

Content available at: <https://www.ipinnovative.com/open-access-journals>

Indian Journal of Pathology and Oncology

Journal homepage: [www.ijpo.co.in](http://www.ijpo.co.in)

## Original Research Article

## Molecular profile &amp; clinical outcome in 121 cases - experience from a tertiary referral centre in South India

Kiruthiga K G<sup>1</sup>, Anne Jennifer Prabhu<sup>2,\*</sup>, Rekha Pai<sup>3</sup>, Leni G Mathew<sup>4</sup>, Dhananjayan S<sup>3</sup>, Selvamani Backianathan<sup>5</sup><sup>1</sup>King Edward Memorial Hospital, Pune, Maharashtra, India<sup>2</sup>Dept. of Pathology, Christian Medical College, Vellore, Tamil Nadu, India<sup>3</sup>Dept. of Molecular Pathology, Christian Medical College, Vellore, Tamil Nadu, India<sup>4</sup>Dept. of Pediatrics, Christian Medical College, Vellore, Tamil Nadu, India<sup>5</sup>Dept. of Radiotherapy, Christian Medical College, Vellore, Tamil Nadu, India

## ARTICLE INFO

## Article history:

Received 02-03-2023

Accepted 22-03-2023

Available online 17-06-2023

## Keywords:

BCoR

CD99

Ewing sarcoma

FLI1

Translocation

## ABSTRACT

**Background:** Ewing sarcoma is the second most common sarcoma involving the bones in children and adolescents. Published data on the clinical features, morphology, translocation and follow-up of patients with Ewing sarcoma from India, is sparse. Objectives of this study were to analyse the clinico-pathologic features of Ewing sarcoma and compare with translocation status, BCoR immunohistochemistry, treatment and survival.

**Materials and Methods:** 406 cases of Ewing sarcoma were diagnosed in the Department of Pathology, Christian Medical College, Vellore during the period 2008-2015. 135 patients underwent molecular testing for common translocations by RT-PCR and were included in this study.

**Results:** Mean age of patients at diagnosis was 22 years. Most common location of tumour was appendicular skeleton (31.4%) and 19% had solid organ involvement. Distant metastasis was present in 21.5% patients. Translocation was seen in 63 (46.7%) cases, EWS-FLI1 type I (87%), EWS-FLI1 type II (11%) and one patient had EWS-ERG translocation. Seven out of 30 patients were classified as "Sarcoma with BCoR genetic alteration". Patients who underwent 6 cycles of chemotherapy had a better mean survival. Mean follow-up was 13.72 months and 3 year event free survival of patients was 93.8%.

**Conclusions:** Percentage of viable tumour <10% was the only significant histologic parameter predicting survival. Age at diagnosis ≤15yrs, female sex, size ≤12cm, extra-osseous site, chemotherapy and translocation positivity predicted an improved survival. This study has analysed the type of mutations/translocations seen in a subset of Indian patients with Ewing sarcoma and correlated the clinical and pathological factors affecting survival.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [reprint@ipinnovative.com](mailto:reprint@ipinnovative.com)

## 1. Introduction

Ewing sarcoma (ES) is the second common sarcoma of bone, in children and adolescents.<sup>1</sup> Ewing family of tumours include classical ES and round cell tumours with different fusion partners or completely different gene

expression profiles, with subtle differences in morphology but same immunohistochemical marker expression and non-random sharing of chromosomal translocations.

ES presents commonly in second decade of life and arises commonly in long tubular bones of extremities.<sup>1</sup> Routine diagnosis is based on morphology as well as immunohistochemistry. However, confirmation of diagnosis is based on identification of specific chromosomal

\* Corresponding author.

