Head and neck rhabdomyosarcoma with TFCP2 fusions and ALK overexpression

Xu, Bin; Suurmeijer, Albert J. H.; Agaram, Narasimhan P.; Zhang, Lei; Antonescu, Cristina R.

Published in:
Histopathology

DOI:
10.1111/his.14323

Publication date:
2021

Histopathology

Histopathology 2021 DOI: 10.1111/his.14323

Head and neck rhabdomyosarcoma with *TFCP2* fusions and ALK overexpression: a clinicopathological and molecular analysis of 11 cases

Bin Xu,[1] Albert J H Suurmeijer,[2] Narasimhan P Agaram,[1] Lei Zhang[1] & Cristina R Antonescu[1]

[1]*Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA, and* [2]*Department of Pathology and Laboratory Medicine, University Hospital Groningen, Groningen, The Netherlands*

Date of submission 4 December 2020
Accepted for publication 29 December 2020
Published online *Article Accepted* 31 December 2020

Xu B, Suurmeijer A J H, Agaram N P, Zhang L & Antonescu C R (2021) *Histopathology* https://doi.org/10.1111/his.14323

Head and neck rhabdomyosarcoma with *TFCP2* fusions and ALK overexpression: a clinicopathological and molecular analysis of 11 cases

Aims: Primary intraosseous rhabdomyosarcoma (RMS) is a rare entity defined by *EWSR1/FUS–TFCP2* or, less commonly, *MEIS1–NCOA2* fusions. The lesions often show a hybrid spindle and epithelioid phenotype, frequently coexpress myogenic markers, ALK, and cytokeratin, and show a striking propensity for the pelvic and craniofacial bones. The aim of this study was to investigate the clinicopathological and molecular features of 11 head and neck RMSs (HNRMSs) characterised by the genetic alterations described in intraosseous RMS.

Methods and results: The molecular abnormalities were analysed with fluorescence *in-situ* hybridisation and/or targeted RNA/DNA sequencing. Seven cases had *FUS–TFCP2* fusions, four had *EWSR1–TFCP2* fusions, and none had *MEIS1–NCOA2* fusions. All except one case were intraosseous, affecting the mandible (n = 4), maxilla (n = 3), and skull (n = 3). One case occurred in the superficial soft tissue of the neck. The median age was 29 years (range, 16–74 years), with an equal sex distribution. All tumours showed mixed epithelioid and spindle morphology. Immunohistochemical coexpression of desmin, myogenin, MyoD1, ALK, and cytokeratin was seen in most cases. An intragenic *ALK* deletion was seen in 43% of cases. Regional and distant spread were seen in three and four patients, respectively. Two patients died of their disease.

Conclusions: We herein present the largest series of HNRMSs with *TFCP2* fusions to date. The findings show a strong predilection for the skeleton in young adults, although we also report an extraosseous case. The tumours are characterised by a distinctive spindle and epithelioid phenotype and a peculiar immunoprofile, with coexpression of myogenic markers, epithelial markers, and ALK. They are associated with a poor prognosis, including regional or distant spread and disease-related death.

Keywords: ALK, *EWSR1–TFCP2* fusion, *FUS–TFCP2* fusion, intraosseous rhabdomyosarcoma, rhabdomyosarcoma

Introduction

The rhabdomyosarcoma (RMS) classification is still evolving, with ongoing discoveries resulting from the wide application of next-generation sequencing (NGS) in clinical practice. In the last 2020 World Health Organization classification, four types of RMS were recognised: embryonal, alveolar, pleomorphic, and spindle cell/sclerosing.[1] Among them, the spindle cell/sclerosing RMS category has witnessed the most significant molecular advances, being now subdivided into a number of genetic subsets, including

Address for correspondence: C R Antonescu, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. e-mail: antonesc@mskcc.org

congenital/infantile spindle cell RMS associated with various gene fusions involving *VGLL2*, *NCOA1/2*, and *SRF*,[2,3] spindle cell/sclerosing RMS with *MYOD1* mutations,[4] and, finally, intraosseous RMS with *EWSR1/FUS–TFCP2* or *MEIS–NCOA2* fusions.[5]

Primary intraosseous RMS has been only recently defined as an extremely rare variant of RMS with mixed spindle and epithelioid morphology and a multiphenotypic immunoprofile. It was initially recognised as a distinct pathological entity on the basis of its recurrent gene fusions, including *TFCP2* fusions with either *EWSR1* or *FUS*, and less commonly *MEIS–NCOA2* fusions.[5,6] To date, <40 cases of intraosseous RMS with confirmed *FUS/EWSR1–TFCP2* or *MEIS–NCOA2* fusions have been reported.[5–14] Whereas intraosseous RMSs with *MEIS–NCOA2* fusions have been reported exclusively in the pelvic bones, RMSs with *TFCP2* fusions show a striking predilection for a craniofacial intraosseous location, although large series are not yet available. In this study, we gathered a cohort of 11 cases of head and neck RMS (HNRMS) characterised by *TFCP2* fusions, with the aim of investigating the comprehensive clinical, histological, immunophenotypic, and molecular profile of this rare tumour.

Materials and methods

CASE SELECTION AND CLINICOPATHOLOGICAL REVIEW

The study was approved by the institutional review board. Eleven cases of HNRMS harbouring *EWSR1/FUS–TFCP2* fusions were retrieved from the archived pathology files and personal consultation files of the authors. The clinical features and outcomes, i.e. age, sex, site of the primary tumour, follow-up period, treatment, local recurrence, and nodal and distant metastasis, were gathered. All slides were centrally reviewed by B.X. and C.R.A. to collect the pathological and immunophenotypic features of each case. The antibodies used for immunohistochemical studies are summarised in Table S1.

