF* V600E mutation-specific antibody VE1 was tried on all cases, but, probably because of the decalcification process, staining was assessable in only one case (case 2); it revealed specific staining only in the stromal component (Figure 3), confirming *BRAF* mutation as driver of the malignant stromal component, and suggesting that the mutation detected in the epithelial component might be due to contamination with the malignant stroma: this needs verification by the use of laser-microdissected samples in future studies. One case showed the *NRAS* p.Gln61Lys mutation. The seventh case (the only paediatric one in this series) was wild-type. *BRAF* and *NRAS* mutations were found to be mutually exclusive in this study.

Discussion

The precise subtyping of mixed epithelial–mesenchymal odontogenic neoplasms is mainly based on the absence (AF) or presence (odontogenic sarcomas) of morphological features of malignancy in the stromal component. Odontogenic sarcomas are further

subdivided on the basis of the absence (AFS) or presence of specialised hard substance (fibrodentinosarcoma and ameloblastic fibro-odontosarcoma).¹ AFS is considered to be the malignant counterpart of AF.¹ AF is rare accounting for 2% of odontogenic tumours. It is composed of odontogenic ectomesenchyme (recapitulating the dental papilla) admixed with ameloblastic epithelial nests and strands. AFS is distinguished from AF by an overtly malignant stromal component defined by high cellularity, unequivocal nuclear atypia, and increased mitoses.¹ Approximately 25% of AFS cases originate from recurrent AF.³ According to a recent review the mean interval between treatment of AF and the development of AFS was 55.1 months.³ However, it is possible that the literature overestimates the rates of recurrence and/or malignant transformation of AF, as unusual and, particularly, more aggressive cases are more likely to be reported.

Owing to its rarity, AFS remains a poorly characterised orphan disease. AFS is defined by the presence of malignant mesenchymal overgrowth of the stromal component.¹ By definition, the epithelial component of AFS lacks features of malignancy, thus allowing AFS to be distinguished from ameloblastic carcinosarcoma, in which both the epithelial and the mesenchymal components are histologically malignant.¹ However, rarely, mild atypia and mitotic activity may be encountered in the ameloblastic epithelial component of AFS without justifying a diagnosis of ameloblastic carcinosarcoma.⁹

A thorough literature review from 2017 uncovered a total of 289 AFs (279 central and 10 peripheral) and 103 AFSs.³ The authors of that review recognised significant differences between AF and AFS with regard to the mean age of affected patients, expansion and cortical perforation of involved bone, and size of the tumour.³ Local recurrence rates were 19.2% and 12.5% for central and peripheral AF, respectively. For AFS, local recurrence rates were 35% for all cases, 28.8% for primary (*de-novo*) AFS, and 50% for secondary AFS occurring following previous AF.³ The mandible, and mainly its posterior part, is a more common site for AFS than the maxilla (4:1).

Larger lesions and older patients were more often treated with surgical resection for central AF. Segmental resection resulted in the lowest rate of recurrence for most of the lesion types.³ The local recurrence rate was lower for those AFS patients treated with radical segmental resection than for those who received marginal resection. Because of its aggressive clinical course, with an overall death rate related to disease complications of 21.3%, radical

Table 1. Clinicopathological and molecular features of ameloblastic fibrosarcomas (*n* = 7)