E-mail address: [annejennifer91@gmail.com](mailto:annejennifer91@gmail.com) (A. J. Prabhu).

translocations by fluorescence in situ hybridisation (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR).<sup>2</sup> Most common translocation in ES involves the EWSR1 gene on chromosome 22 to FLI1 gene on chromosome 11, producing the characteristic translocation, t(11;22) in 85% cases. Of these, around 60% cases involve fusion of exons 1-7 of EWSR1 with exons 6-9 of FLI1 gene (Type -1 fusion), and remaining involve fusion between exons 1-7 EWSR1 with exons 5-9 of FLI1 (Type - 2 fusion). Ten percent of cases involve translocations between EWSR1 and ERG genes.<sup>3</sup> These are detected by RT-PCR in our institution since 2008. Remaining 5% of cases have rare mutations like EWSR1-ETV1, EWSR1-ETV4, EWSR1-FEV which are identified using FISH.<sup>1,2</sup>

“Ewing-like sarcomas” contain fusions between the EWSR1 gene and non-ETS genes like SMARCA5, NFATc2 etc. In other cases, CIC-DUX4 and BCOR-CCNB3 fusion are identified, which have significantly different gene expression profiles and are recently classified as ES, round cell sarcoma with EWSR1-non-ETS fusions, CIC-rearranged sarcoma and sarcoma with BCOR genetic alterations. Tumours with EWS-ETS translocation has a robust response to chemotherapy and good clinical outcomes. Round cell sarcoma with EWSR1-non ETS fusions and CIC rearranged sarcomas have metastatic disease at presentation and poor outcomes. Sarcoma with BCOR genetic alterations respond to ES chemotherapy protocols and have outcomes similar to that group. With the availability of immunohistochemistry, it has become easier to detect this group of tumours, thereby guiding the clinicians with treatment.<sup>1,4-7</sup>

Malignant small round cell tumours include ES / PNET, neuroblastoma, Wilm’s tumour, rhabdomyosarcoma, lymphoma, poorly differentiated synovial sarcoma, mesenchymal chondrosarcoma, desmoplastic small round cell tumour, round cell variant of malignant peripheral nerve sheath tumour (MPNST) and small cell osteosarcoma. Pathogenesis, immunoprofile, treatment and prognosis of these tumours are different although they have almost similar morphology. Hence, it becomes important to diagnose these tumours with accuracy. In this study, we have assessed the accuracy of CD99 and FLI1, compared to RT-PCR based gold standard assay. We have also compared the various clinico-pathological features with treatment and survival and also assessed the BCOR positivity in patients who were negative for the classic translocations involving ETS genes. This study is the first of its kind from the Indian subcontinent and first large scale study that correlates the histopathological & molecular diagnosis and clinical features with survival outcomes from South Asia.

## 2. Materials and Methods

Total of 406 cases of ES were diagnosed in the Department of General Pathology over a period of 8 years. The study

was approved by the Institutional Review Board (IRB Min No: 10302, dated 21.09.2016). Immunohistochemistry for CD99 and FLI1 was used in the initial diagnostic panel along with TLE1, desmin, myogenin, synaptophysin, chromogranin, NSE, CD79a and TdT. Of these, 135 patients underwent additional testing by RT-PCR. Records of patients were obtained from archives of pathology and molecular pathology laboratory. Diagnoses made on immunomorphology were compared with the RT-PCR assay. Blocks of thirty samples that were negative for EWS-FLI and EWS-ERG translocation were retrieved and BCoR (C-10): sc-514576 (SANTA CRUZ Biotechnology) immunostain was used to detect BCOR-CCNB1 mutation.

In the resection specimens, an entire grid of tumour was assessed for response to chemotherapy based on Huvos grading system.<sup>8</sup> Important clinical variables like number of cycles of chemotherapy, recurrence, site of metastasis, death etc was noted from archives.

### 2.1. RT-PCR assay

Representative tumour blocks were chosen and RNA (RecoverAll Total nucleic acid extraction kit, Ambion, USA) was extracted. Total RNA was estimated using the nanodrop (Nanodrop technologies, USA). Quality check was performed and cDNA conversion was done using random primers of cDNA conversion kit (Applied Biosystems, USA). RT PCR and sequencing EWS-FLI gene translocations was amplified using primers published previously in the list below. Following thermal cycling profile was performed: 95 degree C for 8 min, optimized anneal for 30 sec, 62/63 degree C for 1 min and final extension for 72 degree C for 10 min. The PCR product was detected using a 1.5% agarose gel and sequencing was performed with an automated DNA sequencer (ABI PRISM 310 genetic analyzer, Applied Biosystems, USA) using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA).

### 2.2. Statistical analysis

Descriptive data was summarized using frequency along with percentages for categorical variables and mean along with standard deviation for continuous variables using SPSS software Version Stata IC/13. Chi-square/ Fisher’s exact test was used to compare the association between categorical variables and a ‘P’ value of <0.05 was considered significant. Overall survival [OS] and event free survival [EFS] were calculated. Event was described as death. Kaplan-Meir curve was used to depict survival and log rank test was used to compare survival.

