Clinical, Morphologic and Genomic Findings in ROS1 Fusion Spitz Neoplasms.

Pedram Gerami, MD1,2, Daniel Kim, BS1, Elsy V. Compres, BA1, Bin Zhang, MS1, Ayesha U. Khan, MBA1, Joel C. Sunshine, MD, PhD1, Victor L. Quan, MD1, Klaus Busam, MD3
1Department of Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, IL.
2Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL.
3Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York.

Abstract

The presence of a characteristic chimeric fusion as the initiating genomic event is one defining feature of Spitz neoplasms. Characterization of specific subtypes of Spitz neoplasms allows for better recognition facilitating diagnosis. Data on clinical outcomes of the specific tumor types may help in predicting behavior. In this study we present the largest series to date on ROS1 fusion Spitz neoplasms. We present the clinical, morphologic and genomic features of 17 cases. We compared the morphologic features of these 17 cases to a cohort of 99 other non-ROS1 Spitz neoplasms to assess for features that may have high specificity for ROS1 fusions. These tumors consisted of 10 Spitz nevi and 7 Spitz tumors. None of the cases met criteria for a diagnosis of Spitz melanoma. Morphologically, the ROS1 fusion tumors of this series were characterized by a plaque-like or nodular silhouette, often densely cellular intraepidermal melanocyte proliferation, frequent pagetosis, tendency towards spindle cell cytomorphology, low grade nuclear atypia and floating nests with occasional transepidermal elimination. However, there was a significant range in microscopic appearances, including two cases with morphologic features of a desmoplastic Spitz nevus. Different binding partners to ROS1 were identified with PWWP2A and TPM3 being the most common. No case had a recurrence or metastasis. Our findings document that most ROS1 fusion Spitz neoplasms have some typical characteristic microscopic features, while a small proportion will have features overlapping with other genomic subtypes of Spitz neoplasms. Preliminary evidence suggests that they tend to be indolent or low grade neoplasms.
INTRODUCTION

The family of Spitz neoplasms is defined in the most recent edition of the World Health Organization Classification of Skin Tumors (4th edition) as a melanocytic neoplasm with a characteristic Spitz fusion or a mutation in HRAS with Spitzoid morphologic features. Recent studies have attempted to correlate specific clinical and morphologic findings in the various fusion subgroups such as ALK, NTRK1, NTRK3, MAPK, BRAF and ROS1.\(^1\)\(^-\)\(^16\) Genomic fusions involving the ROS1 oncogene are seen in 7 to 17% of Spitz neoplasms.\(^17\),\(^18\) However, thus far only one study of 6 cases has described the morphologic features of ROS1 Spitz neoplasms.\(^13\)

In this study, we report the clinical, histologic and molecular findings in 17 ROS1 fusion Spitz neoplasms in order to better characterize this subset of Spitz neoplasms. We compared a number of morphologic features in this set of ROS1 fusions to a control set of 99 non-ROS1 Spitz melanocytic neoplasms which have also been assessed by next generation sequencing (NGS). We describe characteristic morphologic features and report those morphologic features statistically more frequent in ROS1 Spitz compared to other subtypes of Spitz neoplasms. We also report for the first time the occurrence of ROS1 fusions in two cases of desmoplastic Spitz nevi.

MATERIALS AND METHODS

Case Selection and Genomic Sequencing

Study approval and waiver of consent for use of archived tissue were obtained through the Northwestern Institutional Review Board. The dermatopathology data base at Northwestern was searched for Spitz nevi (SN), atypical Spitz tumor (AST), and Spitz melanomas (SM) in which a ROS1 fusion was identified by NGS. We identified 8 cases matching the above criteria. The paired normal tissue were identified for Case #1, #2, #5 and #6. Additionally nine cases were contributed from the personal consultation files of KJ Busam at Memorial Sloan Kettering Cancer Center in New York. We also identified 99 cases consisting of 20 SN, 53 ST, and 26 SM. Each diagnosis was made at the time of clinical presentation based on morphology with incorporation of FISH or array CGH in select cases. The control group included 59 fusions consisting of the following genes: ALK (n = 14), MAP3K8 (n = 12), BRAF (n = 6), NTRK1 (n = 10), NTRK3 (n = 6), RET (n = 4), MET (n = 1), RASGRF (n = 1), RAF1 (n = 1), MAP3K3 (n = 1), FGFR (n = 1), ERBB4 (n = 1), PRKDC (n = 1). Additionally, there were 5 MAP3K8 truncations. Lastly there were mutations in 19 cases in the following genes: BRAF (n = 8), NRAS (n = 4), HRAS (n = 5), GNAQ (n = 1), ROS1 (n = 1). In 16 cases no known fusions or mutations were identified.