DETECTION OF *EWSR1/FUS–TFCP2* FUSIONS AND OTHER MOLECULAR ANALYSES

The underlying *EWSR1–TFCP2* or *FUS–TFCP2* fusion molecular alterations were investigated with fluorescence *in-situ* hybridisation (FISH) (n = 9), the ARCHER RNA sequencing platform (ArcherDX, Boulder, CO, USA) (n = 3), and/or targeted NGS with either the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform or the FoundationOne platform (Foundation Medicine Inc, Cambridge, MA, USA) (n = 5).

FISH on interphase nuclei from formalin-fixed paraffin-embedded 4-μm sections was performed with custom-designed probes of bacterial artificial chromosomes flanking the target genes *FUS*, *EWSR1*, *TFCP2*, and *ALK*, as previously described.[5,15] Two hundred successive nuclei were examined with a Zeiss Axioplan fluorescence microscope (Zeiss, Oberkochen, Germany), controlled by ISIS 5 software (Metasystems, Waltham, MA, USA). The FISH score was considered to be positive when at least 20% of the nuclei showed a break-apart signal. Nuclei with an incomplete set of signals were omitted from the score.

The ARCHER RNA sequencing platform is a clinical molecular diagnostic assay performed in a Clinical Laboratory Improvement Amendments-accredited laboratory utilising multiplex polymerase chain reaction to detect oncogenic fusion transcripts involving 62 genes as described previously.[9]

Targeted NGS with either MSK-IMPACT or the FoundationOne platform was performed in five cases. MSK-IMPACT is a Food and Drug Administration (FDA)-approved deep-coverage, targeted NGS assay detecting single-nucleotide variants (SNVs), small insertions/deletion (indels), copy number variants, and fusion/structural variants in 468 oncogenes, using custom DNA probes designed for targeted sequencing of all exons and selected introns, including canonical and selected non-canonical transcripts.[16,17] FoundationOne is a commercially available FDA-approved NGS platform detecting SNVs, indels, copy number alterations, and rearrangements in 324 genes.[18]

ALK IMMUNOHISTOCHEMICAL TESTING AND MOLECULAR TESTING FOR *ALK*

Immunohistochemical studies for ALK were performed in all cases. Additionally, molecular alterations of *ALK* were tested in seven cases by the use of FISH (n = 3), the ARCHER RNA sequencing platform (n = 3), MSK-IMPACT (n = 2), and/or the FoundationOne platform (n = 3). In three cases, two or more testing platforms were used.

Results

The clinical, pathological, immunophenotypic and molecular features of the study cohort are

summarised in Table 1. Four cases (cases 5–8 from Table 1) were reported previously by our group.[5,9]

DEMOGRAPHIC AND CLINICAL DATA

HNRMSs with *TFCP2* fusions affected patients of a wide age range, from 16 to 74 years. The median age at diagnosis was 29 years. Two cases (22%) occurred in the paediatric population, affecting a 16-year-old boy and an 18-year-old boy. The male/female ratio was 1:1.2.

Ten of the 11 cases were located intraosseously. The most commonly affected bone was the mandible ($n = 4$, 40%, Figure 1), followed by the maxilla ($n = 3$, 30%), and the skull ($n = 3$, 20%). Computed tomography and/or magnetic resonance imaging were performed at our centre in four patients (cases 2, 7, 8, and 9), and showed large (40–78 mm) heterogeneous expansile lytic intraosseous lesions with destruction of the cortex and soft tissue extension (Figure 1A).

One patient was found to have a *FUS–TFCP2*-fusion positive superficial soft tissue mass involving the dermis and subcutis of the neck/back region, without bone involvement (Figure 2).

HISTOLOGICAL FEATURES

All cases showed a mixture of spindle and epithelioid cytomorphology. The spindle cells formed intervening fascicles (Figure 1C) or were loosely arranged as single cells or small clusters. The epithelioid cells contained abundant, often glassy, eosinophilic cytoplasm arranged as single cells, small clusters, cords, and solid sheets (Figure 1C). The *FUS–TFCP2*-positive superficial soft tissue tumour (case 10) additionally contained areas of small round cell morphology, with uniform round to oval nuclei, scanty cytoplasm, and solid architecture (Figure 2B). No definite rhabdomyoblastic differentiation (i.e. strap cells, concentric paranuclear whorls of filaments, and cross-striations) was seen histologically. Although most of the tumours were composed of uniform monotonous nuclei, two tumours (cases 2 and 3) contained scattered cells with marked nuclear pleomorphism (Figure 1D).

IMMUNOPROFILE

Immunophenotypically, all cases except one showed convincing evidence of rhabdomyoblastic differentiation (Table 1); however, the level of expression varied from rare tumour cells to diffusely positive. Desmin was positive in 10 of the 11 (91%) cases, with a focal staining pattern in three and a diffuse pattern in seven. MyoD1 was positive in all eight cases tested (Figure 1H), and myogenin was positive in nine of 10 tested cases (90%). One case (case 9) was negative for desmin and myogenin; however, no material was available for immunostaining for MyoD1, which was found to be the most sensitive myogenic marker in this subset of RMSs. Moreover, this case was positive for cytokeratin (CK) and ALK, like most other cases in our study group.

Expression of CK AE1/AE3, either diffuse or focal, was commonly seen, being detected in nine cases (82%). The rate of immunopositivity for other keratins was as follows: CAM5.2 was positive in two of four cases tested, CK7 was positive in one of one, CK5/6 was positive in one of two, and CK20 and CK18 were both negative in a single case tested.

ALK was positive in nine of 10 tested cases (90%), mostly in a diffuse cytoplasmic pattern with moderate to strong intensity (Figure 1G).