## 3. Results

Mean (range) age of patients was 22.58(0.75-79) years. Male: female ratio was 1.8:1 (78 males and 43 females).

Most common clinical presentation was pain (44.9%) followed by swelling (32.7%). Other complaints were headache, weakness, abdominal pain, abdominal mass, urinary retention, back ache and chest pain. Tumour dimension was available for 32/43 patients who underwent resection and average (range) size of tumour was 8.2 (0.3-19) cm. Common tumour locations were appendicular skeleton 38 (31.4%), axial skeleton 28 (23.1%), soft tissue 21 (17.5%), solid organs 23 (19%), perinepheral nervous system 4 (3.3%), metastatic site 5 (4.1%) and Askin tumour 2 (1.6%). Distant metastasis was present in 26/121 (21.5%) patients and most common site was lung (62%) and bone (28%). There was one case each with metastasis to lymph node, pleura, rectum and omentum. In our cohort, 15 patients (38.5%) completed 6 cycles of chemotherapy and had a better survival (47.33 months) when compared to patients who did not undergo chemotherapy (n=7) with a mean survival of 16.43 months and patients with 1-4cycles (n=17) with a mean survival of 39.51 months (Figure 3 A).

### 3.1. Results of the RT-PCR

63 (46.67%) were positive for common translocations and 72 (53.33%) were negative. EWS-FLI-1 Type-I mutation was seen in 55 (87.30%) cases, EWS-FLI-1 Type-II mutations in 7 (11.11%) cases and EWS-ERG translocation in one case (1.59%). Sensitivity of CD99 and FLI1 when used alone is 100% and 95.2% respectively, while the specificity is as low as 5.7% and 4.1% (Figure 1 A, C). When CD99 and FLI1 are used in combination, sensitivity, positive predictive value and negative predictive value are 100%, while the specificity is still as low as 11%. Summarised in Table 1.

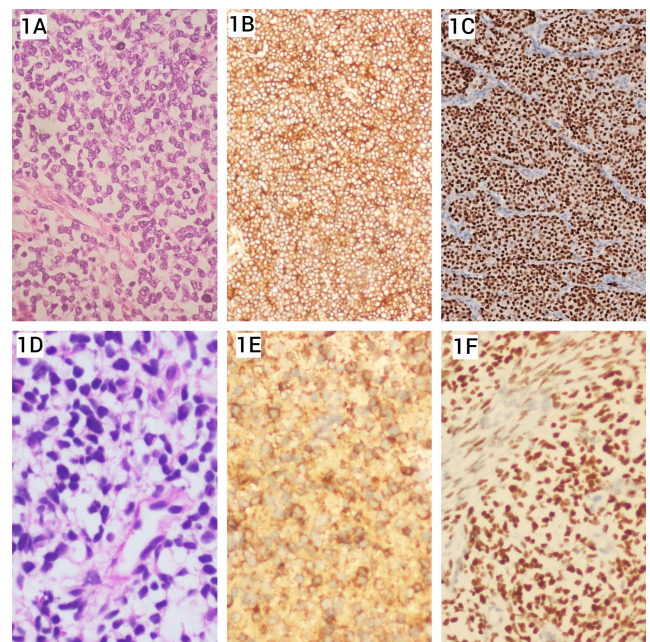
Tumours that were negative for the common mutations included small cell osteosarcoma (3), mesenchymal chondrosarcoma (2), granulocytic sarcoma (1), Non Hodgkinlymphoma (1), Wilm's tumour (1), small cell variant of MPNST (2), poorly differentiated synovial sarcoma (1), angiosarcoma (1) and medulloepithelioma (1). RT-PCR was done on a case of neuroblastoma during follow up, to rule out ES, in view of inadequate treatment response.

