HHS Public Access

Author manuscript

Genes Chromosomes Cancer. Author manuscript; available in PMC 2021 May 01.

Published in final edited form as:

Genes Chromosomes Cancer. 2020 May ; 59(5): 286–294. doi:10.1002/gcc.22828.

Ewing sarcoma with *FEV* gene rearrangements is a rare subset with predilection for extra-skeletal locations and aggressive behavior

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Abstract

The molecular hallmark of Ewing sarcoma (ES) is a fusion involving the *EWSR1* gene and a member of the ETS family of transcription factors. *EWSR1-FLI1* is the most common variant, occurring in 90% of cases, followed by *EWSR1-ERG*. In rare cases the *FUS* gene can substitute for *EWSR1* in these fusions. Only rare case reports have been described to date of ES with *FEV* gene rearrangements. In this study, we investigate the clinicopathologic and molecular features of 10 ES patients with *FEV*-rearrangements, either fused to *EWSR1* (n = 4) or to *FUS* (n = 6). The median age at diagnosis was 38 years (range, 5-61 years) in six males and four females. All tumors were located at extra-skeletal sites, occurring more often in the axial soft tissues. Tumors had a similar morphologic appearance and immunophenotype as ES with more common *EWSR1-ETS* fusions. Of six patients with follow-up data, five patients (83%) developed metastasis and two patients (33%) died of their diseases. The diagnosis was confirmed either by FISH and/or targeted RNA sequencing. In the five cases tested by targeted sequencing, the fusion transcripts were composed of *EWSR1* or *FUS* fused to either exon 1 or 2 of *FEV*, retaining the FEV ETS DNA binding domain. This is the largest study to date investigating the ES subset with *EWSR1/FUS-FEV* fusions showing a predilection for extra-skeletal sites and aggressive behavior.

Keywords

Ewing sarcoma; *EWSR1*; *FUS*; *FEV*; *ETS*; gene fusions

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1. INTRODUCTION

Ewing sarcoma (ES) is the second most frequent bone tumor of childhood and adolescence, with extraskeletal presentation being less common. Genetically, most ES are driven by a canonical fusion between *EWSR1*, or in rare cases *FUS*, and a member of the ETS transcription factor family. The two common variants include *FLI1* or *ERG*, identified in about 90% or 5%, respectively[1,2]. Infrequently, ES are associated with other *ETS* gene members, such as *FEV*[3], *ETV1*[4], or *ETV4*[5]. *EWSR1* and *FUS* are members of the FET family of RNA binding proteins, have similar functions and are interchangeable in translocation-driven sarcomas[6]. A few reports have described ES with *FUS-ERG*[7] and *FUS-FEV*[8]. These fusion oncoproteins act as aberrant transcription factors with neomorphic functions that deregulate hundreds of genes by binding DNA at ETS domain[1].

In addition to the genetically defined *EWSR1-ETS*-positive ES, an Ewing sarcoma-like group of tumors characterized by fusions between *EWSR1* and non-ETS partners such as *PATZ1*[9], *SP3*[10], *NFATC2*[11], and *SMARCA5*[12] is emerging. Preliminary evidence suggests that at least some of these rare molecular subsets may not belong to the conventional *EWSR1-ETS* positive ES, and could represent stand-alone molecular and pathologic entities, similar to *CIC* and *BCOR*-rearranged sarcomas[13,14].

In this study we focus on a group of ES displaying rare *EWSR1/FUS-FEV* gene fusion variants. As only a total of eight ES patients with this fusion have been previously reported, the information regarding this molecular subset is quite limited and it is uncertain if they deserve the current classification under the conventional family of ES[8,10,15,16]. Here, we investigate 10 additional patients by a combined molecular approach and provide detailed clinicopathologic characteristics as well as summarize the existing data on the previously reported patients.

2. Material and Methods

2.1 Patients selection and data collection

The molecular files of the two participating Institutions as well as the personal consultation files of the senior author (CRA) were searched for the diagnosis of ES with *FEV* gene rearrangements. A total of 10 ES patients were identified: four patients with *EWSR1-FEV* and six with *FUS-FEV* gene fusions. For comparison of clinical data, we used 80 patients who were diagnosed with canonical ES with *EWSR1-FLI1* (n = 67) or *EWSR1-ERG* (n = 13) between 2011 and 2018. Furthermore, selected patients had to have been followed for a minimum of one year after treatment.

Hematoxylin and eosin-stained slides and immunohistochemical stains were reviewed in 10 cases. All cases were handled in accordance with the ethical rules of the respective institutions. The tumors were assessed for growth pattern, cytomorphology (round, ovoid, spindle, epithelioid phenotype), cellular pleomorphism, nuclear features including nuclear contour, chromatin pattern and presence of nucleoli, mitotic activity, necrosis, type of stroma and myxoid change. The immunohistochemical stains were re-reviewed including CD99,

cytokeratin and/or EMA, and desmin. However, in most cases a more exhaustive immunohistochemistry work-up was performed.