“Spitzoid” morphology was identified according to the World Health Organization Classification of Skin Tumors (4th edition) and other relevant literature.\(^19\)-\(^22\) NGS with a 1171 cancer related gene panel for DNA and a whole transcriptome sequencing on each case was performed with using the Tempus xO platform and variant-calling.\(^23\),\(^24\) The 1711-gene assay is validated and designed to target therapeutically actionable genes.
Tumor Classification and Clinicopathologic Features

In total there were 17 cases with ROS1 fusions. The clinical features including age, sex and site of the tumors were summarized from the medical record. Morphologic features were assessed by two board certified dermatopathologist experienced in the assessment of melanocytic tumors. The following morphologic features were evaluated: silhouette (plaque, wedge or nodular), cytology (epithelioid, spindled or both), nuclear atypia (mild, moderate or severe), pigmentation (absent, focal, or extensive), host inflammatory reaction (absent, non-brisk, or brisk), cell size (small, intermediate, large), mitotic figures per mm², and for the absence or presence of Kamino body, maturation, ulceration, epidermal hyperplasia, plexiform growth, epithelioid sheets, pagetosis, nesting in the adnexa, and desmoplasia.

Mild nuclear atypia was defined as a slightly larger nucleus than conventional nevomelanocytes. Moderate atypia was defined as a nuclear size similar to the size of keratinocytes with a hyperchromatic nuclear membrane, visible nucleolus, and variable chromatin quality. Severe nuclear atypia was defined as a nuclear size larger than keratinocytes with a hyperchromatic nuclear membrane, prominent and/or multiple nucleoli, and coarse chromatin. For host inflammatory reaction, a brisk response was defined as a diffuse infiltration of lymphocytes across the entire base of the tumor; a non-brisk response was defined as a focal infiltration of lymphocytes that does not cover the entire base. For cell size, the size of melanocytes was compared to the basal keratinocytes. Cells about the size of basal keratinocytes were considered small, those moderately larger than basal keratinocytes were intermediate in size and cells nearly twice the size of basal keratinocytes were considered large. Clinical information including age, gender and site of tumor was also included for analysis.

Statistical Analysis

All statistical analyses were performed in R Studio v1.2.5001 to compare morphologic features across the groups Spitz neoplasms. Fisher’s exact test or Chi square test was used to compare associations in categorical variables. Student’s t-test was used to compare mean values. A p value of < 0.05 was considered statistically significant. All tests were two-sided.

Data Availability

Data Availability—Processed sequencing data (vcf files and count files) can be found through GEO Series accession number GSE142443 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE142443).

RESULTS

Clinical Findings in ROS1 Fusion Spitz Neoplasms

The final diagnosis from the time of clinical care in the set of 17 ROSI Spitz neoplasms was Spitz nevus in 10 cases and Spitz tumor in 7 cases. In none of the cases was a diagnosis of Spitz melanoma favored. The patient ages ranged from 3 to 58 with a mean age of 19 years old. There were 10 female and 7 male patients. The body site of involvement was highly variable with 4 in the head/neck region, 3 on the upper extremities, 3 on the trunk and 7 on the lower extremities. Grossly, all cases were pink to red papules. In 14 cases the clinical
impression was available. In 7 cases the clinician suspected an atypical Spitz nevus and in one of these cases a dermoscopic description of radial streaming was provided. In 2 cases the clinical impression was dermatofibroma, in 2 cases it was benign nevus, in 2 cases it was pyogenic granuloma and in 1 case it was cyst.