### 3.2. Results of BCOR immunohistochemistry

BCOR immunohistochemistry was done on 30 cases where the tissue blocks could be retrieved. Seven cases (23%) showed nuclear staining for BCOR and were classified as BCOR-CCNB3 sarcoma (Table 2, Figure 1 1D-1F). Clinico-pathologic details of the BCOR positive cases (n=7):

### 3.3. Survival analysis

Mean (range) follow-up was 33.72 (0.3 – 71.9) months. Overall mean survival was 66.6 months (5.5 years). Event free survival at 1 year and 3 years was 96.3% and 93.8% respectively. Mean survival of patients with a translocation



**Fig. 1:** Morphology of classic Ewing sarcoma and sarcoma with BCOR genetic alterations; **A):** Morphology of classic Ewing sarcoma, H&E stain, 200X; **B):** CD99 staining, 100X; **C):** FLI1 staining, 100X; **D):** Morphology of BCOR sarcoma, H&E stain, 400X; **E):** CD99 in BcoR sarcoma, 200X; **F):** BCoR staining

was 38.88 months as compared to 36.38 months for those without translocation. Table 3 shows the results of univariate analysis comparing various clinico-pathological factors. Viable tumour  $\leq 10\%$  following chemotherapy is the only statistically significant factor predicting survival (Figure 3 B). Various other features like age at diagnosis  $\leq 15$  yrs, female gender, tumour size  $\leq 12$  cm, extra-osseous site, chemotherapy and translocation positivity predicted an improved survival, although statistically not significant. Five patients in our study died of disease (Table 4).

## 4. Discussion

ES is the second most common sarcoma of bone in young adults, next to osteosarcoma.<sup>9,10</sup> However the incidence of ES is very low in South Asian countries.<sup>11</sup> Confirmation of diagnosis is done on RT-PCR based detection of translocations, the most common ones being EWSR1-FLI1 and EWSR1-ERG. To the best of our knowledge, this is the first and the largest study to describe the clinic-pathological features of ES in detail and analyze the possible factors of prognostic importance including the correlation of outcome with RT-PCR diagnosis from South Asia.

Mean age at diagnosis of our patients was 22 years with a higher incidence in males.<sup>1,12,13</sup> Age at diagnosis has also been correlated to the outcome of disease in ES<sup>12,14,15</sup> and older age is consistently associated with a poor outcome. In our study, age at diagnosis  $\leq 15$  years was found to

**Table 1:** Diagnostic efficacy of CD99 and FLI1

	Sensitivity	Specificity	PPV	NPV
CD99	100%	5.7%	53.4%	100%
FLI1	95.2%	4.1%	45.5%	75%
CD99 + FLI1	100%	11%	100%	100%

PPV: Positive predictivevalue, NPV: Negative predictive value

**Table 2:** Clinico-pathologic details of the BCOR positive cases (n=7)

S. No	Age (years)/ Sex	Site of primary	Gross tumour size (cms)	Viable tumour	Margin involvement	Follow-up
1	13/Male	Proximal femur	NA (Biopsy)	NA	NA	Metastasis to lung
2	26/Female	Sino-nasal tract	NA (Biopsy)	NA	NA	Metastasis to bone
3	13/Male	Femur	NA (Biopsy)	NA	NA	No evidence of disease
4	21/Female	Soft tissue over the back	NA (Biopsy)	NA	NA	NA
5	11/Male	Anterior chest wall	5.5	Nil	No	No evidence of disease
6	48/Female	Lung	12	10%	Yes	No evidence of disease
7	22/Male	Forearm	13.5	75%	No	NA

**Table 3:** Univariate analysis forevent free survival of Ewing sarcoma patients (n=76)

Parameters		Mean survival (months)	Hazard ratio (95% C.I)	Events (No.)	95% C.I	P value
Age at diagnosis	≤15yrs	41.39	1.83 (0.83 – 3.98)	9	32.11 - 50.67	0.12
	>15yrs	33.84		22	23.90 - 43.78	
Sex	Males	35.12	1.04 (0.49 – 2.22)	21	28.03 – 42.22	0.91
	Females	39.97		10	25.69 – 54.26	
Tumour size	≤12cm	44.51	2.65 (0.68 – 10.39)	5	35.33 – 53.69	0.14
	>12cm	27.39		4	7.14 – 47.63	
Site	Skeletal	38.77	2.29 (1.05 – 4.97)	16	31.60 – 45.95	0.07
	Solid organs	52.46		4	36.19 – 68.74	
Chemo	Others	24.01	5.59 (1-31.23)	11	12.01 – 36.01	0.35
	No chemo	16.43		2	5.26 – 27.61	
	1-4 cycles	39.51		4	27.80 – 51.22	
Viable tumour	6 cycles	47.33	5.59 (1-31.23)	4	37.53 – 57.13	0.02*
	≤10%	48.73		4	39.74 – 57.72	
Positive margins	>10%	17.09	0.60 (0.28 – 0.23)	4	11.83 – 22.35	0.27
	No	40.90		26	32.22 – 49.58	
Mets at presentation	Yes	36.22	0.67 (0.33 – 1.36)	5	22.62 – 49.81	0.27
	No	13.28		13	6.16 – 20.39	
RT-PCR	Yes	19.54	0.67 (0.33 – 1.36)	09	7.58 – 31.51	0.27
	Negative	36.38		14	25.01- 47.77	
	Positive	38.88		17	30.89 – 46.88	

