Tumors of Soft Tissues
Update on IHC and Molecular Morphology

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University of London
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Adjunctive Techniques in ST Pathology

- Light Microscopy
- Electron Microscopy
- Immunohistochemistry
- Molecular Diagnosis (FISH, PCR, CGH, NGS)
- Expression Profiling
- Sequencing
- Prognosis
- Classification
- Diagnosis
- Personalisation
Adult STS: Incidence in UK

*50 per million: Per annum 3600*

- Undiff pleomorphic sarcoma: 430
- Leiomyosarcoma: 500
- Liposarcoma: 360
- Synovial sarcoma: 250
- MPNST: 225
- Epithelioid sarcoma: 35

*No of Anatomic Pathologists: 1200*
UK National Institute for Health and Clinical Excellence: Guidelines

A specialist soft tissue pathologist is one who:

- Regularly reports soft tissue sarcomas as a significant part of their workload
- Takes part in an approved External Quality Assessment scheme
- Is a member of a properly constituted Sarcoma Multidisciplinary Management Team

<table>
<thead>
<tr>
<th>Year</th>
<th>Count</th>
<th>Major Discrepancy</th>
<th>B v M</th>
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<tbody>
<tr>
<td>2005</td>
<td>256</td>
<td>11%</td>
<td>5%</td>
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<tr>
<td>2011</td>
<td>250</td>
<td>16.4%</td>
<td>23.5%</td>
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</table>

Thway 2010; Thway 2014
Sarcoma

- A malignant connective tissue tumor
- The term includes many different types of mesenchymal tumor
WHO Consensus Classification

By differentiation
- Adipocytic
- Fibroblastic
- Myofibroblastic
- Fibrohistiocytic
- Smooth muscle
- Skeletal muscle
- Pericytic
- Vascular
- Chondro-osseous
- Neural
- GIST
- Uncertain differentiation

By behavior
- Benign
- Intermediate
  - locally aggressive
    - fibromatosis
  - rarely metastasising
    - solitary fibrous tumor
    - inflamm myofibroblastic tumor
- Malignant
  - grade 1, 2, 3

WHO 2002, 2013
## Uncertain Differentiation

<table>
<thead>
<tr>
<th>Synovial sarcoma</th>
<th>E/S myxoid chondrosarcoma</th>
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<tr>
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</tr>
<tr>
<td>Epithelioid sarcoma</td>
<td>Alveolar soft part sarcoma</td>
</tr>
<tr>
<td>epithelial (part)</td>
<td>?</td>
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<tr>
<td>Clear cell sarcoma</td>
<td>PEComa</td>
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<tr>
<td>melanocytic</td>
<td>myomelanocytic</td>
</tr>
<tr>
<td>Ewing sarcoma/PNET</td>
<td>Gastrointestinal stromal tumor</td>
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<tr>
<td>neuroectodermal</td>
<td>?interstitial cell of Cajal</td>
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Adipocytic

Endothelial

Spindle cell

Epithelioid

Small round cell

Pleomorphic
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<th>Spindle</th>
<th>Epithelioid</th>
<th>Small round</th>
<th>Pleomorphic</th>
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<tr>
<td>CK</td>
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<td>CD34</td>
<td>EMA</td>
<td>Desmin</td>
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<td>CD31</td>
<td>CD99</td>
<td>S100 pr</td>
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<td>S100 pr</td>
<td>ERG</td>
<td>FLI-1</td>
<td>p16</td>
</tr>
<tr>
<td>MUC4</td>
<td>SMA</td>
<td>WT1</td>
<td>MDM2</td>
</tr>
<tr>
<td>STAT6</td>
<td>Desmin</td>
<td>S100 pr</td>
<td></td>
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<td></td>
<td>S100 pr</td>
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</tr>
<tr>
<td></td>
<td>INI1</td>
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</table>
Natural History of a New Diagnostic Ab

Highly specific and sensitive for one tumor type

Occasionally positive in similar tumor types

Often positive in different tumor types

Used only as part of a larger panel of Abs

No longer used
IHC and Molecular Genetics

- Markers of specific genetic changes
  - Gain, loss, mutation
    MDM2, CDK4, INI1, CD117, b-catenin, MYC
  - From fusion genes
    FLI1, WT1, TFE3, ALK, CAMTA-1, NTRK1, BCOR, CCNB3, ETV4
- Antibodies derived from expression profiling
  TLE1, DOG1, MUC4, GR1A2
- Antibodies derived from sequencing
  STAT6
Molecular Abnormalities in STT

- Translocations
- Non-translocational abnormalities
  - Somatic mutations
    - activating – *KIT, PDGFRA*
    - non-activating – *SMARCB, TSC*
  - Copy number abnormalities
    - gene amplification – *MDM2*
    - gene deletion – *SDH, SMARCB*
- Complex unbalanced karyotypes
Translocation-associated Sarcomas