Follow up was available for 13 of 17 cases (Table 1). The average follow up time was 23 months and ranged from 4 months to 95 months. In 12 cases the lesions were re-excised with clear margins with no evidence of recurrence. One of these 12 cases also had a sentinel lymph node biopsy (SLNB) which was negative. In one case the original biopsy was incisional and no further re-excision was performed. There was persistent tumor at a follow exam 4 months later.

**Morphologic and Immunohistochemical Findings in ROS1 Fusions Spitz Neoplasms**

The low power silhouette on the 16 ROS1 cases was mostly that of either a plaque like (n = 7) or nodular pattern (n = 7). Two cases had a wedge shaped silhouette and 1 was polypoid. In 12 cases the cytomorphology was a mixed pattern of epithelioid and spindle cells while in 4 cases there was a predominance of spindle cells. In all cases the atypia was mild or moderate with none of the cases having high grade nuclear atypia (P = 0.006) (Figure 1). This was statistically significant with ROS1 cases being less likely to have high grade nuclear atypia than the group of non-ROS1 Spitz neoplasms. The cell sizes were also all small to intermediate with none of the cases having large cells and this was also statistically significant (P = 0.001). Maturation was present in all cases and this was also statistically significant (P = 0.044). There was also a tendency for lower mitotic rate 1.3/mm² (P = 0.001) (Table 2). Kamino bodies were also more common in this type of Spitz (8/17) than non-ROS1 Spitz (P = 0.025).

Thirteen of 17 cases had overlying epidermal hyperplasia. Fourteen of 17 cases were completely amelanotic. Lobulated nests were seen in 2 cases and nesting in the adnexa in 5 cases. Five cases had notable pagetosis in the epidermis. None of these features were statistically significant compared to non-ROS1 Spitz neoplasms. Nine of 17 cases had floating nests defined as nests situated above the basal layer and in 3 cases there was transepidermal elimination of nests (Figure 1 and 2). Myxoid changes were not identified in any of the cases. Two cases were characterized by prominent stromal desmoplasia, and were morphologically best characterized as a desmoplastic Spitz nevus (Figure 3).

Immunohistochemical Staining for ROS1 was performed in 16 cases. Fifteen of the 16 cases showed strong positive staining (Figure 4). In one case only a blush staining was seen which was not convincingly positive.

**Genomic Findings in ROS1 Fusion Spitz Neoplasms**

The fusion partner was identified in 16 of the 17 cases in the study. The most common genomic fusions among the 16 ROS1 cases were a PWWP2A-ROS1 fusion seen in 6 cases and a TPM3-ROS1 fusion also seen in 5 cases. Other recurrent fusion partners included a PPFIBP1-ROS1 fusion seen in 2 cases, and fusions partners involving MYH9-ROS1, CAPRINI1-ROS1 and MYO5A-ROS1 were each seen in 1 case (Table 3).
Three cases had copy number aberrations identified by NGS and SNP arrays. Two cases had
copy number aberrations identified by NGS and 1 case had a copy number aberration
identified by SNP array. Copy number loss of BCL11B, FGF3, CARD11, FBXO11, FLT4,
GRIN2A, HGF, MGMT, MYCN, MYOD1, NPM1, NTRK3, PLAG1, PTPRT, RET, TERT
and TLX1 were identified in case 2. This case was negative for copy number alterations
when tested by a SNP array platform. Copy number gains of HOXA9, JUN, MDM2 were
identified in case 6. Case 10 had an isolated loss at 6q.

**DISCUSSION**

Among two studies sequencing a large number of Spitz neoplasms the frequency of ROS1
fusions varied from 7 to 17%. The vast majority of these cases were diagnosed as either
Spitz nevus or Spitz tumor. In this study 10 were diagnosed as Spitz nevus and 7 as Spitz
tumor. We did not identify any cases that met the criteria of a Spitz melanoma. In the study
from Wiesner et al where kinase fusions in Spitz neoplasms were first described, 3 of 24
ROS1 fusions were designated as Spitz melanoma, but no adverse clinical outcome was
reported. This study from Wiesner et al is the larger series on ROS1 fusions but does not
discuss morphologic features. Thus far there is only one study involving 6 cases of ROS1
fusions which were all designated as Spitz tumors by Donati et al which discusses
morphologic features.