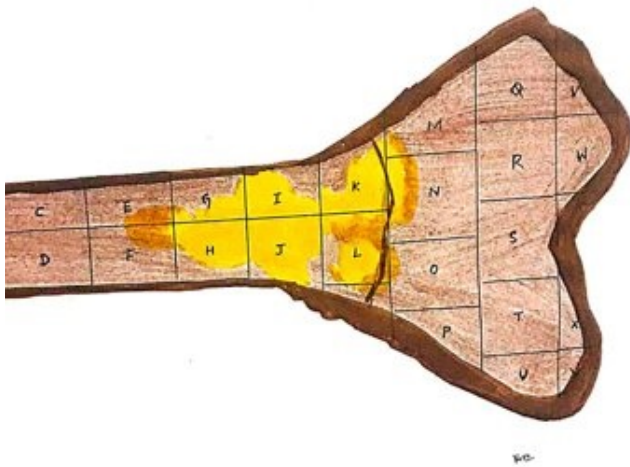
have a higher mean survival time when compared to >15 years of age, with a HR of 1.83. Unlike osteosarcoma, ES is not associated with any of the known genetic cancer syndromes<sup>13</sup> and our study did not find any patient with a syndromic association. Most of our patients presented with pain and swelling of the limb and the most common location of the tumour was appendicular (31.4%) followed by axial skeleton (23.1%). Solid organ involvement in ES has been reported in various organs like lung, kidneys, pancreas,

colon, uterus, and ovaries.<sup>16</sup> Most common solid organs involved were kidney, urinary bladder, ovary and rectum and accounted for 19% of cases.

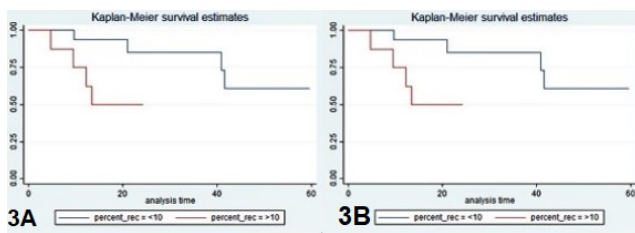
Tumour size has been found to be an important prognostic factor.<sup>11,13,14</sup> A cut off value of ≤12cm and >12cm was found to have a prognostic significance with a hazard ratio of 2.65 (95% C.I 0.68 – 10.39, p = 0.17). Most common site of metastasis was lung (62%) followed by bone (28%) as in other studies. A large study group from

**Table 4:** Clinico-pathological details of deceased patients (n=5)

	Case 1 (Biopsy)	Case 2 (Resection)	Case 3 (Resection)	Case 4 (Biopsy)	Case 5 (Biopsy)
Age (years)	25	19	12	10	10
Sex	Female	Male	Female	Male	Male
Complaints	Swelling	Swelling	Pain	Swelling	Pain
No of cycles of chemotherapy	NA	3	6	NA	NA
Site of primary	Vertebra (L2)	Tibia	Tibia	Femur	Soft tissue around nape of neck
RT-PCR	EWSR-FLI1 (Type 1)	EWSR-FLI1 (Type 1)	EWSR-FLI1 (Type 1)	EWSR-FLI1 (Type 2)	Negative
Gross tumour size	-	11cm	10.6cm	-	-
Viable tumour	-	60%	30%	-	-
Margin involvement	-	Yes	No	-	-
Distant metastasis	No	Yes (Lung)	No	No	Yes (Lung)