- >30% of sarcomas
- Balanced or unbalanced
  - non-pleomorphic but mostly G3
- Non-random, leads to new fusion gene
  - transcriptional regulators
  - tyrosine kinases
  - growth factors
# Malignant Soft Tissue Tumors

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Translocation or rearrangement</th>
<th>Gene or Fusion gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar soft part sarcoma</td>
<td>der(17)(X;17)(p11;q25)</td>
<td>ASMPR1-TFE3</td>
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<tr>
<td>Angiomatoid fibrous histiocytoma</td>
<td>t(12;22)(q13;q12)</td>
<td>EWSR1-ATF1</td>
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<tr>
<td>Clear cell sarcoma</td>
<td>t(12;22)(q13;q12)</td>
<td>EWSR1-ATF1</td>
</tr>
<tr>
<td>Clear cell sarcoma (GIT)</td>
<td>t(2;22)(q33;q12)</td>
<td>EWSR1-CREB1</td>
</tr>
<tr>
<td>Dermatofibrosarcoma protuberans</td>
<td>t(17;22)(q21;q13)</td>
<td>COL1A1-PDGF</td>
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<tr>
<td>Desmoplastic SRCT</td>
<td>t(11;22)(p13;q12)</td>
<td>EWSR1-WT1</td>
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<tr>
<td>Endometrial stromal sarcoma Low-grade</td>
<td>t(7;17)(p5;q21)</td>
<td>JAZF1-SUZ12</td>
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<td></td>
<td>der(7)(t;7)(p21;p22)</td>
<td>JAZF1-PHF1</td>
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<tr>
<td></td>
<td>t(10;17)(q22;p13)</td>
<td>YWHAE-FMA22A/B</td>
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<td></td>
<td>t(X;17)(p11.2;q21.33)</td>
<td>MBTD1-CXorf67</td>
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<td>t(10;17)(q22;p13)</td>
<td>YWHAE-NUTM2A/B</td>
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<td>Epithelioid inflammatory myofibroblastic sarcoma</td>
<td>t(2;2)(p23;q13)</td>
<td>RANBP2-ALK</td>
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<tr>
<td>Ewing sarcoma/PNET family and ES family-like tumors</td>
<td>t(11;22)(q24;q12)</td>
<td>EWSR1-FLI1</td>
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<td></td>
<td>t(21;22)(q12;q12)</td>
<td>EWSR1-ERG</td>
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<td>t(2;22)(q33;q12)</td>
<td>EWSR1-FEV</td>
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<td>t(7;22)(p22;q12)</td>
<td>EWSR1-ETV1</td>
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<td></td>
<td>t(17;22)(q12;q12)</td>
<td>EWSR1-ETV4</td>
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<td>inv(22)(q12;q12)</td>
<td>EWSR1-PATZ1</td>
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<td>t(2;22)(q31;q12)</td>
<td>EWSR1-SP3</td>
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<td>t(20;22)(q13;q12)</td>
<td>EWSR1-NFATC2</td>
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<td>t(4;22)(q31;q12)</td>
<td>EWSR1-SMARCA5</td>
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<td></td>
<td>t(16;21)(p11;q22)</td>
<td>FUS-ERG</td>
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<td></td>
<td>t(2;16)(q36;p11)</td>
<td>FUS-FEV</td>
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<td>t(4;19)(q35;q13.1)tJCB</td>
<td>CIC-DUX4</td>
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<td>t(X;X)(p11;p11)</td>
<td>BCOR-CCNB3</td>
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<td>Extraskeletal myxoid chondrosarcoma</td>
<td>t(9;22)(q22;q12)</td>
<td>EWSR1-NR4A3</td>
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<td>t(9;17)(q22;q11)</td>
<td>TAF15N-NR4A3</td>
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<td>t(9;15)(q22;q21)</td>
<td>TCF12-NR4A3</td>
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<td>t(3;9)(q12;q22)</td>
<td>TFG-NR4A3</td>
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<td>Hemangioendothelioma Epithelioid</td>
<td>t(1;3)(p36.3;q25)</td>
<td>WWTR1-CAMTA1</td>
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<td>t(X;11)(p11.2;q13)</td>
<td>YAP1-TFE3</td>
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<tr>
<td>Pseudomyogenic</td>
<td>t(7;19)(q22;q13)</td>
<td>SERPINE1-FOSB</td>
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# Malignant Soft Tissue Tumors