While there is limited clinical outcomes information available on Spitz tumors with ROS1
fusions, among the 13 cases with follow up in this study and the 6 cases from Donati et al,
there are no reported recurrences or metastases after complete excision of the primary
tumors. One case in our series had a SLNB which was also negative. Thus, preliminary
evidence suggests that most Spitz tumors with ROS1 fusions are likely indolent or at least in
a much lower risk category compared to Spitz neoplasms with BRAF or MAP3K8 fusions
which seem to constitute much of the more aggressive variants of Spitz neoplasms.

We did not identify morphologic features which could allow for a definitive diagnosis of a
ROS1 fusion by microscopic review alone but there were some characteristic features. This
included a tendency for plaque-like or nodular silhouette without a deeply infiltrative
component with a combination of epithelioid and spindle cell cytomorphology. Statistically
significant features included lack of high grade cytologic atypia in all cases, lack of larger
cell type, presence of maturation, frequent Kamino bodies and lower mitotic rate. These
findings are consistent with the fact that all cases were diagnosed as Spitz nevus or Spitz
tumor and none were thought to be Spitz melanoma.

In our cases, 13/17 had epidermal hyperplasia and 5/17 had notable epidermal pagetosis.
Two cases had lobulated nests and 4 had nesting in the adnexa. None of these features were
statistically significant as they can be seen in a broad spectrum of Spitz subtypes. In
particular many of these features can overlap with NTRK1 fusion Spitz neoplasms. Donati et
al reported transepidermal elimination of nests and myxoid changes as being present in all 6
cases. Another highly characteristic feature was floating nests seen in 9 of 17 cases with
transepidermal elimination of nests in 3 cases. We did not identify significant mucinous

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changes though a colloidal iron was not performed. Although none of these features are totally specific, one might anticipate a ROS1 fusion in compound plaque like Spitz neoplasm with prominent intraepidermal component, Kamino bodies, with small to intermediate sized cells with low grade cytology, pagetosis and floating nests within the epidermis.

An interesting and novel observation is the detection of a ROS1 fusion in two desmoplastic Spitz nevi. This illustrates the wide spectrum of microscopic features associated with ROS1 fusions, but it also documents that the desmoplastic phenotype among Spitz nevi is not limited to HRAS aberrations. Gains of 11p (location of HRAS) and/or HRAS mutations have previously been thought to be typical of desmoplastic Spitz nevi. While they likely represent the most common aberration associated with a desmoplastic Spitz nevus, we hereby document two cases with a ROS1 kinase fusion associated with a desmoplastic phenotype.

In the 17 cases in this series, 6 different fusion partners were identified. This included PWWP2A (n = 6), TPM3 (n = 5), PPFIBP1 (n = 2), MYO5A (n = 1), CAPRINII (n = 1) and MYH9 (n = 1). PWWP2A was also the most frequent fusion partner in the series from Donati et al. A figure showing the chimeric protein model and the breakpoint of the fusions can be found in Figure 5. Previous in vivo studies show rising levels of phosphorylation produced by this fusion protein indicating that the ROS1 kinase is being constitutively activated.17

ROS1 fusions have been identified in 9% Spitz melanomas and 1.3% in melanomas from previous studies.17, 30 There are no cases of ROS1 fusion melanoma in the TCGA database. ROS1 fusions are also seen in a subset of 1 to 2% non small cell lung cancers. More recently ROS1 fusions were identified in 9 of 130 gliomas from an infant population.31 Also, rare cases of ROS1 fusions in angiosarcoma, thyroid and breast cancer have been reported.32–34 Interestingly in melanocytic neoplasms with ROS1 fusions the tumors seem to have an indolent clinical behavior.

In conclusion, this study describes the largest series to date on ROS1 fusion Spitz neoplasms. They seem to represent a lower grade group of tumors with generally indolent behavior. We could not find specific morphologic aberrations that were predictive of the molecular aberration but identified a number of features that were enriched in the group of ROS1 fusion tumors. They included a plaque or nodular silhouette with a cellular intraepidermal component, frequent Kamino bodies, a slight predisposition towards spindle cytology, a lower grade of cytologic atypia and floating nests/transepidermal elimination of nests. We also report for the first time the association of a desmoplastic phenotype with ROS1 fusions.