**Fig. 2:** Huvos grading to assess the percentage of viable tumour



**Fig. 3:** A): Kaplan Meir graph comparing survival with number of chemotherapy cycles; B): Kaplan Meir graph comparing the survival with percentage of viable tumour

South Korea have reported lymph nodes as being the most common site of metastasis in patients with extraskelatal ES.<sup>17</sup>

There was an improving trend in survival of patients in patients who underwent 6 cycles of chemotherapy who had a mean survival of 47.3 months as compared to patients with 1-4cycles (39.5months) and patients who did not receive

chemotherapy (16.4months). ES being a highly proliferative tumour, classically responds well to chemotherapy and best outcomes are seen in patients who have completed six cycles of chemotherapy especially in a shorter duration of time.<sup>18</sup>

The only known histological factor of prognostic significance is the response of the tumour to chemotherapy. Several grading systems have been used in the past, with the best system being the one developed by Huvos et al. (Figure 2).<sup>8,19</sup> It is graded by calculating the percentage of viable tumour in resected specimen.<sup>15,20,21</sup> With a cut off of ≤10% and >10%, there was a drastic difference in the survival of our patients, with 48.7 months and 17.1 months respectively for the two groups (HR= 5.6, 95% C.I 1 – 31.23, p = 0.04).

In our study, the EWSR-FLI1 (Type 1 fusion) accounted for 87% of cases, while 11% had type 2 fusion of EWSR-FLI1 genes. Only one patient had EWS-ERG translocation. A study by de Alava et al. have found that the presence of EWSR-FLI1 (type 1) mutation has a lower proliferation rate and is an independent factor of prognosis.<sup>22,23</sup> We found that the mean survival of patients with a translocation (38.88months) was more than those without (36.38months). However, the survival between the different types of translocations could not be compared because of small number of patients with EWSR-FLI1 type-2 fusion and EWSR-ERG fusions.

In our study, a primary diagnosis of ES was made based on morphology complemented by immunohistochemistry for CD99 and FLI1. Sensitivity of both markers in combination was 100% while the specificity was as low as 11%. When used alone, CD99 had a sensitivity of 100% while the specificity was as low as 5.7%. CD99 is positive in a huge spectrum of small round cell tumours such as lymphoblastic lymphoma, desmoplastic small round cell tumour and rhabdomyosarcoma to name a few. Similarly, when FLI1 was used alone, the specificity was only 4%. Previous studies have also reported similar findings.<sup>24–26</sup>

One has to remember that the fact that although EWS/FLI1 fusion gene is specific for ES, FLI1 protein expression is not. To conclude, a diffuse and strong membranous positivity for CD99 in combination with FLI1 positivity is sensitive in the diagnosis of ES. In this study, 23% of cases showed BCOR staining by immunohistochemistry and were classified as 'BCOR-CCNB3 fusion sarcoma'. Out of the 7 cases, three presented in the bone, three in the soft tissue and one case in the lung. Most patients were in second to third decade of life at presentation. This is similar to the study by Puls et al.<sup>4,5</sup> These patients are also known to present with metastatic lesions, most common site being the lung. In our study, two patients had metastasis, three had no evidence of disease and follow up was not available in two patients. Morphologically, these tumours displayed a round to spindle cell morphology with varying amounts of myxoid stroma. CD99 expression was either weak or focal in these cases. Although BCOR-CCNB3 tumours have a different genetic alteration and have been classified separately, they respond to ES based treatment regimens and have similar survival outcomes.<sup>1,6,7</sup> Due to the small sample size in our study, the survival data could not be compared between the classical Ewing and BCOR-CCNB3 cases.