Retrospective chart review or contacting the outside pathologists was conducted to collect clinical information such as maximum tumor size, tumor location, stage at diagnosis (primary versus distant metastasis at diagnosis), modality of initial therapy, local recurrence or metastasis, vital status at last follow-up and survival time. Thus we obtained complete follow-up data in 6 patients. One case with *FUS-FEV* fusion has been previously reported[17].

2.2 Fluorescence in situ hybridization (FISH)

FISH was conducted for *EWSR1, FUS* and *FEV* in eight cases (patients# 1-8). FISH for break-apart assay was applied on formalin-fixed and paraffin-embedded 4-micron sections as previously described[18]. Custom probes using bacterial artificial chromosomes (BACs) covering and flanking the *EWSR1*, *FUS*, and *FEV* genes were utilized[17]. The BAC clones were selected according to the UCSC genome browser (http://genome.ucsc.edu) and obtained from the BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI) (Oakland, CA) (http://bacpac.chori.org). DNA from individual BACs was isolated in line with the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI. Two hundred tumor nuclei were evaluated using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A cut-off of >20% nuclei showing a break-apart signal was considered to be positive for rearrangement. Nuclei with incomplete set of signals were omitted from the score.

2.3 MSK-IMPACT assay

Three cases (patient# 3, 6, 9) were also investigated with the MSK-IMPACT assay. MSK-IMPACT assay is an FDA approved hybridization capture-based next-generation sequencing assay for targeted deep sequencing. A detailed description of MSK-IMPACT workflow and data analysis is described elsewhere[19,20]. This assay involves all exons and selected introns of up to 468 oncogenes and tumor-suppressor genes, allowing the detection of point mutations, small and large insertions or deletions, and rearrangements. In addition to capturing all exons of the genes, the assay also captures >1000 intergenic and intronic single-nucleotide polymorphisms (tiling probes), interspersed homogenously across the genome, aiding the accurate assessment of genome-wide copy number. In total, the probes target approximately 1.2 megabases of the human genome.

2.4 Targeted RNA Sequencing

Four cases were subjected to RNA targeted sequencing for fusion detection. RNA was extracted from FFPE tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, MA). Two cases (patient# 6, 9) were studied by ARCHER [21,22] and two cases (patient# 4, 10) were analyzed by targeted RNA sequencing using the TruSight RNA Fusion Panel (Illumina, San Diego, CA)[23], as previously described. Targeted RNA sequencing was performed on an Illumina MiSeq platform. Reads were independently aligned with STAR (version 2.3) against the human reference genome (hg19) and analyzed

by STAR-Fusion. In one patient (patient# 3) the fusion was also confirmed by the clinical MSK-IMPACT platform[19].

2.5 Statistical analysis

Kaplan-Meier curves were used to estimate disease-specific survival (DSS). DSS was defined as the time from diagnosis to disease-related death and was censored at the date of the latest follow-up or death. Log-rank tests were used to compare cumulative survival. Categorical variables were compared between groups using chi-square tests. Numerical variables were compared using Mann–Whitney U test. Statistical analyses were performed using SPSS version 21 (IBM), with significance set at $p < 0.05$.

3. RESULTS

3.1 Clinical findings and follow-up data

Of 286 ES cases confirmed in the authors' database to have *EWSR1* or *FUS* gene rearrangements and a known gene partner, *FEV*-rearrangement was found in 10 (3.5%) cases. The clinical and molecular features of ES with *FEV*-rearrangements are summarized in Table 1. Briefly, the patients had a median of 38 years of age (range, 5-61) at diagnosis. Only three patients were in the pediatric age group (age <18 years). There were six males and four females. All tumors were located at extra-skeletal sites, with six being axial, including two each in the para-spinal soft tissue and chest wall, and one in the subclavicular soft tissue and anterior mediastinum. Three tumors were located in the deep soft tissue of the extremities, including two in the arm and one in the shoulder. One patient presented with a visceral lesion, involving the uterus. The largest tumor diameter obtained from either imaging studies at presentation or gross descriptions had a mean of 9.6 cm (range, 4.5 to 18.0 cm).

Follow-up data were available in six of our patients, summarized in Table 2, which also summarizes the clinical data from the six previously reported patients. Two of our patients (# 1 and 6) presented with metastases at diagnosis and were treated with palliative chemotherapy, succumbing of disease after 25 and 30 months, respectively. One patient (#3) presenting with localized disease was treated with pre- and post-operative chemotherapy using the Ewing sarcoma protocol, vincristine/doxorubicin/cyclophosphamide (VDC) and ifosfamide/etoposide (IE). The patient is alive with no evidence of disease 28 months after diagnosis. Patient# 5 presented with localized disease and underwent surgery and chemotherapy (vincristine, doxorubicin, ifosfamide, and etoposide). However, she developed lung metastases, 11 months after diagnosis, and is alive with disease at 18 months follow-up. Patient# 8 presented with localized disease and underwent surgery. However, he developed a late bone metastasis to the humerus, 23 years after diagnosis, which was confirmed by core biopsy. The last patient with available follow-up (patient# 9) presented with localized tumor and underwent definitive surgery, but developed lung metastases 22 months after diagnosis and received palliative chemotherapy. In total, five of six patients with available clinical follow-up developed metastases and two patients died of disease.