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Figure 1.
A) At 40X one can appreciate the plaque like silhouette of this ROS1 Fusion Spitz Neuvs B) At 100X the epidermal hyperplasia with a predominance of nests with spindle shaped melanocytes can be seen C) At 200X one can appreciate the transepidermal elimination of small nests into the stratum corneum. D) 400X demonstrates the Spitzoid cytomorphology with relatively low grade nuclear atypia.
Figure 2).
A and B) At 40x and 100X, respectively, a plaque like Spitz nevus with epidermal hyperplasia. C) At 200X one can appreciate some floating nests in the epidermis D) At 400X one can appreciate the relatively bland cytology of the Spitzoid melanocytes.
Figure 3).  
A) Low power shows a symmetric paucicellular Spitzoid neoplasm in a desmoplastic stroma. B) Higher magnification shows small nests and individual units of Spitzoid melanocytes entrapped in a sclerotic stroma consistent with a diagnosis of desmoplastic Spitz nevus.
Figure 4). A) Low power showing plaque like silhouette of a ROS1 Fusion Spitz nevus. B) IHC staining for ROS1 shows strong and uniform staining throughout the nevus. C) Higher magnification shows nests of epithelioid and spindle shaped melanocytes with bland cytomorphology lacking significant atypia.
Figure 5).
Diagrams of the chimeric fusion proteins in the *ROS1* fusion Spitz neoplasms
Table 1. Summary of clinical data in 16 cases of Spitz neoplasms with *ROS1* fusions

| Case | Age  | Gender | Location                | Diagnosis                  | Clinical impression                                                                 | Surgical Treatment                              | SLNB | Metastasis | Follow up | Recurrence |
|------|------|--------|-------------------------|----------------------------|------------------------------------------------------------------------------------|-----------------------------------------------|------|-------------|------------|------------|
| 1    | 34   | F      | Right lower medial leg  | Atypical Spitz tumor       | Rule out atypical nevus vs Spitz nevus vs malignant melanoma; 6 x 4 mm color variated pink brown papule with radial streaming pattern at edges | Complete excision                              | No   | No          | 24 months | No         |
| 2    | 37   | F      | Left anterior medial thigh | Atypical Spitz tumor      | 5.5 mm erythematous papule; dermatofibroma – check margins                        | Complete excision                              | No   | No          | 95 months | No         |
| 3    | 28   | F      | Left thigh              | Atypical Spitz tumor       | Cyst                                                                               | Complete excision                              | No   | No          | 64 months | No         |
| 4    | 13   | F      | Left upper arm          | Spitz nevus               | Re-excision; rule out Spitz nevus                                                  | Complete excision                              | No   | Not available | Not available | Not available |
| 5    | 20   | F      | Left buttock            | Spitz nevus               | Intradermal nevus, rule out atypia                                                  | None                                          | No   | No          | Not available | Not available |
| 6    | 6    | M      | Right buttock           | Spitz nevus               | Changing nevus, rule out Spitz nevus                                                | Complete excision                              | No   | No          | 35 months | No         |
| 7    | 36   | F      | Left shin               | Atypical Spitz tumor       | Melanocytic lesion, rule out atypical Spitz tumor                                    | Not available                                  | No   | Not available | Not available | Not available |
| 8    | 12   | F      | Right ear               | Atypical Spitz tumor       | None provided                                                                       | Incisional biopsy without further re-excision  | No   | No          | 4 months   | Persistent tumor 4 months later |
| 9    | 15   | M      | Left neck               | Spitz nevus               | Rule out Spitz nevus                                                                 | Complete excision                              | No   | No          | 15 months  | No         |
| 10   | 15   | M      | Right upper arm         | Atypical Spitz tumor       | Pyogenic granuloma versus hypertrophic scar                                          | Complete excision                              | No   | No          | 11 months  | No         |
| 11   | 9    | M      | Right ear               | Spitz nevus               | Nevus, rule out atypia                                                               | Complete excision                              | No   | No          | 10 months  | No         |
| 12   | 18   | M      | Left mid back           | Spitz nevus               | Spitz nevus                                                                         | Complete excision                              | No   | No          | 9 months   | No         |
| 13   | 17   | M      | Left upper back         | Desmoplastic Spitz nevus   | Dermatofibroma                                                                      | Complete excision                              | No   | No          | 5 months   | No         |
| 14   | 3    | M      | Left ear                | Spitz nevus               | Rule out pyogenic granuloma                                                          | Complete excision                              | No   | No          | 13 months  | No         |
| 15   | 4    | F      | Left knee               | Spitz nevus               | Rule out Spitz nevus                                                                 | Complete excision                              | No   | No          | 13 months  | No         |
| 16   | 58   | F      | Left arm                | Atypical Spitz tumor       | None provided                                                                       | Complete excision                              | Yes, negative | No          | 6 months   | No         |
| Case | Age | Gender | Location  | Diagnosis               | Clinical impression | Surgical Treatment | SLNB | Metastasis | Follow up | Recurrence |
|------|-----|--------|-----------|-------------------------|---------------------|--------------------|------|------------|-----------|------------|
| 17   | 6   | F      | Abdomen   | Desmoplastic Spitz      | None provided       | Complete excision  | No   | No         | Not available | No         |