No.	Age (year)/sex	Site	Pre-existing benign precursor?	Treatment	Outcome	Ki67 index (%)	TST15 gene panel results	VE1 IHC
1	37/F	Right mandible	Ameloblastoma versus adenomatoid odontogenic tumour at age 31 years, AFS at age 37 years	Surgical resection with free margins	ANER (37 months)	NA	Mesenchymal component: <i>NRAS</i> p.GLN61Lys Epithelial component: failed	NA
2	25/F	Right mandible (completely involved)	Initially AF with two recurrences, AFS 47 months from initial diagnosis	Surgical resection with free margins	Two recurrences of AF, the second with AFS 47 months after initial diagnosis; then lost to follow-up	AF component: 1% AFS: 70%	Mesenchymal component: <i>BRAF</i> V600E Epithelial component: <i>BRAF</i> V600E	Positive in mesenchymal component only
3	23/M	Mandible (bilateral, completely involved)	No	Surgical resection with free margins	ANER (344 months)	NA	<i>BRAF</i> V600E	NA
4	57/F	Left mandible	No	Surgical resection with free margins, adjuvant chemotherapy (not specified)	ANER (193 months)	NA	<i>BRAF</i> V600E	NA
5	26/F	Left maxilla causing massive soft tissue mass and ulceration, root resorption, and displacement of teeth	Focal areas of possible transition from AF	Surgical resection with free margins	Diagnosed during pregnancy, resected after delivery, ANER at 19 months	AF component: 10% AFS: 45%	<i>BRAF</i> V600E	NA
6	16/M	Right maxillary sinus, invaded the soft tissues anteriorly to the maxilla, the ethmoid, and the orbit	Transition from AF	Surgical resection with free margins	ANER (26 months)	AF component: 3% AFS: 90%	<i>BRAF</i> V600E	Not done

Table 1. (Continued)

No.	Age (year)/sex	Site	Not specified	Pre-existing benign precursor?	Treatment	Outcome	Ki67 index (%)	TST15 gene panel results	VE1 IHC
7	27/M			Unspecified ameloblastic tumour since age 7 years (three recurrences)	Surgical resection with free margins	Follow-up not available	AF component: 2% AFS: 65%	All genes wild-type	Not done

AF, Ameloblastic fibroma; AFS, Ameloblastic fibrosarcoma; ANER, Alive with no evidence of recurrence; F, Female; IHC, Immunohistochemistry; M, Male; NA, Not assessable, owing to poor staining quality after decalcification.

segmental resection with free margins is recommended for AFS.³

In this study, which is the first to address the molecular pathogenesis of AFS, we found a high rate of oncogenic *BRAF* mutations (five of seven cases) in AFS. All *BRAF* mutations were the classic *BRAF* V600E mutation. The presence of the *BRAF* mutation in both components of the tumour on separate analysis, and the unequivocal immunoexpression of the mutant *BRAF* VE1 in the stromal component of one case, confirm the presence of *BRAF* mutation in the sarcomatous mesenchymal component of AFS. The question of whether *BRAF* mutation is present in both components of a single tumour could not be conclusively addressed in this study, given the scarcity of the epithelial component in some cases, which precluded reliable separate molecular testing without laser microdissection. These findings make targeted therapy using available *BRAF* inhibitors an option that merits clinical testing in patients with this aggressive rare disease.

Very sparse data are available on the genetic features of AF. Muller *et al.* analysed the DNA ploidy status in AF as compared with that in AFS. They found diploid status in all three AFs, but also in 80% of AFSs.¹⁰ Brunner *et al.* detected *BRAF* V600E mutations in six of 18 ameloblastic fibro-odontomas and in two of five AFs.¹¹ However, microdissected stromal components of four ameloblastic fibro-odontomas and one AF with a known *BRAF* V600E mutation were *BRAF* wild-type.¹¹ The study by Brown *et al.* identified a *BRAF* V600E mutation in one ameloblastic fibro-dentinomas and two AFs.¹² Although the authors reported 100% concordance with the VE1 IHC, there was no mention of the presence or absence of reactivity in the stromal components of the two AFs. A recent study published in Chinese (only abstract reviewed) reported the *BRAF* V600E mutation in 16 of 16 AFs (100%).¹³

According to defined morphological criteria, our cases are consistent with previous observations that at least a subset of AFSs do originate from pre-existing recurring AFs or contain AF-like bland-looking areas indicating development within a background of AF. In our series, five of the seven cases had either AF-like areas ($n = 2$) or developed in a background of previously treated odontogenic tumour (reported as AF in one case, as ameloblastoma versus adenomatoid odontogenic tumour in another case, and as unspecified ameloblastic tumour in the third case).