3.2 Pathologic findings

All tumors showed a primitive round cell morphology arranged in solid sheets and nests, separated by limited stromal component. The lesional cells had scant cytoplasm and round, uniform nuclei with smooth nuclear contour, fine chromatin and inconspicuous nucleoli (Figure 1). One case each had some peculiar histologic features, including a microcystic growth, loose myxoid stromal component, and areas of ovoid to short spindle cell growth (Figure 1). No variation in nuclear size or shape was noted. Overall features recapitulated the morphologic spectrum seen in conventional Ewing sarcomas.

By immunohistochemistry, all tumors showed strong and diffuse membranous staining pattern for CD99 (Fig. 1, Table 1). One case (Patient# 6) which occurred in the anterior mediastinum and was submitted with a presumed diagnosis of NUTM1-negative midline carcinoma, showed in addition to diffuse membranous CD99 staining, strong nuclear expression for P40 as well as positivity for cytokeratins (AE1:AE3, Cam5.2), synaptophysin and chromogranin (Fig. 2). The tumor was negative for TTF1, CD56, WT1 and PAX8. Some of these immunohistochemical findings, corroborated with the presence of *FUS-FEV* fusion, might be in keeping with the alternative terminology of a so-called ‘adamantinoma-like Ewing sarcoma’. One additional case showed positivity for synaptophysin and focally for chromogranin (patient#7).

3.3 Molecular Findings

Eight patients were tested by FISH showing gene rearrangements in *EWSR1* and *FEV* genes in four cases or *FUS* and *FEV* in four cases. In one case, a reciprocal *FEV* exon 1 and *EWSR1* exon 9 fusion was also confirmed by the MSK-IMPCT assay (Table 1). In one case, targeted RNA sequencing revealed fusion of *EWSR1* exon 8 and *FEV* exon 2. Three other cases showed fusion of *FUS* exon 3, 7, or 10 with *FEV* exon 2 (Fig. 3). All cases retained *FEV* exon 3 which encodes the FEV ETS DNA binding domain. Representative rearrangements are shown in Figure 3.

By MSK-IMPACT, three missense mutations of *CCND1*, *ICOSLG*, *MSH6*, and one deletion of *ZFHX3* were detected in patient# 6 (*FUS-FEV*). Three missense mutations of *ARIDA1*, *KMT2D*, and *PREX2* were found in patient# 9 (*FUS-FEV*). MSK-IMPCT assay showed no other mutation aside from *EWSR1-FEV* in patient# 3, who was the only patient with no evidence of disease at last follow-up.

3.4 Meta-analysis of the eight ES with FEV-rearrangement reported in the literature

As shown in Table 1, there were seven patients with *EWSR1-FEV* fusion and one patient with *FUS-FEV* fusion, with median age of 20 years (range, 2 to 40 years). All tumors occurred in the axial location, and all except one were located in soft tissues. Two of the six patients with available clinical follow-up had metastasis at presentation (Table 2). At last follow-up, three patients died of diseases and two patients were alive with metastatic diseases.

Microscopically, of the eight cases previously reported in the literature, five tumors were composed of uniform small round cells, two cases contained large round polygonal cells,

and one showed poorly differentiated features. All patients had immunohistochemical data for CD99 and all tumors showed positive staining.

Three cases had more detailed molecular findings including the fusion transcript type, including two cases with *EWSR1* exon 7 fused to *FEV* exon 2 and one case with *FUS* exon 10 fused to *FEV* exon 2 (Table 1).

All together, including present and published data, of eight cases with available data, the *EWSR1* breakpoints included exon 7 in two cases and exon 8 or 9 in one; while the *FUS* breakpoints were exon 10 in two cases and exon 3 or 7 in one. Of note, in 7 of 8 cases the breakpoint was in *FEV* exon 2.

3.5 ES patients with FEV rearrangements showed a higher extraskeletal location and a worse clinical outcome compared to patients with EWSR1-FLI1/ERG fusions

The control group included 80 ES patients from our prospective research database, harboring the canonical *EWSR1-FLI1* (n = 67) or *EWSR1-ERG* (n = 13) fusions. ES patients with *FEV* gene rearrangements (n=18) had a significantly higher proportion of extra-skeletal tumors, compared to patients with the typical *EWSR1-FLI1* or *EWSR1-ERG* fusions (94% vs 35%, respectively, $p < 0.001$, Table 3). Moreover, the 5-year disease specific survival of patients with *EWSR1-FLI1* or *EWSR1-ERG* was 74% (95% CI: 52% to 87%) compared to 43% (95% CI: 11% to 72%) for patients with *EWSR1-FEV* ($p = 0.035$, Figure 4).

4. DISCUSSION

In this study we investigated 10 Ewing sarcoma (ES) patients characterized by *EWSR1/FUS-FEV* fusions. This rare molecular subset had a prevalence of 3.5% of all ES available in our research database with comprehensive fusion data available. ES with *FEV* gene rearrangements were associated with distinct clinical features compared to the common ES with canonical *EWSR1-FLI1* or *EFSR1-ERG* fusions. First, all our cases occurred at extra-skeletal location. Applebaum et al[24] reported that extraskeletal Ewing sarcoma involved 31% of a total of 2202 Ewing sarcoma patients detected in the Surveillance, Epidemiology, and End Results Program (SEER) database. Second, our cohort had a median of 38 years of age at diagnosis. In contrast, nearly 80% of ES patients with the common *FLI1/ERG* variants are younger than 20 years of age, with a peak age incidence in the second decade of life. Patients older than 30 are uncommon[25].

