A Unique Sarcoma with Dual Morphologic, Cytogenetic, and Molecular Signatures
Pertinent history and symptoms

- 43 year old woman presented in 2011 with recurrent soft tissue tumor in the left arm.
- In 2004, at age 35, she presented at another institution with a 5-year history of a left arm mass (7.5 x 5.5 x 4 cm), which was resected and diagnosed as a sarcoma.
- Because she lost her medical insurance, she did not receive any further therapy after surgical resection.
- At re-presentation in 2011, she had a large, protuberant and firm soft tissue mass (9.6 x 7.6 x 6.5 cm) located along the posterior arm/triceps muscle.
- Based on diagnosis of high-grade sarcoma, patient received induction chemotherapy followed by surgical resection with intraoperative and subsequent external beam radiation.

**Diagnoses will be revealed later**
External and cut surfaces of recurrent tumor (9.6 x 7.6 x 6.5 cm)
cytokeratin cocktail
Summary of Morphologic and Immunohistochemical (IHC) Features

- Variably cellular and multinodular neoplasm
- Hypercellular areas with spindled cells in streaming to storiform growth pattern
- Hypocellular areas with round cells embedded in fibromyxoid to hyalinized stroma
- Hypercellular areas exhibit subtle transition to hypocellular areas
- Round cells with eccentrically placed nuclei, irregular nuclear contours, hyperchromasia, multiple chromocenters, and moderate amount of dense eosinophilic cytoplasm
- Mitotic activity up to 12 mf/10 high power fields
- Post-treatment necrosis approximately 20%
- Lymphovascular space invasion present

- IHC positive for S-100 (patchy), cytokeratin cocktail AE1/AE3 + CAM5.2 (patchy), EMA (patchy), MUC4 (focal), and bcl-2 (diffuse)
- IHC negative for CD99, synaptophysin, chromogranin, SMA, calponin, GFAP, desmin, myogenin
Differential Diagnosis

- Soft tissue myoepithelioma (aka soft tissue mixed tumor or parachordoma)
- Extraskeletal Myxoid Chondrosarcoma (EMC)
- Synovial Sarcoma (SS)
Further cytogenetic and molecular testing performed on original and recurrent tumors

- FISH
- RT-PCR to elucidate the gene fusion partners for *EWSR1* and *SYT* rearrangements in tumors
Fluorescence In-situ Hybridization results

- **EWSR1** (22q12) rearrangement in 188 of 200 cells (94%)
- **SYT** (18q11) rearrangement in 70 of 200 cells (35%)

Courtesy of Pauline Brenholz, MD
RT-PCR fusion results

- **EWS:NR4A3** fusion product from \( t(9;22)(q22;q12) \) characteristic of EMC

```
AGAGGCTTTATGGATATGACCAG   ATCATGCCCAAG
EWS exon 12               NR4A3 exon 3
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- **SS18-SSX2** fusion product from \( t(X;18)(p11;q11) \) characteristic of SS