MOLECULAR PROFILE

Seven cases had *FUS–TFCP2* fusions, whereas the remaining four cases showed *EWSR1–TFCP2* fusions. *MEIS1–NCOA2* fusion, a molecular alteration that has previously been reported in intraosseous RMS,[5] was not detected in any HNRMS. The *FUS–TFCP2* rearrangement was confirmed by use of the ARCHER RNA sequencing platform in three cases, all of which showed an in-frame fusion between *FUS* exon 6 and *TFCP2* exon 2.

Among the seven cases that were tested for *ALK* alterations with MSK-IMPACT ($n = 2$), the FoundationOne platform ($n = 3$), the ARCHER RNA sequencing platform ($n = 3$), and/or FISH ($n = 3$), *ALK* deletion was detected in three (43%) cases. The platforms used to detect *ALK* deletion in these three cases were: the FoundationOne platform ($n = 1$), the ARCHER RNA sequencing ($n = 1$), and MSK-IMPACT ($n = 1$). The three different *ALK* deletions identified spanned from intron 1 to intron 16, from exon 2 to exon 17, and from exon 2 to exon 19, respectively. None of the deletions included the kinase domain of ALK, which corresponds to exons 22–25. The remaining four cases did not show an *ALK* alteration. ALK was immunohistochemically positive in nine of 10 tested cases, and it was negative in one of the three cases with an *ALK* intragenic deletion.

All five cases subjected to MSK-IMPACT or FoundationOne NGS sequencing (cases 2, 7, 9, 10, and 11) additionally showed *CDKN2A* loss.

Table 1. Clinicopathological, immunophenotypic and molecular findings of the study cohort

Case no.	Age (years) /sex	Location	Cytological features	Myogenin	MyoD1	Desmin	CK AE1/3	ALK IHC	Molecular alterations	Metastasis	Outcome
1	22/M	Mandible	Spindle and epithelioid	Positive (focal)	Positive	Positive (focal)	Positive	Positive	*FUS–TFCP2* *ALK*: WT	Lymph node	NA
2	34/M	Mandible	Spindle, epithelioid, and rhabdoid	NA	Positive (patchy)	Positive	Negative	Negative	*FUS–TFCP2* *ALK* deletion (exons 2–17)	No	AWD (10 months)
3	16/M	Mandible	Spindle, epithelioid, and rhabdoid	Positive (focal)	Positive (focal)	Positive (focal)	Positive	Positive	*FUS–TFCP2* *ALK* deletion (exons 2–19)	Bone, lung, lymph node	DOD (20 months)
4	43/F	Mandible	Spindle and epithelioid	Positive (rare cells)	Positive	Positive	Positive	Positive	*FUS–TFCP2* *ALK*: ND	NA	NA
5*	20/F	Maxilla	Spindle and epithelioid	Positive	NA	Positive (focal)	Positive	Positive	*EWSR1–TFCP2* *ALK*: ND	Bone	NA
6*	33/F	Maxilla	Spindle and epithelioid	Positive	Positive	Positive (focal)	Positive	Positive	*EWSR1–TFCP2* *ALK*: ND	NA	NED (108 months)
7*	74/F	Maxilla/ gingiva	Spindle and epithelioid	Positive (focal)	Positive (patchy)	Positive	Negative	Positive	*FUS–TFCP2* *ALK*: WT	Lymph node	DOD (21 months)
8*	27/F	Skull	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	Positive	*EWSR1–TFCP2* *ALK*: ND	Bone	AWD (1 month)
9	18/M	Skull	Spindle and epithelioid	Negative	NA	Negative	Positive	Positive	*FUS–TFCP2* *ALK*: WT	NA	NA
10	29/M	Skull (base)	Spindle and epithelioid	Positive	Positive	Positive	Positive	NA	*EWSR1–TFCP2* *ALK*: WT	Lung	AWD (2 months)
11	40/F	Neck superficial soft tissue	Spindle, epithelioid, and round	Positive (rare cells)	NA	Positive	Positive	Positive	*FUS–TFCP2* *ALK* deletion (introns 1–16)	NA	NA

AWD, alive with disease; CK, cytokeratin; DOD, dead of disease; F, female; IHC, immunohistochemistry; M, male; NA, not available; ND, not done; NED, no evidence of disease; WT, wild type.

*Cases 5–8 were reported previously.[5,9]