Histologically and immunohistochemically, the *FEV*-positive subset shared similar features to the ES with the canonical gene fusions, including a monomorphic primitive round cell neoplasm with strong and diffuse CD99 reactivity. However, rare features such as microcystic change, myxoid stromal component or ovoid to short spindle cytomorphology were also noted in one case each. In addition, one case, presenting in the anterior mediastinum of an 23 year-old male, showed an immunophenotype in keeping with the so-called adamantinoma-like Ewing sarcoma[26].

The predicted structure of EWSR1-FEV or FUS-FEV fusion oncoprotein is consistent with other fusion variants in ES. It includes the required N-terminal serine-tyrosine-glutamine-glycine transactivation domain of EWSR1 or FUS and the DNA-binding domain of FEV. However, in one of our cases (patient# 6), the fusion transcript consists of a shorter *FUS* transcript (*FUS* exons 1-3), which predicts a breakpoint located within the FUS transactivation domain, compared to the previously reported *FUS* exons 1-10 transcript in one of the *FUS-FEV* cases[15].

The FEV protein belongs to the ETS transcription factor family and is closely related to the FLI1/ERG subgroup, that have a highly conserved 85-amino acid ETS domain binding purine-rich DNA sequences[8]. This suggests that EWSR1-FEV may alter the transcription of targeted genes similar to EWSR1-FLI1 or EWSR1-ERG. In our analysis, all fusions included *FEV* exon 3 which encodes the ETS domain. However, FEV demonstrates a unique structure aside from the DNA binding domain. FLI1 and ERG have large N-terminal domains which are involved in transcription activation of reporter genes containing ETS binding sites[27,28]. The absence of an N-terminal domain in FEV indicates that this protein does not share the transcriptional activation properties of FLI1 and ERG[8]. Moreover, the high content in alanine residues of the C-terminal part of FEV, a feature which was observed in various transcription repressors, may suggest that FEV is a repressor of the FLI1/ERG subfamily[29]. Further studies are needed to determine whether EWSR1/FUS-FEV protein has unique effects on transcription of targeted genes compared to EWSR1-FLI1/ERG protein. Moreover, further molecular studies, including gene expression signatures or methylation profiling, are warranted to confirm whether this minor molecular subset of ES with *FEV*-rearrangement groups together with the *EWSR1-ETS* positive conventional ES or may represent a separate pathologic entity. Recent molecular studies have suggested that *EWSR1-NFATC2* or *EWSR1-PATZ1* positive sarcomas have distinct genomic and/or methylation signatures and thus likely represent separate subsets, given their unique clinical behavior and molecular characteristics[30–32].

Of the patients in our cohort with *FEV* gene rearrangements and available follow-up, 33% presented with disseminated disease at diagnosis and died of disease, while 83% of patients overall developed distant metastases. Our data also suggested that ES with *FEV*-rearrangement has aggressive features compared to ES with canonical *EWSR1-FLI1* or *EWSR1-ERG* fusions. In the SEER database, 30% of ES patients had metastasis at presentation. The 5-year overall survival in localized skeletal and extra-skeletal ES has been reported to be 63% and 70%, respectively[24]. Although our clinical data are limited, one patient presenting with localized disease and treated with conventional ES regimens had no evidence of disease at last follow-up. Furthermore, the MSK-IMPACT results on this tumor did not reveal any additional secondary mutations aside from the *EWSR1-FEV* fusion. This result is in keeping with recent data that the pattern and extent of mutation including driver and passenger mutation may have significant value in predicting the aggressiveness of ES[33].

In conclusion, this is the largest study to date investigating one of the rarest ES molecular subsets, characterized by *FEV* gene rearrangements. This is also the first molecular investigation to establish the prevalence of ES with *FEV*-related fusions among Ewing sarcoma (3.5%). Our data combined with previously reported cases suggest that the *FEV*-

positive ES are associated with axial soft tissue locations, older age at diagnosis and aggressive clinical behavior. Despite these clinical differences, the morphologic and immunoprofile of this subset recapitulates the pathologic spectrum of conventional ES.

Acknowledgments

Disclosures: Supported in part by: P50 CA 140146-01 (CRA), P50 CA217694 (CRA), P30 CA008748, Cycle for Survival (CRA), Kristin Ann Carr Foundation (CRA), St Baldrick Foundation (CRA).