```
TGGTTTGATG   ATATGCCCTGCGTCCAAG
SS18 exon 10       SSX2 exon 6
```
Diagnosis

- Sarcoma with dual morphologic, cytogenetic, and molecular signatures of extraskeletal myxoid chondrosarcoma and synovial sarcoma
Patient diagnosed with EMC in 2004 based on histomorphology and immunohistochemical (IHC) characteristics
Underwent surgical resection, margin positive
Lost to follow-up
Re-presented in 2011 with tumor recurrence
FNA performed on recurrent tumor and FISH study showed SYT (18p11) rearrangement → prompted a more thorough investigation of EMC vs SS
Pathology review of original tumor and recurrent tumor disclosed identical histologic and IHC characteristics
FISH studies on original and recurrent tumors performed for EWSR1 (22q12) and SYT (18p11) rearrangements
RT-PCR and direct automated sequencing performed on original and recurrent tumors
Primarily because of SS diagnosis, patient received induction chemotherapy with ifosfamide-epirubicin → followed by surgical resection with intraoperative and subsequent external beam radiation
## Comparison of Typical Clinical Features and Clinical Behavior of EMC vs SS

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>EMC</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>Rare, accounts for &lt;3% of all soft tissue sarcomas</td>
<td>Rare, but accounts for 5-10% of all soft tissue sarcomas</td>
</tr>
<tr>
<td>Affects</td>
<td>Middle aged and elderly individuals; M:F ratio 2:1</td>
<td>Adolescents and young adults; M:F ratio 1.2:1</td>
</tr>
<tr>
<td>Location</td>
<td>Deep-seated; &gt;66% in proximal extremities, limb girdles, thigh, popliteal fossa</td>
<td>Deep-seated; 85-95% in extremities, in vicinity of large joints, especially knee region</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Relatively slow-growing; 5 year survival rate 90%; late recurrence and metastasis common</td>
<td>Aggressive; 5 year survival rate range from 36-76% based on prognostic factors</td>
</tr>
<tr>
<td>Therapy</td>
<td>Radiation therapy responsive; standard of care surgical resection ± adjuvant radiotherapy</td>
<td>Chemosensitive (ifosfamide + doxorubicin/epirubicin); induction chemotherapy + resection + adjuvant radiotherapy</td>
</tr>
</tbody>
</table>
## Comparison of Histopathologic and Immunohistochemical Features of EMC vs SS

<table>
<thead>
<tr>
<th>Features</th>
<th>EMC</th>
<th>Monophasic SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spindle cell population</td>
<td>Sometimes</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Abundant myxoid matrix</td>
<td>Characteristic</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Multinodularity</td>
<td>Characteristic</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Epithelioid cells</td>
<td>Characteristic</td>
<td>Focally</td>
</tr>
<tr>
<td>S-100</td>
<td>± (18-50%)</td>
<td>± (30%)</td>
</tr>
<tr>
<td>Cytokeratin cocktail</td>
<td>-</td>
<td>+ (69%)</td>
</tr>
<tr>
<td>EMA</td>
<td>± (30%)</td>
<td>+ (97%)</td>
</tr>
<tr>
<td>MUC4</td>
<td>-</td>
<td>± (30%)</td>
</tr>
<tr>
<td>bcl-2</td>
<td>+ (&gt;95%)</td>
<td>+ (&gt;95%)</td>
</tr>
</tbody>
</table>
Comparison of Molecular Signatures Associated with EMC vs SS

<table>
<thead>
<tr>
<th></th>
<th>Translocations</th>
<th>Genes</th>
<th>Incidence</th>
</tr>
</thead>
</table>
| Extraskeletal myxoid chondrosarcoma | t(9;22)(q22;q12)  
tag(9;17)(q22;q11)  
tag(9;15)(q22;q21) | NR4A3*;EWS  
NR4A3;RBP56**  
NR4A3;TCF12 | 70%  
20% |
| Synovial Sarcoma      | t(X;18)(p11;q11)        | SS18-SSX1  
SS18-SSX2  
SS18-SSX4 | >95% |

*NR4A3, also known as CHN, NOR1, TEC

**RBP56, also known as TAF15
Significance of this finding

- First sarcoma reported in the literature with two translocation-defining abnormalities (EWS-NR4A3 & SS18-SSX2)
- Tumor displays morphologic and immunophenotypic overlap between EMC & SS
- Reinforces idea that specific genetic alterations can drive tumor morphology
- The discovery of SS18-SSX2 fusion (in addition to EWS-NR4A3 fusion) allowed clinical team to institute a more effective chemoradiation regimen directed toward synovial sarcoma
- As molecular testing for tumors becomes more widespread, perhaps more tumors with dual differentiation will be discovered → lead to greater understanding of the link between specific gene rearrangements and morphology and clinical behavior
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  - Balaram Puligandla, MD

**All figures used in this presentation are original and were not used for publication.**
References