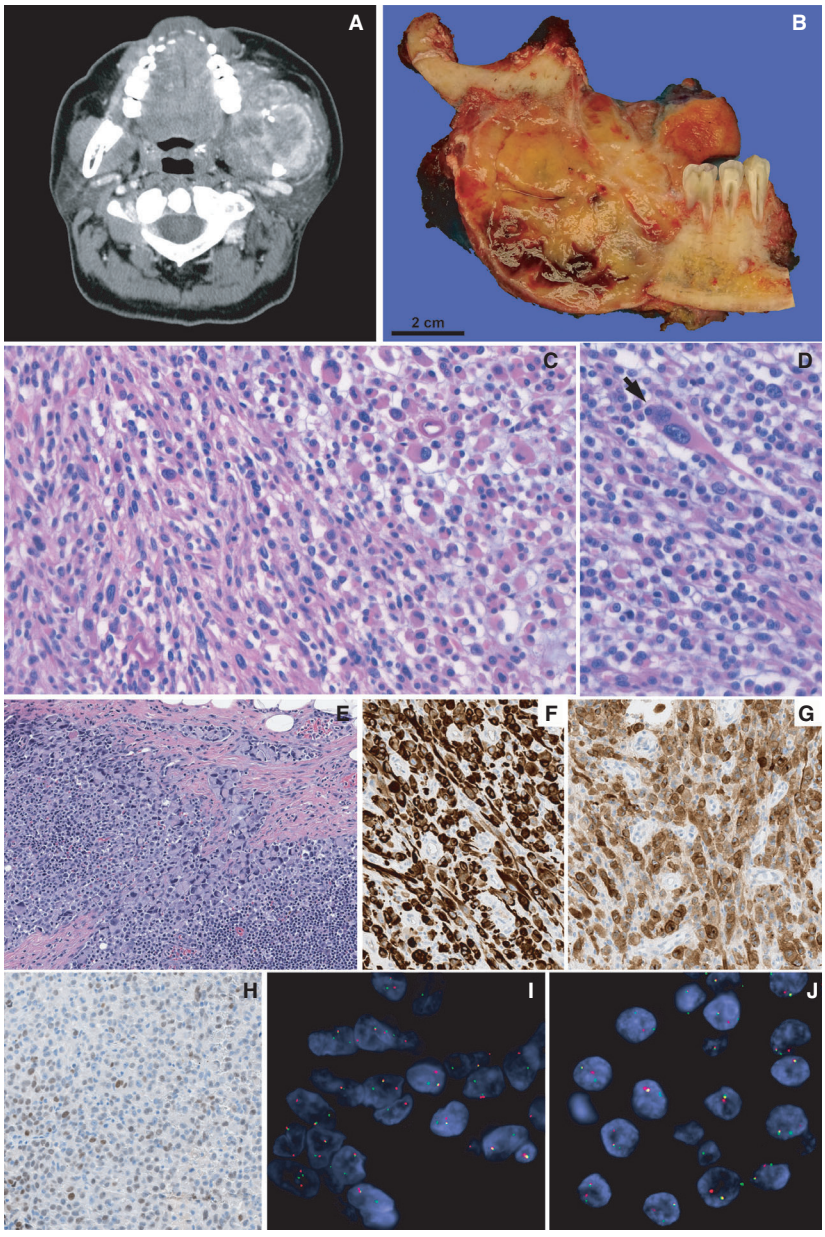

Figure 1. An intraosseous rhabdomyosarcoma of the mandible with *FUS–TFCP2* fusion in a 34-year-old man. **A**, A computed tomography scan shows a large lytic destructive lesion centred on the posterior body, angle, and ramus of the left mandible with expansile soft tissue extension. **B**, Grossly, the tumour has a tan–yellow soft cut surface. Areas of haemorrhage, bone destruction, and soft tissue extension are evident. **C**, Histologically, the tumour is highly cellular, showing hybrid spindle (left) and epithelioid (right) cytological features. The spindle cells form loose intervening fascicles, whereas the epithelioid cells contain eccentric nuclei and abundant eosinophilic cytoplasm. **D**, Scattered tumour cells show marked nuclear pleomorphism (arrows). **E**, Nodal metastasis to regional cervical lymph nodes was present at the time of primary resection. **F–H**, The tumour is diffusely positive for desmin (**F**) and ALK (**G**), and focally positive for MyoD1 (**H**). **I,J**, Fluorescence *in-situ* hybridisation with custom break-apart probes for *FUS* (**I**) and *TFCP2* (**J**) demonstrates split signals, in keeping with gene rearrangements (red, centromeric; green, telomeric).

TREATMENT AND CLINICAL OUTCOME

Four patients had undergone lymph node sampling or dissection at the time of initial diagnosis. Among them, two (cases 1 and 7) had lymph node metastasis (Figure 1E). Additionally, one patient developed regional lymph node recurrence (case 3).

Six patients had follow-up data available. Among them, three patients developed local recurrence 7, 10, and 11 months after the initial resection with