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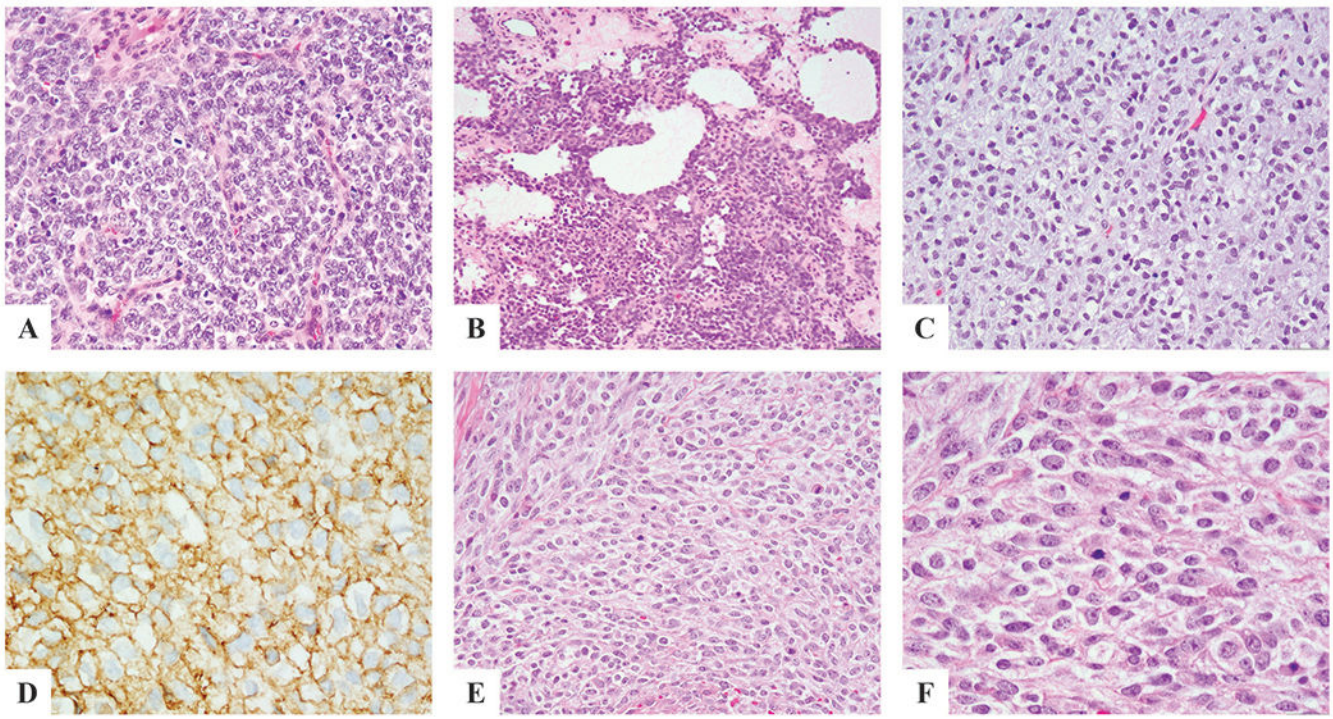

Figure 1. The morphologic spectrum of Ewing sarcoma with *FEV* gene rearrangements.

A. Undifferentiated round cell sarcoma composed of solid sheets of primitive uniform round cells with ill-defined cell borders, scant cytoplasm and monomorphic nuclei with smooth nuclear contours and vesicular chromatin (patient# 2, *EWSR1-FEV*). **B**. Same case showing focal microcystic areas. **C**. Round cell sarcoma in a myxoid stromal component (patient# 5, *FUS-FEV*). **D**. Same case showing strong, crisp membranous immunostaining for CD99. **E, F**. One case showed areas with more ovoid to short spindle cells arranged in vague streaming pattern (patient# 1, *EWSR1-FEV*). High magnification shows nuclei round to ovoid nuclei with smooth contours, fine chromatin, inconspicuous nucleoli and increased mitotic activity.

