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Commentary

Ewing-like sarcomas: New molecular diagnoses in need of optimized treatment approaches

In this issue, Rekhi *et al*[1] reported a case series of non-Ewing small round cell sarcomas (SRCSs), including confirmed cases of *CIC*- and *BCOR*-rearranged sarcomas. These valuable data add to the burgeoning global experience of these rare entities and highlight important clinical and research challenges that must be faced as the effort to define the optimal treatment approach begins.

The advent of widely available techniques for the identification of recurrent genomic abnormalities has significantly improved the specificity of diagnosis of a growing number of cancer types and enabled the focussed development of disease-specific management approaches. Such techniques have been of particular use in the differential diagnosis of SRCS, a group of rare, heterogeneous cancers, where variable site of anatomical origin and significant overlap in histopathological characteristics had previously confounded conventional morphological and immunophenotypic classification[2]. Ewing sarcoma (ES) family of tumours (EFT) are a group of SRCS defined by chromosomal rearrangements that result in the fusion of the ES gene *(EWSR1)* to one of the ETS families of transcription factor-encoding genes (*FLI-1* in 90-95%; *ERG* in 5-10%; *ETV1, E1A-F* or *FEV* in <1%)[3]. The molecular classification of EFT has led to the successful conduct of a series of international, collaborative clinical trials in a robustly defined patient population[4]. However, while the typical presentation of EFT is that of classic ES (*i.e.*, primary tumour of a long bone presenting in childhood; monotonous cytomorphology with diffuse and strong CD99 expression; sensitivity to intensified chemotherapy and radiotherapy), there is significant variability in the histopathological characteristics and clinical behaviour of EFT that remains incompletely described by any association with specific *EWSR1* rearrangements[5]. Meanwhile, the subset of SRCSs that bear histopathological similarity to EFT but do not harbour *EWSR1* fusions have been classified as Ewing-like sarcomas, a heterogeneous group of cancers that frequently exhibit clinical characteristics atypical for classic ES and are associated with primary chemoresistance and poor survival outcomes[2].

The use of modern cytogenetic, genomic and transcriptomic analysis in the study of Ewing-like sarcomas has led to the identification of several new molecularly defined disease entities that are increasingly considered as distinct diagnoses[6,7]. Fusions of *CIC* with *DUX4* or, less commonly, *FOXO4* or *DUX4L*, have now been reported in almost 200 cases of SRCS[8]. An older age of presentation, soft tissue sites of origin and more aggressive clinical course distinguish these *CIC*-rearranged sarcomas from classic ES and are now thought to account for as much as two-thirds of *EWSR1*-negative SRCS[8]. Gene expression profiling of *CIC*-rearranged sarcomas has shown little overlap at the transcriptomic level with classic ES, supporting their classification as a separate cancer entity[9]. Meanwhile, tumours with a recurrent *BCOR-CCNB3* fusion resulting from paracentric inversion in the X-chromosome appear to account for a much smaller proportion (<5%) of Ewing-like sarcomas[7]. *BCOR-CCNB3* fusion sarcomas have a strong male predilection but otherwise have so far been seen to exhibit close clinical similarities to classic ES, including high rates of response to chemotherapy and favourable clinical outcomes[10]. However, despite clinicopathological similarity to classic ES, *BCOR*-rearranged sarcomas have been shown to be biologically distinct at the gene expression level[7].

In the newly reported Indian series, the authors retrospectively identified cases of SRCS reported

within their centre across an 11-yr period[1]. After exclusion of tumours with diagnostic features of EFT or other characteristic sarcoma subtypes, a total of 51 ostensible Ewing-like sarcomas with available archival formalin-fixed, paraffin-embedded (FFPE) tumour samples were defined. Patient and tumour clinicopathological parameters were reported alongside the results of contemporaneously performed immunohistochemistry (IHC) panels and, when not already performed, retrospective IHC assessment of CCNB3 nuclear expression. Assessment for *EWSR1* rearrangement was performed using fluorescent *in situ* hybridization (FISH) and/or reverse transcription polymerase chain reaction (RT-PCR) for fusions with *FLI* or *ERG* in 40 of 51 tumours. RT-PCR to detect *BCOR-CCNB3* and *CIC-DUX4* fusion transcripts was attempted in all 51 tumours. However, the quantity and/or quality of RNA extracted from FFPE tissue resulted in a lack of interpretable RT-PCR data for 17 cases. Of a total of 32 tumours confirmed negative for *EWSR1* rearrangement, for which interpretable RT-PCR results were available, fusion transcripts of *CIC-DUX4* and *BCOR-CCNB3* were detected in seven (21.8%) and five (15.6%) cases, respectively[1].