From a differential diagnostic point of view, AFS should be distinguished from the stroma-rich variant of AF, recurrent AF, and undifferentiated sarcomas. AFS differs from cellular AF by the presence of unequivocal malignant features such as significant

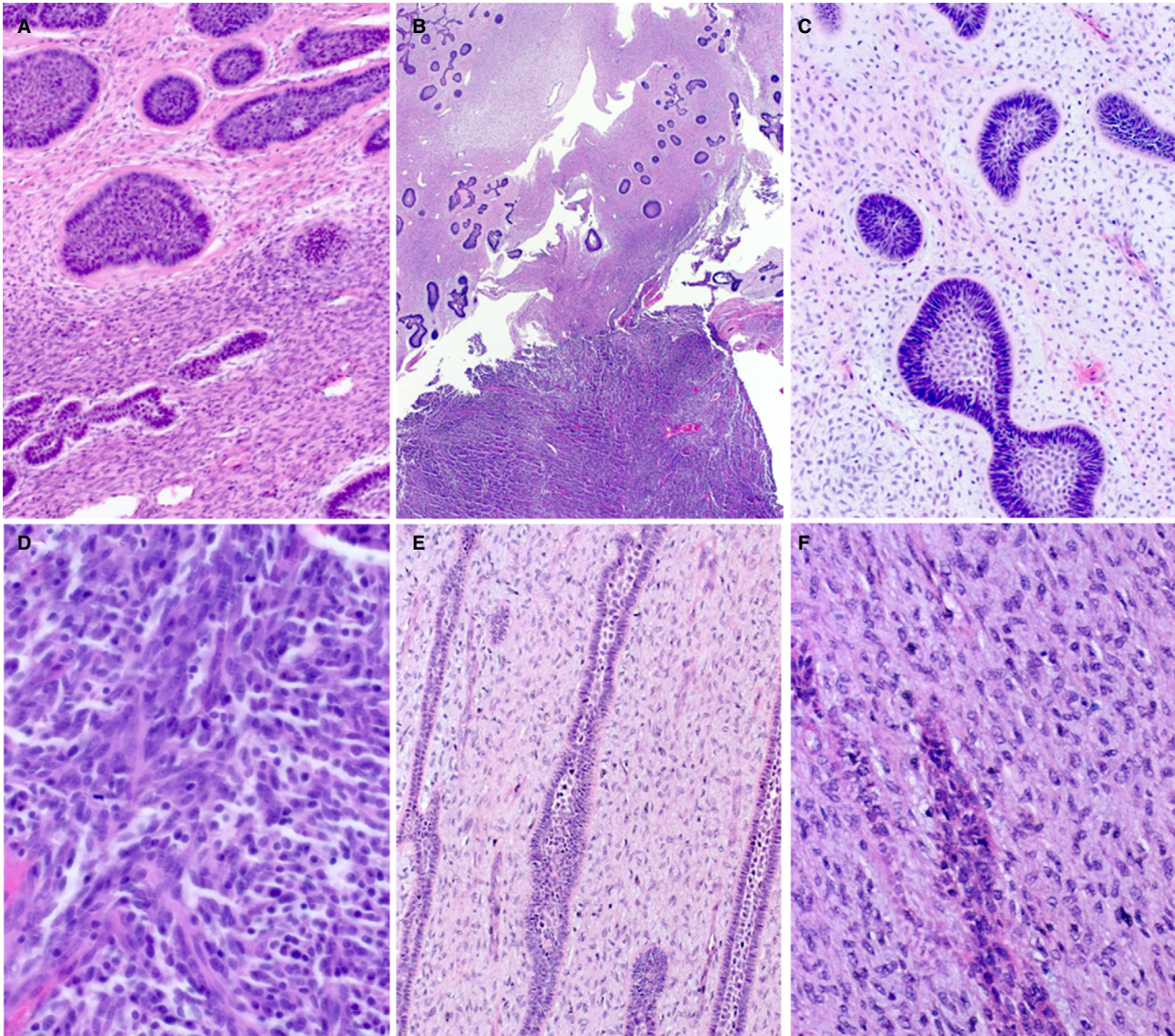


Figure 1. Representative examples of the diverse proportional patterns of odontogenic stroma in ameloblastic fibrosarcoma (AFS). A, Transition from low cellularity to cellular stroma is seen also in the ameloblastic fibroma (AF)-like components without other features of malignancy. B, Transition of AF-like areas (upper field) to highly cellular malignant stromal overgrowth (lower field) is seen in this case. C, Higher magnification of the AF component. D, Higher magnification of the malignant stromal component. E, F, In some areas, stromal overgrowth resulted in separation of parallel arrays of ameloblastic epithelial strands (E), and in other areas it showed disruption of the peripheral palisades of the epithelium partially obscuring the ameloblastic pattern (F).

stromal overgrowth, moderate to high-grade atypia, brisk mitotic activity, and the presence of necrosis. Particularly in recurrences and in tumours with significant stromal overgrowth, the epithelial component may be missing, justifying consideration of unclassified spindle cell sarcoma or spindle cell carcinoma. In contrast to spindle cell carcinoma, AFS lacks a surface epithelial component, cytokeratin expression, and features of true epithelial differentiation, as determined by light microscopic and ultrastructural examination. Distinction from unclassified spindle cell

sarcoma or fibrosarcoma of bone is based mainly on the demonstration of ameloblastic epithelium, an AF component, or a history of AF. Our findings suggest that, in the appropriate clinicopathological context, molecular testing for *BRAF* and *NRAS* mutations in malignant intra-osseous spindle cell lesions might be a useful adjunct in the subtyping of difficult-to-classify sarcomatoid lesions. Central spindle cell rhabdomyosarcoma shows features of a rhabdomyoblastic lineage, including expression of desmin, MyoD1, and, variably, myogenin.¹⁴