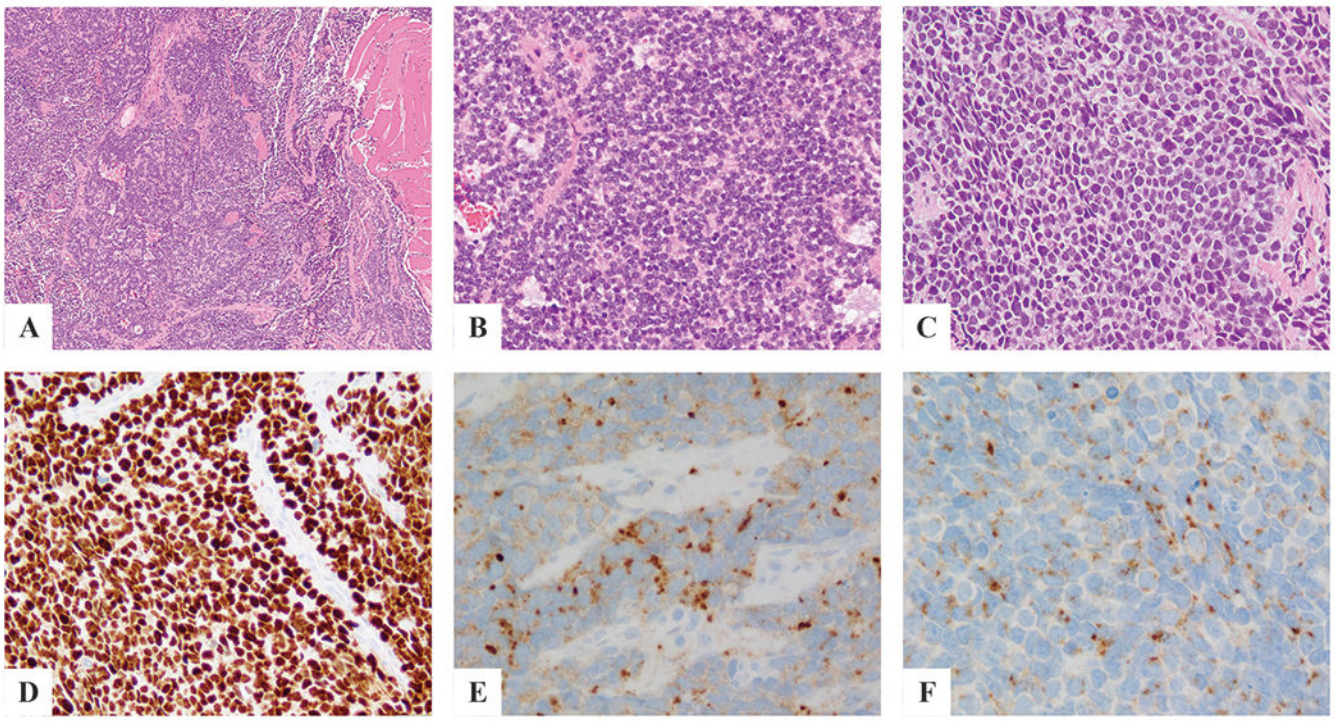

Figure 2. Additional pathologic findings in ES with *FUS-FEV* gene fusions.

A,B Low power view showing a markedly cellular round cell neoplasm involving skeletal muscle, while at high power it shows a uniform cytomorphology with round nuclei and ill-defined cell membranes, and vague rosette formation (patient# 7). **C-F**. A primitive round cell tumor arranged in diffuse sheets, which by immunohistochemistry in addition to CD99 and CK expression (not shown), had diffuse nuclear staining for P40 (D), and focal staining for synaptophysin (E) and chromogranin (F) (patient# 6).