SLNB, sentinel lymph node biopsy; F, female; M, male;
Table 2.
Comparison of Clinical and Morphologic Findings in ROSI and non-ROSI fusion Spitz neoplasms

|                  | All (n = 116) | Non-ROSI (n = 99) | ROSI (n = 17) | P    |
|------------------|---------------|-------------------|--------------|------|
| **Clinical**     |               |                   |              |      |
| Age, years       |               |                   |              | 0.75 |
| Mean             | 20.5          | 20.7              | 19.5         |      |
| Range            | 1–65          | 1–65              | 3–58         |      |
| Gender           |               |                   |              | 0.80 |
| Female           | 64            | 54                | 10           |      |
| Male             | 52            | 45                | 7            |      |
| Location         |               |                   |              | 0.68 |
| Head/Neck        | 23            | 19                | 4            |      |
| Upper Extremity  | 33            | 30                | 3            |      |
| Trunk            | 15            | 12                | 3            |      |
| Lower Extremity  | 45            | 38                | 7            |      |
| **Histologic**   |               |                   |              |      |
| Tumor Subtype    |               |                   |              | 0.008|
| SN               | 29            | 20                | 9            |      |
| AST              | 61            | 53                | 8            |      |
| SM               | 26            | 26                | 0            |      |
| Tumor depth, mm  |               |                   |              | 0.32 |
| Mean             | 2.04          | 1.88              | 2.93         |      |
| Range            | 0.25–17.0     | 0.25–12.2         | 0.40–17.0    |      |
| Tumor Diameter, mm|              |                   |              | 0.71 |
| Mean             | 4.78          | 4.74              | 5.02         |      |
| Range            | 0.69–16.5     | 0.69–16.5         | 2.90–14.0    |      |
| Characteristic       | All (n = 116) | Non-ROS1 (n = 99) | ROS1 (n = 17) | P    |
|---------------------|---------------|-------------------|---------------|------|
| Silhouette          |               |                   |               |      |
| Plaque              | 49            | 42                | 7             | 0.16 |
| Wedge               | 31            | 29                | 2             |      |
| Nodular             | 34            | 27                | 7             |      |
| Polypoid            | 2             | 1                 | 1             |      |
| Cytology            |               |                   |               | 0.17 |
| Epithelioid         | 25            | 24                | 1             |      |
| Spindled            | 30            | 26                | 4             |      |
| Both                | 61            | 49                | 12            |      |
| Nuclear Atypia      |               |                   |               | 0.006|
| Mild                | 11            | 10                | 1             |      |
| Moderate            | 74            | 58                | 16            |      |
| Severe              | 31            | 31                | 0             |      |
| Kamino body         |               |                   |               | 0.025|
| Absent              | 89            | 80                | 9             |      |
| Present             | 27            | 19                | 8             |      |
| Pigmentation        |               |                   |               | 0.10 |
| Absent              | 66            | 52                | 14            |      |
| Focal               | 29            | 27                | 2             |      |
| Extensive           | 21            | 20                | 1             |      |
| Maturation          |               |                   |               | 0.044|
| Absent              | 18            | 18                | 0             |      |
| Partial             | 26            | 19                | 7             |      |
| Present             | 72            | 62                | 10            |      |
| Ulceration          |               |                   |               | 0.62 |
| Absent              | 107           | 92                | 15            |      |