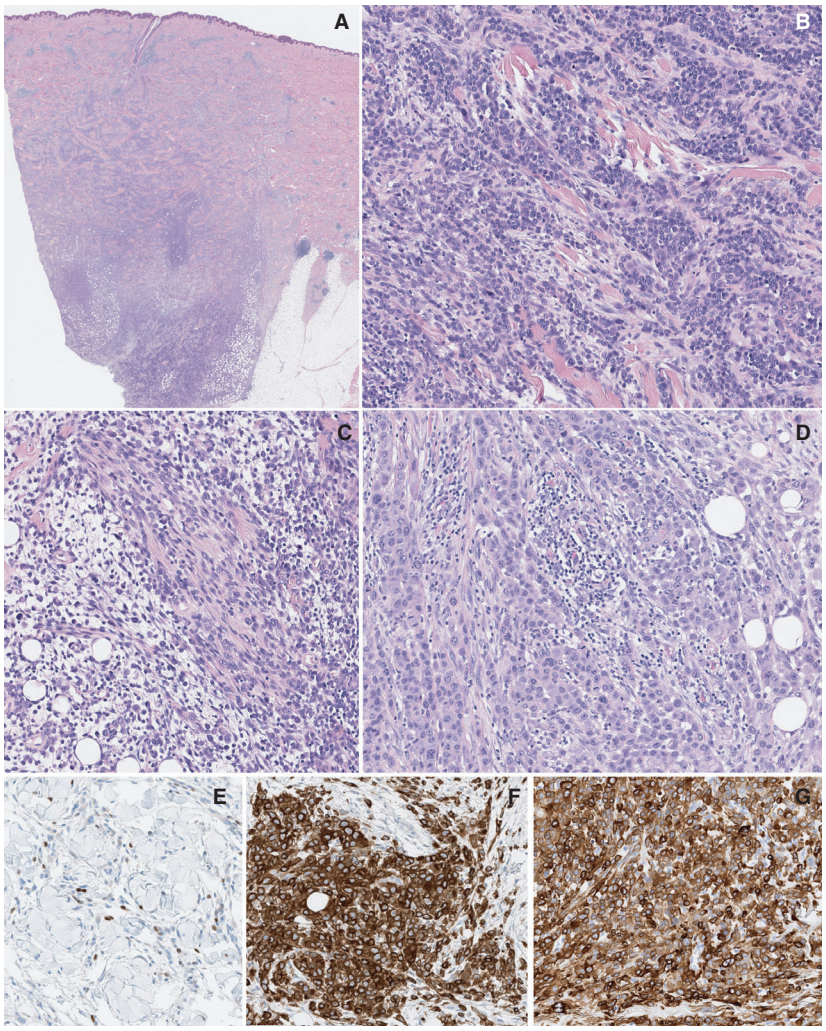

Figure 2. A rhabdomyosarcoma with *FUS–TFCP2* fusion originated in the superficial soft tissue of the neck. **A**, This hypercellular tumour shows infiltrative growth within the dermis and subcutis. **B–D**, It is associated with areas of round (**B**), spindle (**C**), and epithelioid (**D**) morphology. **E**, Rare cells are positive for myogenin. **F**,**G**, Desmin (**F**) and cytokeratin AE1/AE3 (**G**) are diffusely and strongly positive in this tumour.

curative intent, two patients had incomplete initial resection, and one patient was disease-free without evidence of recurrence after 105 months.

Four patients received adjuvant chemoradiation therapy, including three treated with a vincristine, dactinomycin, and cyclophosphamide regimen and one with a vincristine, dactinomycin, and ifosfamide (VAI) regimen.

Four patients developed distant metastases to bone ($n = 3$) and/or lung ($n = 2$), including two patients with metastasis to other bones or lung within a month of the initial presentation (cases 5, 8, and 9), and another patient developed metastases to bone and lung 14 months after the initial resection (case 3).

Two patients died of their disease 20 and 21 months after the initial diagnosis. Both tumours had *FUS–TFCP2* fusions. A 16-year-old male patient with a mandibular RMS died of local recurrence and disease that was widely metastatic to the lung, lymph node, and vertebra, and was unresponsive to chemoradiation therapy with the VAI regimen (case 3); a 74-year-old woman with a maxillary tumour died of local recurrence (case 7).

Discussion

A literature review of HNRMSs with *TFCP2* fusions is provided in Table 2. To date (including the current

Table 2. Literature review: head and neck rhabdomyosarcoma with *TFCP2* fusions

Reference	Age (years) /sex	Location	Cytological features	Myogenin	MyoD1	Desmin	CK AE1/3	Other positive keratins	ALK IHC	*TFCP2* fusion	Metastasis	Outcome
Dashti *et al.*[6]	72/M	Mandible	Spindle	Positive (limited)	Positive (+++)	Positive (+++)	Positive (+++)	CAM5.2	Positive (+++)	*FUS–TFCP2*	NA	NA
Le Loarer *et al.*[7]	32/M	Mandible	Spindle	Positive (focal)	Positive	Positive (focal)	Positive	NA	Positive	*FUS–TFCP2*	Lung	AWD (14 months)
Le Loarer *et al.*[7]	58/F	Mandible	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive	*FUS–TFCP2*	No	NED (21 months)
Le Loarer *et al.*[7]	12/F	Mandible	Spindle and epithelioid	Positive	Positive	Positive	Positive	NA	Positive	*FUS–TFCP2*	No	NED (21 months)
Le Loarer *et al.*[7]	25/M	Mandible	Epithelioid	Negative	Positive	Negative	Positive	NA	Positive	*EWSR1–TFCP2*	No	NED (20 months)
Koutlas *et al.*[8]	15/M	Mandible	Spindle, epithelioid, and round	Positive (focal)	Positive	Positive	Positive	NA	Positive	*EWSR1–TFCP2*	Lymph node, bone	AWD (NA)
This study	22/M	Mandible	Spindle and epithelioid	Positive (focal)	Positive	Positive (focal)	Positive	NA	Positive	*FUS–TFCP2*	Lymph node	NA
This study	34/M	Mandible	Spindle, epithelioid, and rhabdoid	NA	Positive (patchy)	Positive	Negative	NA	Negative	*FUS–TFCP2*	No	AWD (10 months)
This study	16/M	Mandible	Spindle, epithelioid, and rhabdoid	Positive (focal)	Positive (focal)	Positive (focal)	Positive	NA	Positive	*FUS–TFCP2*	Bone, lung, lymph node	DOD (20 months)
This study	43/F	Mandible	Spindle and epithelioid	Positive (rare cells)	Positive	Positive	Positive	NA	Positive	*FUS–TFCP2*	NA	NA
Le Loarer *et al.*[7]	11/F	Maxilla	Epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive (5%)	*EWSR1–TFCP2*	No	DOD (NA)
This study; Agaram *et al.*[5]	20/F	Maxilla	Spindle and epithelioid	Positive	NA	Positive (focal)	Positive	Positive	Positive	*EWSR1–TFCP2*	Bone	NA
This study; Agaram *et al.*[5]	33/F	Maxilla	Spindle and epithelioid	Positive	Positive	Positive (focal)	Positive	CK5/6	Positive	*EWSR1–TFCP2*	NA	NED (108 months)

Table 2. (*Continued*)