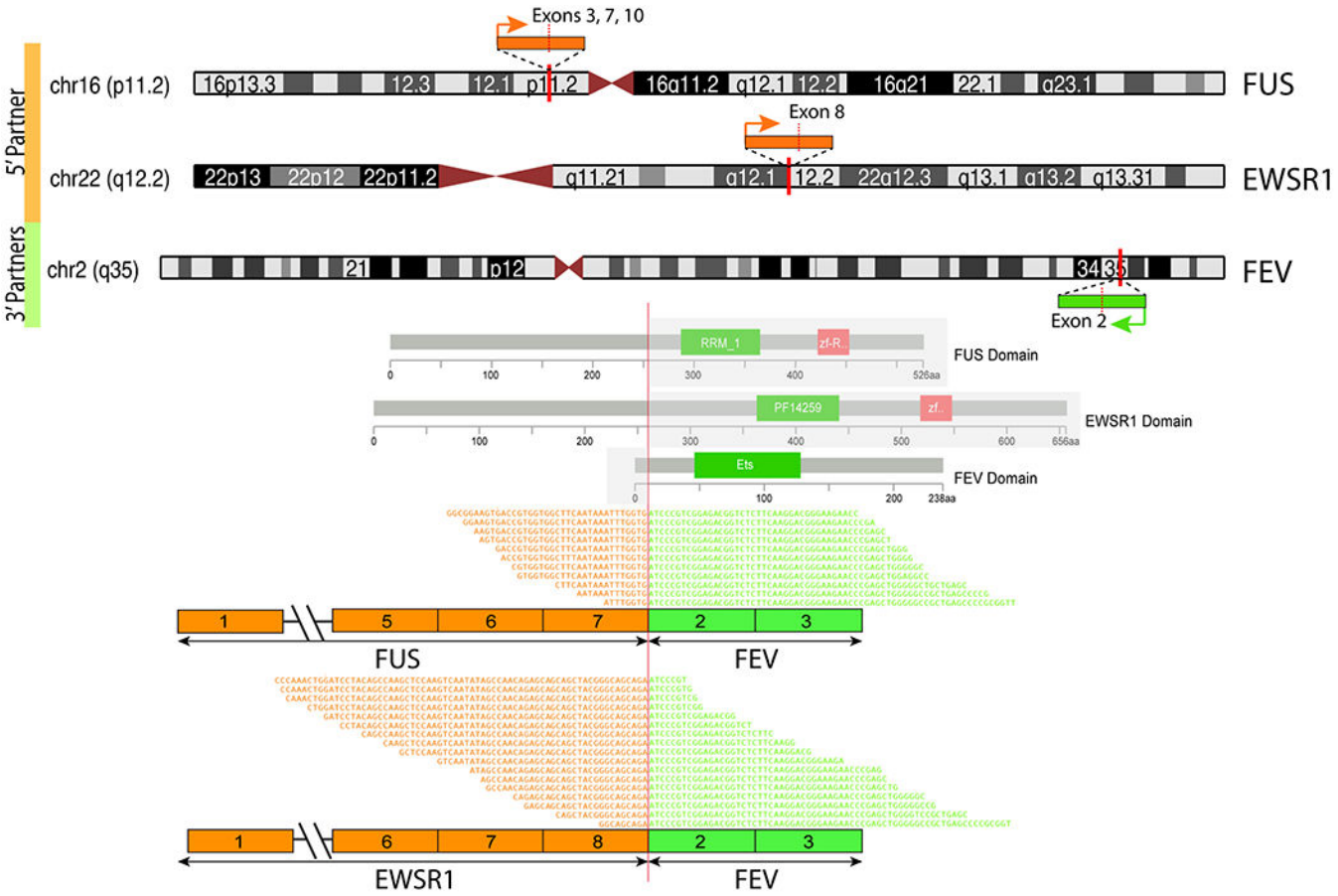

Figure 3. Diagrammatic representation of the *EWSR1-FEV* and *FUS-FEV* fusions studied with RNA sequencing.

Upper panel shows chromosomal localization of *FUS* gene on 16p11.2, *EWSR1* on 22q12.2 and *FEV* on 2q35. Red vertical lines depict the genomic breakpoints. Orange arrows and bars show the direction of transcription of each gene and the exonic variants breaks, respectively. The protein domains of each gene are shown in the mid-panel. Representative fusion variants are depicted on the lower panel showing *FUS* exon 7 fused to *FEV* exon 2 and *EWSR1* exon 8 to *FEV* exon 2.

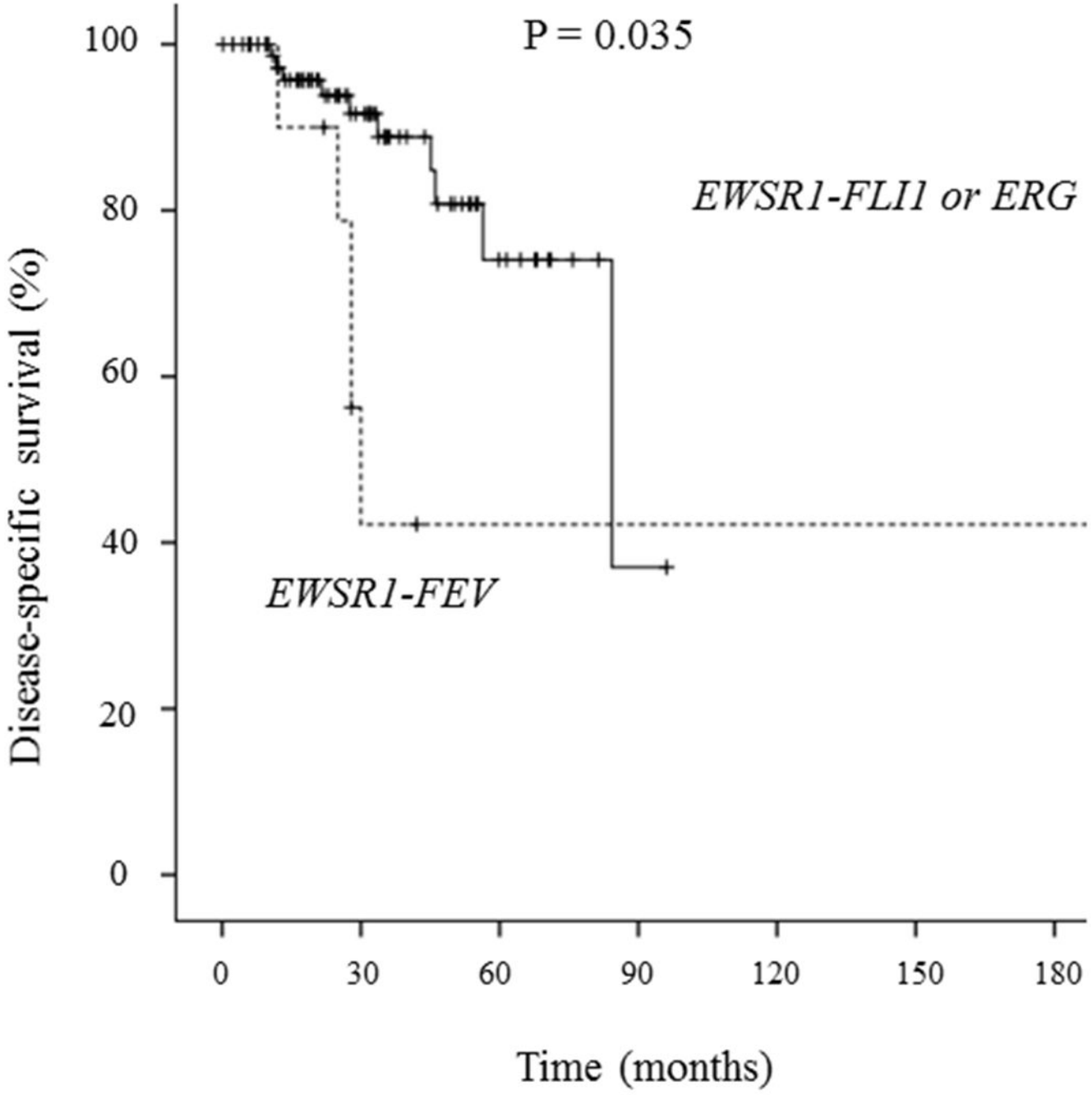

Figure 4. Kaplan-Meier curve of disease-specific survival stratified by fusion type.