Clinicopathological and IHC features of the identified *CIC*-rearranged tumours were consistent with those reported in other case series - a wide range of age of presentation was seen (8-53 yr), although 6 of 7 patients were aged 20 or above; all seven cases were of soft tissue origin, with anatomical sites varying between trunk, pelvis and extremities; WT1 expression was seen in 6 of 7 tumours, CD99 in 5 of 7 and FLI-1 in 4 of 4 cases, for which staining was performed. Of note, focal expression of cytokeratins was seen in three cases. Survival data were available for 5 of 7 patients - after a median of six month follow up, two patients had died of disease, two were alive with disease and one was alive with no evidence of disease.

Consistent with other series, the five reported cases of *BCOR*-rearranged sarcomas had younger age of presentation (range 6-29 yr) than *CIC*-rearranged cases, a male preponderance (4/5 cases) and tumours arising from either soft tissue or bone in the extremities or trunk. CD99 expression was present in 4 of 5 cases but was generally patchier and/or weaker than that seen in the *CIC*-rearranged cases. Strong CCNB3 expression was seen in 4 of 5 and patchy positivity in the remaining one case. Survival follow up was available for 3 of 5 patients, with only one patient alive and disease free after six months of follow up.

The authors met with a number of challenges in their study. First, the use of archival FFPE tissue for molecular diagnostic testing imposed unavoidable technical limitations - in this series, one in three tumours did not yield RNA of sufficient quality or quantity for successful RT-PCR. Second, there were limits to the sensitivity of PCR-based approaches in detecting fusion transcripts. The authors used two pairs of PCR primer pairs to detect *CIC-DUX4* transcripts. However, given the recognized variation in *CIC* rearrangements, both in terms of specific fusion breakpoints and fusion partner, such an approach will have imperfect sensitivity for all previously reported *CIC* fusion genes. This may have contributed to the comparatively low incidence of *CIC*-rearranged sarcomas detected in this series of *EWSR1*-negative SRCS. FISH has been used in previous reports of *CIC*- or *BCOR*-rearranged sarcomas and should be considered as a technique complimentary to RT-PCR in future studies[11,12]. The investigation for accurate immunophenotypic correlates for these molecular diagnoses should continue, with the aim of broadening access to the ability to diagnose *CIC*- and *BCOR*-rearranged sarcomas in routine practice. Meanwhile, the ever-increasing accessibility of next-generation sequencing has accelerated the discovery of gene fusions with SRCS[13]. Chimeric transcripts may be sequenced *de novo* without prior knowledge of either fusion partner, while the preparation of targeted sequencing libraries using FFPE-compatible techniques such as multiplexed anchored PCR has enabled high throughput identification of gene fusions where only one of the involved genes is known[14]. Such methods promise to facilitate individualized approaches to defining the genomic landscape of SRCS.

In this series from India, the use of molecular diagnostic techniques enabled the identification of tumours with *CIC-DUX4* or *BCOR-CCNB3* fusions that conformed to the histological and clinical characteristics reported by previous series. These data add to the nascent global experience of these new sarcoma subtypes and should increase awareness of the importance of molecular diagnostics in de-convoluting the clinical heterogeneity of Ewing-like sarcomas.

At present, the clinical utility of prospectively diagnosing *CIC*- or *BCOR*-rearranged sarcomas is limited to prognostication, although this alone provides important and valuable information for patients. It is, however, now imperative that investigators aim to define optimal management approaches for each of these new molecularly-defined diagnoses. Reported

evidence indicates that *CIC*-rearranged sarcomas are resistant to the combination chemotherapy schedules used in EFT. Meanwhile, despite evidence of disparate molecular pathology, *BCOR*-rearranged sarcomas appear to share clinical characteristics with EFT including chemo-sensitivity and favourable long-term outcomes. This early experience indicates that the benefit of intensified multimodality therapies seen in EFT may be shared in *BCOR*-rearranged sarcomas. Meanwhile, it may be the case that the pronounced toxicity of such approaches are not balanced by any incremental benefit in *CIC*-rearranged tumours and that effort should be focussed on the development of novel therapies informed by an improved understanding of fusion-related molecular pathology. A retrospective study of clinically annotated, archival tissue cohorts collected as part of completed studies of SRCS will provide important data on the true incidence and treatment outcomes of these fusion-associated sarcomas. Meanwhile, new international collaborations will begin to prospectively assess the differential benefit of current treatment approaches in these diseases and provide a platform for the investigation of novel approaches. Given the rarity of these cancers, contribution throughout the global sarcoma community will be crucial to meeting these challenges.

***Conflicts of Interest*:** None.

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