|                                | All (n = 116) | Non-ROS1 (n = 99) | ROS1 (n = 17) | P  |
|--------------------------------|---------------|-------------------|---------------|----|
| Present                        |               |                   |               |    |
| Inflammatory Reaction          |               |                   |               |    |
| Absent                         | 7             | 4                 | 3             | 0.04|
| Non-Brisk                      | 68            | 57                | 11            |    |
| Brisk                          | 41            | 38                | 3             |    |
| Epidermal Hyperplasia          |               |                   |               |    |
| Absent                         | 20            | 16                | 4             | 0.49|
| Present                        | 96            | 83                | 13            |    |
| Plexiform                      |               |                   |               |    |
| Absent                         | 73            | 64                | 9             | 0.42|
| Present                        | 43            | 35                | 8             |    |
| Epithelioid Sheet              |               |                   |               |    |
| Absent                         | 94            | 77                | 17            | 0.04|
| Present                        | 22            | 22                | 0             |    |
| Pagetosis                      |               |                   |               |    |
| Absent                         | 93            | 81                | 12            | 0.32|
| Present                        | 23            | 18                | 5             |    |
| Cell Size                      |               |                   |               | 0.001|
| Small                          | 21            | 20                | 1             |    |
| Intermediate                   | 65            | 49                | 16            |    |
| Large                          | 30            | 30                | 0             |    |
| Nesting Adnexa                 |               |                   |               | 0.15|
| Absent                         | 97            | 85                | 12            |    |
| Present                        | 19            | 14                | 5             |    |
| Lobulated Nests                |               |                   |               | 0.73|
|                     | All (n = 116) | Non-ROS1 (n = 99) | ROS1 (n = 17) | P     |
|---------------------|--------------|------------------|--------------|-------|
| Absent              | 95           | 80               | 15           |       |
| Present             | 21           | 19               | 2            |       |
| **Mitotic index (per mm²)** |              |                  |              | 0.001 |
| Mean                | 2.2          | 2.34             | 1.30         |       |
| Range               | 0–20         | 0–20             | 0–4          |       |
| Case | Fusion       | Copy Number Variation                      |
|------|--------------|--------------------------------------------|
| 1    | PWWP2A-ROS1  | None identified                            |
| 2    | TPM3-ROS1    | Copy loss: BCL11B, CARD11, FBXO11, FGFR3, FLT4, GRIN2A, HGF, MGMT, MYCN, MYOD1, NPM1, NTRK3, PLAG1, PTPRT, RET, TERT, TLX1 |
| 3    | TPM3-ROS1    | None identified                            |
| 4    | MYH9-ROS1    | None identified                            |
| 5    | PPFIBP1-ROS1 | None identified                            |
| 6    | MYOSA-ROS1   | Copy gain: HOXA9, JUN, MDM2                |
| 7    | TPM3-ROS1    | None identified                            |
| 8    | Identified by FISH Breakapart Probe | None identified |
| 9    | PWWP2A-ROS1  | None identified                            |
| 10   | PWWP2A-ROS1  | Copy loss: 6q22.1*                         |
| 11   | PWWP2A-ROS1  | None identified                            |
| 12   | PPFIBP1-ROS1 | None identified                            |
| 13   | PWWP2A-ROS1  | None identified                            |
| 14   | TPM3-ROS1    | None identified                            |
| 15   | TPM3-ROS1    | None identified                            |
| 16   | CAPRIN1-ROS1 | Not assessed                               |
| 17   | PWWP2A-ROS1  | None identified                            |

*Identified by SNP array