To conclude, immunohistochemical markers like CD99 and FLI1 although sensitive, are not specific for ES. Diffuse, circumferential and strong membranous staining pattern for CD99 is more likely to be in favour of ES. FLI1 will be negative in tumours without FLI1 translocation. Although FLI1 was considered specific for ES, this is not true anymore.<sup>25</sup> Grid examination of the tumour to assess percentage of viable tumour is the most important exercise for a histopathologist while evaluating a specimen of ES. BCoR immunohistochemistry is a valuable tool in cases that are negative for conventional translocations. It is important to identify BCoR sarcomas since they have a similar response to Classic ES based chemotherapy protocol with better survival outcomes. Although this study could not detect statistical significance, age at diagnosis  $\leq 15$  yrs, female gender, size  $\leq 12$  cm, extra-osseous site, chemotherapy and translocation positivity predicted an improved survival rate. Less number of patients with positive EWSR-FLI1 and EWSR-ERG translocation could be related to the varied genetics of our population as compared to the West and these patients might harbour other translocations like the EWSR1-ETV1, EWSR1-ETV4, EWSR1-FEV which are routinely not tested. These patients might also harbour translocations like BCOR-CCNB1, CIC-DUX4 etc. Given the paucity of studies comparing molecular diagnostics and clinical features on ES from South East Asia, this study will serve as a baseline for future studies. It is an attempt to analyse ES in detail, and we believe that this will help in better understanding the biology of the disease. However, large multi centre collaborative studies are needed to decipher the factors of

prognostic significance that will help improve the survival of our patients.

## 5. Source of Funding

None.

## 6. Conflict of Interest


None.

## References

1. Soft Tissue and Bone Tumours. 5th ed. Lyon, France: IARC Press; 2020.
2. Machado I, Noguera R, Pellin A, Lopez-Guerrero JA, Piqueras M, Navarro S, et al. Molecular diagnosis of Ewing sarcoma family of tumors: a comparative analysis of 560 cases with FISH and RT-PCR. *Diagn Mol Pathol*. 2009;18(4):189–99.
3. Lewis TB, Coffin CM, Bernard PS. Differentiating Ewing's sarcoma from other round blue cell tumors using a RT-PCR translocation panel on formalin-fixed paraffin-embedded tissues. *Mod Pathol*. 2007;20(3):397–404.
4. Puls F, Niblett A, Marland G, Gaston CLL, Douis H, Mangham DC, et al. BCOR-CCNB3 (Ewing-like) sarcoma: a clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. *Am J Surg Pathol*. 2014;38(10):1307–18.
5. Henderson-Jackson EB, Bui MM. Molecular Pathology of Soft-Tissue Neoplasms and Its Role in Clinical Practice. *Cancer Control*. 2015;22(5):186–92.
6. Thway K, Folpe AL. Update on selected advances in the immunohistochemical and molecular genetic analysis of soft tissue tumors. *Virchows Arch*. 2020;476(1):3–15.
7. Schaefer IM, Cote GM, Hornick JL. Contemporary Sarcoma Diagnosis, Genetics, and Genomics. *J Clin Oncol*. 2018;36(2):101–10.
8. Wunder JS, Paulian G, Huvos AG, Heller G, Meyers PA, Healey JH. The histological response to chemotherapy as a predictor of the oncological outcome of operative treatment of Ewing Sarcoma. *J Bone Joint Surg Am*. 1998;80(7):1020–33.
9. Ottaviani G, Jaffe N. The Epidemiology of Osteosarcoma. In: Jaffe N, Bruland OS, Bielack S, editors. Pediatric and Adolescent Osteosarcoma. Cancer Treatment and Research. vol. vol 152. US: Springer; 2009. p. 3–13. doi:10.1007/978-1-4419-0284-9\_1.
10. Parkin DM, Stiller CA, Nectoux J. International variations in the incidence of childhood bone tumours. *Int J Cancer*. 1993;53(3):371–6.
11. Obata H, Ueda T, Kawai A, Ishii T, Ozaki T, Abe S, et al. Clinical outcome of patients with Ewing sarcoma family of tumors of bone in Japan. *Cancer*. 2007;109(4):767–75.
12. Burningham Z, Hashibe M, Spector L, Schiffman JD. The Epidemiology of Sarcoma. *Clin Sarcoma Res*. 2012;2(1):14. doi:10.1186/2045-3329-2-14.
13. Orr WS, Denbo JW, Billups CA, Wu J, Navid F, Rao BN. Analysis of prognostic factors in extraosseous Ewing sarcoma family of tumors: review of St. Jude Children's Research Hospital experience. *Ann Surg Oncol*. 2012;19(12):3816–22.
14. Lee CY, Yen C, Yen HJ, Yen CY, Chao TC, Wu PK, et al. Outcomes of 50 Patients With Ewing Sarcoma Family of Tumors Treated at a Single Institution in Taiwan. *Medicine (Baltimore)*. 2016;95(22):e3830.
15. Maldegem AMV, Hogendoorn PCW, Hassan AB. The clinical use of biomarkers as prognostic factors in Ewing sarcoma. *Clin Sarcoma Res*. 2012;2:7.
16. Takahashi D, Nagayama J, Nagatoshi Y, Inagaki J, Nishiyama K, Yokoyama R, et al. Primary Ewing's sarcoma family tumors of the lung a case report and review of the literature. *Jpn J Clin Oncol*. 2007;37(11):874–7.