ES patients with *FEV* gene rearrangements follow a significantly more aggressive clinical course compared to ES patients harboring the more common, canonical fusions.

Table 1.

Clinicopathologic features of Ewing sarcoma with *FEV* gene rearrangements in our cohort and previous literature

Patient# (current study)	Fusion type	Fusion Transcript	Age	Sex	Site	Skeletal/Extra-skeletal	Location	CD99
1	EWSR1-FEV	NA	32	M	Chest wall	Extra-skeletal	Axial	Diffuse membranous
2	EWSR1-FEV	NA	5	F	Subclavicular	Extra-skeletal	Axial	Diffuse membranous
3	EWSR1-FEV	EWSR1 exon 9 / FEV exon 1 [*μ]	16	M	Para-spinal soft tissue	Extra-skeletal	Axial	Diffuse membranous
4	EWSR1-FEV	EWSR1 exon 8 / FEV exon 2 [α]	61	F	Uterus	Extra-skeletal	Axial	NA
5	FUS-FEV	NA	46	F	Arm	Extra-skeletal	Extremity	NA
6	FUS-FEV	FUS exon 3 / FEV exon 2 [β,μ]	23	M	Anterior mediastinum	Extra-skeletal	Axial	Diffuse membranous
7	FUS-FEV	NA	54	M	Forearm	Extra-skeletal	Extremity	Diffuse membranous
8	FUS-FEV	NA	13	M	Shoulder	Extra-skeletal	Extremity	Multifocal
9	FUS-FEV	FUS exon 10 / FEV exon 2 [β,μ]	40	F	Chest wall	Extra-skeletal	Axial	Diffuse membranous
10	FUS-FEV	FUS exon 7 / FEV exon 2 [α]	51	M	Spinal epidural	Extra-skeletal	Axial	NA
Milione et al.	EWSR1-FEV	NA	28	F	Ileum	Extra-skeletal	Axial	Strong
Wang et al.	EWSR1-FEV	NA	2	F	Para-spinal	Extra-skeletal	Axial	Positive
	EWSR1-FEV	NA	15	M	Maxillary	Extra-skeletal	Axial	Positive
	EWSR1-FEV	NA	15	M	Para-testicular	Extra-skeletal	Axial	Positive
	EWSR1-FEV	NA	24	M	Retroperitoneal	Extra-skeletal	Axial	Positive
	EWSR1-FEV	EWSR1 exon 7 / FEV exon 2	40	M	Retroperitoneal	Extra-skeletal	Axial	Positive
Llombart-Bosch A et al.	EWSR1-FEV	EWSR1 exon 7 / FEV exon 2	15	M	Inguinal area	Extra-skeletal	Axial	Strong
Ng et al.	FUS-FEV	FUS exon 10 / FEV exon 2	33	M	Clavicle	Skeletal	Axial	Strong membranous

NA; not available,

[*] Reciprocal fusion was detected by MSK-IMPACT;

cases confirmed by targeted RNA sequencing[α] or ARCHER[β], and cases studied by IMPACT[μ]

Table 2.

Clinical data and follow-up information of patients with *FEV* gene fusions from current study and published literature

Patient# (current study)	Fusion type	Metastasis at presentation	Metastatic sites	Status	FU (months)
1	EWSR1-FEV	Yes	Lung	DOD	25
3	EWSR1-FEV	No	No	NED	28
5	FUS-FEV	No	Lung	AWD	18
6	FUS-FEV	Yes	Bone	DOD	30
8	FUS-FEV	No	Bone	AWD	276
9	FUS-FEV	No	Lung	AWD	22
Milione et al.	EWSR1-FEV	Yes	Liver, lung, stomach and peritoneum	AWD	204
Wang et al.	EWSR1-FEV	Yes	NA	DOD	28
	EWSR1-FEV	No	No	DOD	12
	EWSR1-FEV	No	Lung, lymph node	AWD	42
Llombart-Bosch et al.	EWSR1-FEV	No	Central nerve system, cutaneous	DOD	28
Ng et al.	FUS-FEV	No	No	NED	10

NA; not available; FU, follow-up; DOD, dead of disease; NED, no evidence of disease; AWD, alive with disease.

Table 3.

Clinicopathologic comparison of ES with *FEV* rearrangements versus ES with the canonical *EWSR-FLI1/ERG* fusion

		EWSR1-FLI1/ERG	**%**	***EWSR1/FUS-FEV***	**%**	**p value**
Total number of patients		80		18		
Age (median, IQR, years)		22 (15 to 35)		30 (15 to 42)		0.192
Sex	Male	44	55	12	67	0.652
	Female	36	45	6	33	
Site	Extremity	27	34	3	17	0.257
	Axial	53	66	15	83	
Skeletal/Extra-skeletal	Skeletal	52	65	1	6	**<0.001**
	Extra-skeletal	28	35	17	94	
5-year disease-specific survival (%)		74.0		42.6		**0.035**