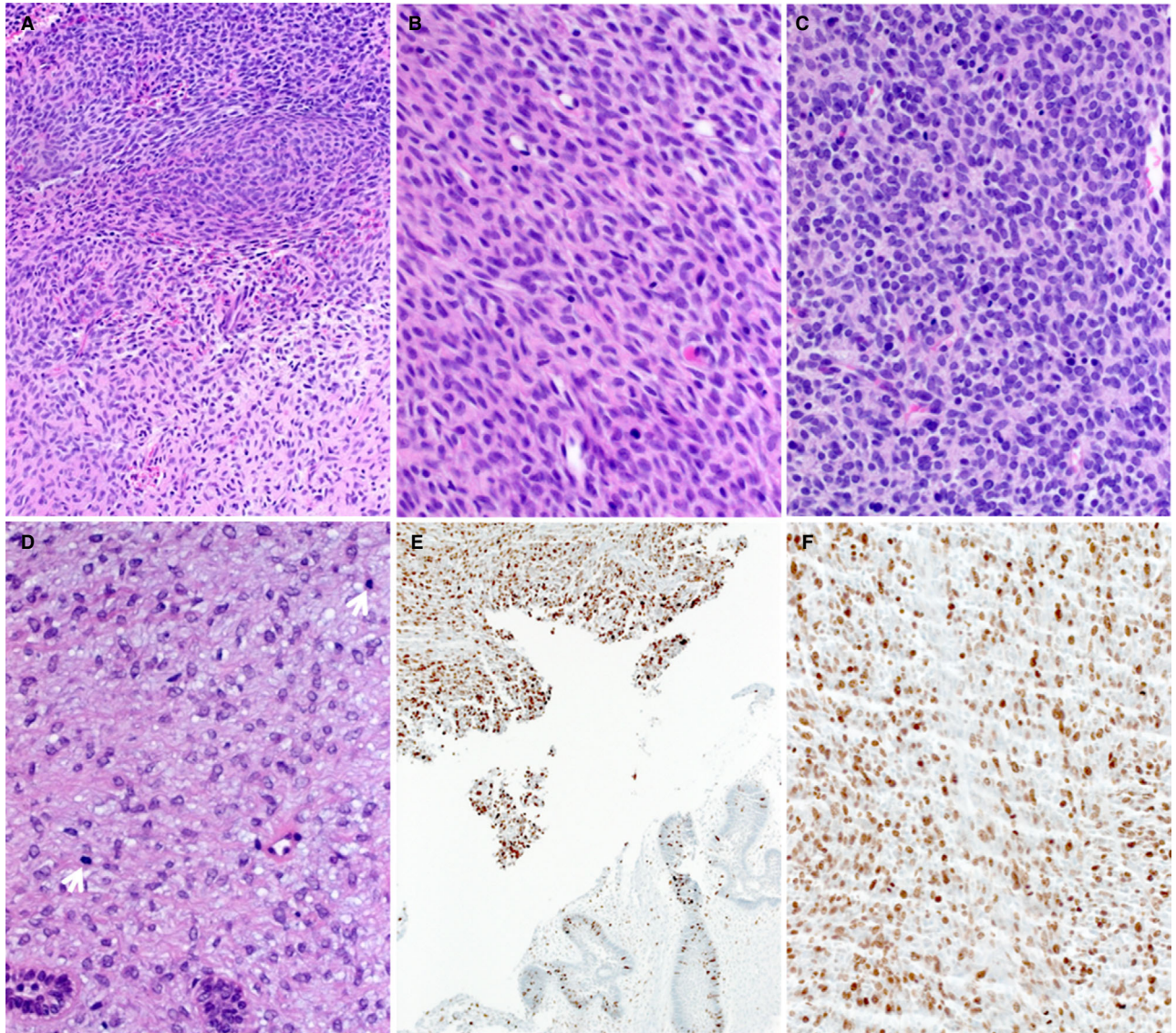


Figure 2. A–D, The stromal component varied from dense whorls closely juxtaposed to hypocellular collagenised areas (A), cellular fibrosarcoma-like (B), small undifferentiated cells (C), to a hypocellular but mitotically active (D, white arrows = mitoses) pattern. E, A striking difference in the Ki67 fraction between the AF-like component (lower field) and the malignant stromal component (upper field). F, Higher magnification of the Ki67 stain in the malignant component of same tumour as in (E).

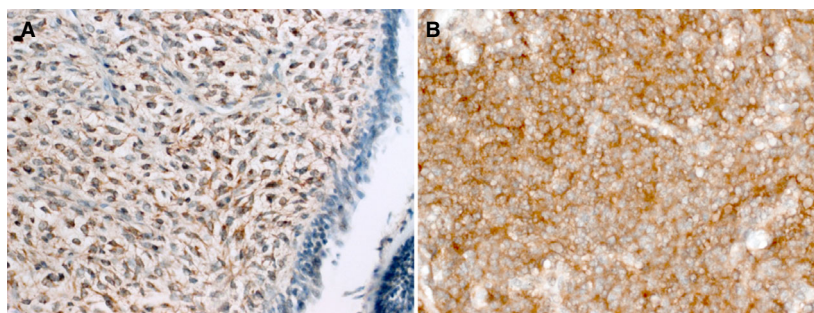


Figure 3. In the one case with successful testing, the VEGF immunohistochemistry showed positive staining in the bland-looking less cellular stroma (A) and the highly cellular malignant stroma (B), but no specific staining in the epithelial component.

In summary, this is the first study of the molecular features of AFS highlighting frequent *BRAF* and occasional *NRAS* mutations. There is no difference in the distribution of *BRAF* mutations among tumours originating from AF or *de novo*. AFS should be tested for these mutations, given the availability of approved promising drugs targeting mutant *BRAF*.

Conflicts of interest

The authors state that they have no conflicts of interest.

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Author contributions

Study conception and design: A. Agaimy and S. Bauer. Data collection and interpretation: A. Agaimy, A. Skalova, A. Franchi, R. Alshagroud, A. J. Gill, R. Stoehr, D. Baumhoer, and S. Bauer. Drafting of the manuscript: A. Agaimy. Discussion of results, critical reading of the manuscript, intellectual editing and comments, and approval of the manuscript: A. Agaimy, A. Skalova, A. Franchi, R. Alshagroud, A. J. Gill, R. Stoehr, D. Baumhoer, and S. Bauer.

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