Reference	Age (years) /sex	Location	Cytological features	Myogenin	MyoD1	Desmin	CK AE1/3	Other positive keratins	ALK IHC	*TFCP2* fusion	Metastasis	Outcome
This study; Zhu *et al.*[9]	74/F	Maxilla/ gingiva	Spindle and epithelioid	Positive (focal)	Positive (patchy)	Positive	Negative	CK7	Positive	*FUS–TFCP2*	Lymph node	DOD (21 months)
Le Loarer *et al.*[7]	32/M	Palate/lip	Spindle and epithelioid	NA	Positive	Positive	Positive	NA	Negative	*EWSR1–TFCP2*	No	DOD (8 months)
Wong *et al.*[10]; Lewin *et al.*[11]	23/M	Nasal	Spindle and epithelioid	Positive (rare cells)	Positive	Positive (patchy)	Negative	NA	Positive (3+)	*FUS–X*	NA	NA
Le Loarer *et al.*[7]	16/F	Sphenoid bone	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive	*FUS–TFCP2*	Bone	DOD (15 months)
Watson *et al.*[12]	NA (16–25)/F	Sphenoid bone	Epithelioid	Positive	Positive	NA	NA	NA	Positive	*FUS–TFCP2*	NA	DOD (<5 months)
This study; Agaram *et al.*[5]	27/F	Skull	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive	*EWSR1–TFCP2*	Bone	AWD (1 month)
Chrisinger *et al.*[13]	Mid-20s to 30s/F	Skull	Spindle and epithelioid	Negative	NA	Positive (focal)	Positive	CK7	Positive (patchy)	*EWSR1–TFCP2*	Bone	DOD (17 months)
This study	18/M	Skull	Spindle and epithelioid	Negative	NA	Negative	Positive	NA	Positive	*FUS–TFCP2*	NA	NA
This study	29/M	Skull	Spindle and epithelioid	Positive	Positive	Positive	Positive	CAM5.2	NA	*EWSR1–TFCP2*	Lung	AWD (2 months)
Brunac *et al.*[14]	16/F	Craniovertebral junction	Epithelioid	Positive	Positive	Positive	Negative	CK7	Positive	*FUS–TFCP2*	NA	AWD (19 months)
Le Loarer *et al.*[7]	20/M	Orbito-temporal-sphenoid	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive (<5%)	*FUS–TFCP2*	No	DOD (6 months)
Le Loarer *et al.*[7]	17/F	Cervico-occipital junction	Round	Negative	Positive	Positive (focal)	NA	NA	Positive	*FUS–TFCP2*	No	AWD (15 months)
Le Loarer *et al.*[7]	31/M	Occipital bone	Spindle and epithelioid	Positive (focal)	Positive	Positive	Positive	NA	Positive	*FUS–TFCP2*	Lung, mediastinum	DOD (6 months)
This study	40/F	Neck superficial soft tissue	Spindle, epithelioid, and round	Positive (rare cells)	NA	Positive	Positive	CAM5.2	Positive	*FUS–TFCP2*	NA	NA

AWD, alive with disease; CK, cytokeratin; DOD, dead of disease; F, female; IHC, immunohistochemistry; M, male; NA, not available; NED, no evidence of disease.

study), 27 cases of HNRMS with *TFCP2* fusions have been reported.[5–14] The tumours mostly affect young adults but may also occur in elderly patients. The median age at diagnosis is 25 years (range, 11–74 years). Paediatric patients (defined as 21 years of age or younger) account for 37% of all cases. There is a slight male predominance, with a male/female ratio of 1.25:1.[5–14]

All but one case (96%) had an intraosseous component.[5–14] The mandible was the most common site of the tumour. The bones affected, in descending order, were the mandible (n = 10, 37%), the maxilla (n = 4, 15%), the skull (n = 4, 15%), the sphenoid bone (n = 2, 7%), the occipital bone (n = 1, 4%), the palate (n = 1, 4%), the nasal cavity (n = 1, 4%), the orbito-temporal-sphenoid bone (n = 1, 4%), the craniovertebral junction (n = 1, 4%), and the cervico-occipital junction (n = 1, 4%).

We herein report the first case of HNRMS with *TFCP2* fusion occurring in the superficial soft tissue of the neck/back without bone involvement as determined by radiological and histological examination. This case represents the second extraskeletal *TFCP2* fusion-positive RMS reported. The initial case occurred in the inguinal soft tissue of an 86-year-old male,[7] thus expanding the anatomical location of this tumour beyond osseous sites.

Histologically, the majority of cases (20/27, 74%) showed a mixed spindle and epithelioid phenotype.[5–14] Pure epithelioid or spindle features were present in four (15%) and two (7%) cases, respectively. Two cases (7%) also contained round cell morphology, either in its pure form (n = 1) or admixed with spindle and epithelioid areas (n = 1). Herein, we also report two cases with marked nuclear pleomorphism.

Immunophenotypically, all reported tumours showed evidence of myogenic differentiation. The rates of positivity for myogenin, MyoD1, and desmin were 84% (21/25), 100% (25/25), and 92% (24/26), respectively.[5–14] It is worth mentioning that the extent of myogenin positivity may be limited, being seen in rare tumour cells (n = 3) or focally (n = 12) within the tumour. In contrast, MyoD1 immunoexpression and desmin immunoexpression were commonly more extensive or diffuse. Therefore, a combination of immunohistochemical studies using myogenin, MyoD1, and desmin may be needed in small biopsy material to establish rhabdomyoblastic differentiation.

RMSs associated with *TFCP2* fusions are characteristically positive for CK AE1/AE3, many with diffuse and strong staining patterns. The rate of CK AE1/AE3 positivity in this tumour is 88% (21/24).[5–14] Epithelial membrane antigen and other keratins, including CK7, CAM5.2, and CK5/6, can also be positive in this tumour. RMS with *TFCP2* fusion joins an expanding spectrum of mesenchymal tumours showing keratin positivity (e.g. alveolar rhabdomyosarcoma,[19] adamantinoma-like Ewing sarcoma,[20] and mesenchymal tumour with *GLI1* alteration[21]), challenging the traditional view that keratin positivity can distinguish epithelial neoplasms from mesenchymal tumours.