17. Huh J, Kim KW, Park SJ, Kim HJ, Lee JS, Ha HK, et al. Imaging Features of Primary Tumors and Metastatic Patterns of the Extraskelatal Ewing Sarcoma Family of Tumors in Adults: A 17-Year Experience at a Single Institution. *Korean J Radiol.* 2015;16(4):783–90.
18. Jain S, Kapoor G. Chemotherapy in Ewing's sarcoma. *Indian J Orthop.* 2010;44(4):369–77.
19. García-Castellano JM, Yordi NA, Reyes C, Healey JH. Histopathologic and Radiologic Assessment of Chemotherapeutic Response in Ewing's Sarcoma: A Review. *Sarcoma.* 2012;2012:357424. doi:10.1155/2012/357424.
20. Bacci G, Ferrari S, Bertoni F, Rimondini S, Longhi A, Bacchini P. Prognostic factors in nonmetastatic Ewing's sarcoma of bone treated with adjuvant chemotherapy: analysis of 359 patients at the Istituto Ortopedico Rizzoli. *J Clin Oncol Off J Am Soc Clin Oncol.* 2000;18(1):4–11.
21. Venkitaraman R, George MK, Ramanan SG, Sagar TG. A single institution experience of combined modality management of extra skeletal Ewings sarcoma. *World J Surg Oncol.* 2007;5:3. doi:10.1186/1477-7819-5-3.
22. De Alava E, Kawai A, Healey JH, Fligman I, Meyers PA, Huvos AG. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J Clin Oncol Off J Am Soc Clin Oncol.* 1998;16(4):1248–55.
23. De Alava E, Panizo A, Antonescu CR, Huvos AG, Pardo-Mindán FJ, Barr FG, et al. Association of EWS-FLI1 Type 1 Fusion with Lower Proliferative Rate in Ewing's Sarcoma. *Am J Pathol.* 2000;156(3):849–55.
24. Louati S, Senhaji N, Chbani L, Bennis S. EWSR1 Rearrangement and CD99 Expression as Diagnostic Biomarkers for Ewing/PNET Sarcomas in a Moroccan Population. *Dis Markers.* 2018;2018:7971019.
25. Tomlins SA, Palanisamy N, Brenner JC, Stall JN, Siddiqui J, Thomas DG, et al. Usefulness of a Monoclonal ERG/FLI1 Antibody for Immunohistochemical Discrimination of Ewing Family Tumors. *Am J Clin Pathol.* 2013;139(6):771–9.
26. Mhaweche-Fauceglia P, Herrmann F, Penetrante R, Beck A, Sait S, Block AM, et al. Diagnostic utility of FLI-1 monoclonal antibody and dual-colour, break-apart probe fluorescence in situ (FISH) analysis in Ewing's sarcoma/primitive neuroectodermal tumour (EWS/PNET). A comparative study with CD99 and FLI-1 polyclonal antibodies. *Histopathology.* 2006;49(6):569–75.


### Author biography

**Kiruthiga K G**, Consultant Histopathologist  <https://orcid.org/0000-0002-5554-3606>

**Anne Jennifer Prabhu**, Professor  <https://orcid.org/0000-0003-2893-5812>

**Rekha Pai**, Professor

**Leni G Mathew**, Professor of Pediatrics  <https://orcid.org/0000-0003-1386-6316>

**Dhananjayan S**, Senior Demonstrator  <https://orcid.org/0000-0002-6284-4503>

**Selvamani Backianathan**, Professor  <https://orcid.org/0000-0002-9394-7249>

**Cite this article:** Kiruthiga K G, Prabhu AJ, Pai R, Mathew LG, Dhananjayan S, Backianathan S. Molecular profile & clinical outcome in 121 cases - experience from a tertiary referral centre in South India. *Indian J Pathol Oncol* 2023;10(2):163-169.