Overexpression of ALK has been detected at the transcriptional and protein levels in RMSs with *TFCP2* fusions.[7] Among the 26 reported HNRMSs with *TFCP2* fusions and with ALK immunostaining results, 23 (88%) were shown to be immunohistochemically positive for ALK, including 20 cases with diffuse staining patterns.[5–14]

In our cohort, 43% (3/7) of tumours showed intragenic deletion of *ALK*. Similarly, Wong *et al.* reported a large *ALK* deletion skipping exons 1–16.[10] Using array comparative genomic hybridisation (aCGH), Le Loarer *et al.*[7] also showed that *TFCP2* fusion-positive RMSs frequently harbour coexisting *ALK* genomic deletions. Together, these data suggest that the observed overexpression of ALK may be a result of a truncated isoform originating from intragenic deletion of *ALK*.

Given the presence of ALK up-regulation in this tumour, ALK inhibitors that suppress kinase activity have been used as potential targeted therapies in two patients harbouring RMSs with *TFCP2* fusions.[11,14] The treatment effects were inconclusive: one patient had no response to crizotinib treatment,[11] whereas the other showed a partial response and stable disease when treated with radiation therapy and the ALK inhibitors crizotinib, alectinib, and lorlatinib, after two cycles of vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide chemotherapy had failed.[14]

The fusion partners for HNRMS with *TFCP2* translocation are *FUS* (in our cohort, 7/11, 64%; in all reported cases, 18/27, 67%) and *EWSR1* (in our cohort, 4/11, 36%; in all reported cases, 9/27, 33%).[5–14] *TFCP2* encodes the LSF oncoprotein, which is a transcription factor that functions as a coactivator for YAP, a key transcription factor downstream of the Hippo and Wnt signalling pathway.[22,23] LSF overexpression has been observed in multiple cancers, e.g. hepatocellular, breast, pancreatic, and colorectal carcinomas, and is associated with a poor prognosis.[22,24] Factor quinolinone inhibitors (FQIs), which constitute a family of small-molecule inhibitors of

LSF, was recently identified to inhibit tumour growth of hepatocellular carcinoma *in vitro* and in animal models.[22] It remains to be determined whether patients with RMSs with *TFCP2*-based translocations will benefit from targeted FQI strategies.

MEIS1–NCOA2 fusion, a molecular event that has recently been described in intraosseous RMS,[5] has not been reported in intraosseous HNRMS (including our cohort).

We found *CDKN2A* deletion in all cases tested with the MSK-IMPACT NGS platform, confirming the initial results of *CDKN2A* homozygous deletion in *TFCP2* fusion-positive RMS found by Le Loarer *et al.* using an aCGH platform.[7]

Like other types of RMS, in particular alveolar RMS,[1,25,26] RMS with *TFCP2* fusion has the potential to spread both lymphatically to regional lymph nodes and haematogenously to distant sites, such as the lung and other bones. Among the 19 patients with *TFCP2*-translocated HNRMS with metastasis data available in the literature,[5–14] four (22%) patients developed regional lymph node metastasis and nine (47%) developed distant metastases to other bones and/or the lungs. Overall, HNRMS with *TFCP2* fusion has a dismal prognosis. Among the 19 patients with documented relatively short follow-up (1–108 months), nine (47%) suffered disease-related death.[5–14] The 1- and 2-year disease-specific survival rates of patients with HNRMS with *TFCP2* fusion calculated on the basis of reported cases with follow-up data are 74% and 35%, respectively.[5–14]

Conclusions

HNRMS with *TFCP2*-related fusions is a rare RMS subtype, with a predilection for skeletal involvement, in particular for the mandibular and maxillary bones. Although this genetic alteration appears to be prone to develop within the bone microenvironment, we also report herein a *TFCP2* fusion-positive HNRMS originating in the superficial soft tissue. The tumours show unique pathological characteristics, including their mixed spindle and epithelioid morphology and polyphenotypic immunoprofile, that distinguish them from other spindle cell RMS molecular variants. These distinct pathological and molecular features are in support of a novel, stand-alone RMS genetic category, separate from all other histological subtypes. The clinical impact of ALK overexpression remains undetermined, as the underlying recurrent genetic alteration consists of large intragenic deletions, which spare ALK kinase domain. Moreover, associated *CDKN2A* deletions were detected in all cases tested, and may contribute to its aggressive pathogenesis. The limited follow-up available showed its propensity for distal and regional lymph node metastasis, and a dismal outcome.

Acknowledgements

The research reported in this publication was supported in part by Cancer Center Support Grants of the National Institutes of Health/National Cancer Institute under award numbers P30CA008748 (B. Xu, N. P. Agaram, and C. R. Antonescu), P50 CA140146 (C. R. Antonescu), and P50 CA217694 (C. R. Antonescu), and Cycle for Survival (C. R. Antonescu). The content is solely the responsibility of the authors, and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest

The authors state that they have no competing financial interests.

Author contributions

B. Xu: reviewed cases, managed the database, and drafted the manuscript. A. J. H. Suurmeijer: provided cases and edited the manuscript. N. P. Agaram: providing cases and edited the manuscript. L. Zhang: performed FISH. C. R. Antonescu: designed the study, provided and reviewed cases, and edited the manuscript.

References

1. WHO Classification of Tumours Editorial Board eds. *World Health Organization classification of tumours of soft tissue and bone*. 5th ed. Lyon: IARC Press, 2020.
2. Alaggio R, Zhang L, Sung YS *et al.* A molecular study of pediatric spindle and sclerosing rhabdomyosarcoma: identification of novel and recurrent *VGLL2*-related fusions in infantile cases. *Am. J. Surg. Pathol.* 2016; **40**; 224–235.
3. Karanian M, Pissaloux D, Gomez-Brouchet A *et al. SRF-FOXO1* and *SRF-NCOA1* fusion genes delineate a distinctive subset of well-differentiated rhabdomyosarcoma. *Am. J. Surg. Pathol.* 2020; **44**; 607–616.
4. Agaram NP, LaQuaglia MP, Alaggio R *et al. MYOD1*-mutant spindle cell and sclerosing rhabdomyosarcoma: an aggressive subtype irrespective of age. A reappraisal for molecular classification and risk stratification. *Mod. Pathol.* 2019; **32**; 27–36.
5. Agaram NP, Zhang L, Sung YS *et al.* Expanding the spectrum of intraosseous rhabdomyosarcoma: correlation between 2

distinct gene fusions and phenotype. *Am. J. Surg. Pathol.* 2019; **43**; 695–702.
6. Dashti NK, Wehrs RN, Thomas BC *et al*. Spindle cell rhabdomyosarcoma of bone with *FUS–TFCP2* fusion: confirmation of a very recently described rhabdomyosarcoma subtype. *Histopathology* 2018; **73**; 514–520.
7. Le Loarer F, Cleven AHG, Bouvier C *et al*. A subset of epithelioid and spindle cell rhabdomyosarcomas is associated with *TFCP2* fusions and common ALK upregulation. *Mod. Pathol.* 2020; **33**; 404–419.
8. Koutlas IG, Olson DR, Rawwas J. FET(EWSR1)-TFCP2 rhabdomyosarcoma: an additional example of this aggressive variant with predilection for the gnathic bones. *Head Neck Pathol.* 2020. https://doi.org/10.1007/s12105-020-01189-1
9. Zhu G, Benayed R, Ho C *et al*. Diagnosis of known sarcoma fusions and novel fusion partners by targeted RNA sequencing with identification of a recurrent *ACTB-FOSB* fusion in pseudomyogenic hemangioendothelioma. *Mod. Pathol.* 2019; **32**; 609–620.
10. Wong DD, van Vliet C, Gaman A, Giardina T, Amanuel B. Rhabdomyosarcoma with FUS re-arrangement: additional case in support of a novel subtype. *Pathology* 2019; **51**; 116–120.
11. Lewin J, Desai J, Smith K, Luen S, Wong D. Lack of clinical activity with crizotinib in a patient with FUS rearranged rhabdomyosarcoma with ALK protein overexpression. *Pathology* 2019; **51**; 655–657.
12. Watson S, Perrin V, Guillemot D *et al*. Transcriptomic definition of molecular subgroups of small round cell sarcomas. *J. Pathol.* 2018; **245**; 29–40.
13. Chrisinger JSA, Wehrli B, Dickson BC *et al*. Epithelioid and spindle cell rhabdomyosarcoma with FUS-TFCP2 or EWSR1-TFCP2 fusion: report of two cases. *Virchows Arch.* 2020; **477**; 725–732.
14. Brunac AC, Laprie A, Castex MP *et al*. The combination of radiotherapy and ALK inhibitors is effective in the treatment of intraosseous rhabdomyosarcoma with *FUS-TFCP2* fusion transcript. *Pediatr. Blood Cancer* 2020; **67**; e28185.
15. Chang JC, Zhang L, Drilon AE *et al*. Expanding the molecular characterization of thoracic inflammatory myofibroblastic tumors beyond *ALK* gene rearrangements. *J. Thorac. Oncol.* 2019; **14**; 825–834.
16. Morris LGT, Chandramohan R, West L *et al*. The molecular landscape of recurrent and metastatic head and neck cancers: insights from a precision oncology sequencing platform. *JAMA Oncol.* 2017; **3**; 244–255.
17. Cheng DT, Mitchell TN, Zehir A *et al*. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J. Mol. Diagn.* 2015; **17**; 251–264.
18. FDA Summary of Safety and Effectiveness Data of FoundationOne CDx™. 2017. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf. Accessed 21 October 2019.
19. Thompson LDR, Jo VY, Agaimy A *et al*. Sinonasal tract alveolar rhabdomyosarcoma in adults: a clinicopathologic and immunophenotypic study of fifty-two cases with emphasis on epithelial immunoreactivity. *Head Neck Pathol.* 2018; **12**; 181–192.
20. Bishop JA, Alaggio R, Zhang L, Seethala RR, Antonescu CR. Adamantinoma-like Ewing family tumors of the head and neck: a pitfall in the differential diagnosis of basaloid and myoepithelial carcinomas. *Am. J. Surg. Pathol.* 2015; **39**; 1267–1274.
21. Xu B, Chang K, Folpe AL *et al*. Head and neck mesenchymal neoplasms with GLI1 gene alterations: a pathologic entity with distinct histologic features and potential for distant metastasis. *Am. J. Surg Pathol.* 2020; **44**; 729–737.
22. Willoughby JLS, George K, Roberto MP *et al*. Targeting the oncogene LSF with either the small molecule inhibitor FQI1 or siRNA causes mitotic delays with unaligned chromosomes, resulting in cell death or senescence. *BMC Cancer* 2020; **20**; 552.
23. Zhang X, Sun F, Qiao Y *et al*. TFCP2 is required for YAP-dependent transcription to stimulate liver malignancy. *Cell Rep.* 2017; **21**; 1227–1239.
24. Kotarba G, Krzywinska E, Grabowska AI, Taracha A, Wilanowski T. TFCP2/TFCP2L1/UBP1 transcription factors in cancer. *Cancer Lett.* 2018; **420**; 72–79.
25. Rhee DS, Rodeberg DA, Baertschiger RM *et al*. Update on pediatric rhabdomyosarcoma: a report from the APSA Cancer Committee. *J. Pediatr. Surg.* 2020; **55**; 1987–1995.
26. Leiner J, Le Loarer F. The current landscape of rhabdomyosarcomas: an update. *Virchows Arch.* 2020; **476**; 97–108.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Antibodies for immunohistochemical studies.