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Translocation or rearrangement</th>
<th>Gene or Fusion gene</th>
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<tbody>
<tr>
<td>Infantile fibrosarcoma</td>
<td>t(12;15)(p13;q26)</td>
<td>ETV6-NTRK3</td>
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<td></td>
<td>Trisomies 8, 11, 17, and 20</td>
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<tr>
<td>Liposarcoma:</td>
<td>12q14-15 amplification</td>
<td>MDM2, CDK4, HMGA2</td>
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<td>Well-differentiated</td>
<td>t(12;16)(q13;p11)</td>
<td>FUS-DDIT3</td>
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<tr>
<td>Myxoid/round cell</td>
<td>t(12;22)(q13;q12)</td>
<td>EWSR1-DDIT3</td>
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<tr>
<td>Epithelioid pleomorphic</td>
<td>t(12;16)(q13;p11)</td>
<td>FUS-DDIT3</td>
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<td>Low-grade fibromyxoid sarcoma and sclerosing epithelioid fibrosarcoma</td>
<td>t(7;16)(q33;p11)</td>
<td>FUS-CREB3L2</td>
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<td>t(11;16)(p13;p11)</td>
<td>FUS-CREB3L1 (rare)</td>
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<td>Mesenchymal chondrosarcoma</td>
<td>t(8;8)(q21;g13.3)</td>
<td>HEY1-NCOA2</td>
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<td>Mesothelioma</td>
<td>t(14;22)(q23;q12)</td>
<td>EWSR1-YY1</td>
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<td>Myoepithelial tumor</td>
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<td>t(1;22)(q23;q12)</td>
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<td>t(6;22)(p21;q12)</td>
<td>EWSR1-POU5F1</td>
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<td>t(12;22)(q13;q12)</td>
<td>EWSR1-ATF1</td>
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<td>t(9;22)(q33;q12)</td>
<td>EWSR1-PBX3</td>
</tr>
<tr>
<td></td>
<td>t(6;16)(p21;p11)</td>
<td>FUS-POU5F1</td>
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<td>t(1;23)(p34;q12)</td>
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<td>t(1;16)(p34;p11)</td>
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<td>TGFB3-MGEA5</td>
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<td>3p11-12 ampri</td>
<td>VGLL3, CHMP2B</td>
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<td>Primary pulmonary myxoid sarcoma</td>
<td>t(2;22)(q33;q12)</td>
<td>EWSR1-CREB1</td>
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<td>Rhabdomyosarcoma:</td>
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<td>Embryonal</td>
<td>Trisomies 2q, 8, &amp; 20</td>
<td>LOH at 11p15</td>
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<td>Alveolar</td>
<td>t(1;13)(p36;q14)</td>
<td>PAX7-FOXO1</td>
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<td>t(2;13)(q35;q14)</td>
<td>PAX3-FOXO1</td>
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<td>t(X;2)(q13;q35)</td>
<td>PAX3-FOXO4</td>
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<td>t(2;2)(q35;p23)</td>
<td>PAX3-NCOA1</td>
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<td>t(2;8)(q35;q13)</td>
<td>PAX3-NCOA2</td>
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<td>t(6;8)(p21;q13)</td>
<td>SRF-NCOA2</td>
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<td>Spindle cell (infantile)</td>
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<td>Synovial sarcoma</td>
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<td>SS18-SSX4 (rare)</td>
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<td>SS18L1-SSX1</td>
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Benign Soft Tissue Tumors

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<th>Histological type</th>
<th>Translocation or rearrangement</th>
<th>Gene or Fusion gene</th>
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<tbody>
<tr>
<td>Aggressive angiomyxoma</td>
<td>12q15</td>
<td>HMGI-C</td>
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<tr>
<td>Angiofibroma of soft tissue</td>
<td>t(5;8)(p15;q13) t(7;8;14)(q11;q13;q31)</td>
<td>AHRR-NCOA2, GTF2I-NCOA2</td>
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<tr>
<td>Cellular angiofibroma</td>
<td>del 13q14</td>
<td>RB1 loss</td>
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<tr>
<td>Chondroid lipoma</td>
<td>t(11;16)(q13;p12–13)</td>
<td>CT10r95-MKL2</td>
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<tr>
<td>Fibromatosis</td>
<td>+8, +20 5q21–22 loss</td>
<td>APC loss, CTNNB1 mutations</td>
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<tr>
<td>Desmoplastic fibroblastoma</td>
<td>t(2;11)(q31;q12) t(11;17)(q12;p11.2) t(2;11)(q31–32;q12)</td>
<td>Unknown</td>
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<tr>
<td>Fibroma of tendon sheath</td>
<td>t(2;11)(q31–32;q12)</td>
<td>Unknown, USP6</td>
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<tr>
<td>Giant cell tumor of tendon sheath</td>
<td>t(1;2)(p13;q37)</td>
<td>CSF1-COL6A3</td>
</tr>
<tr>
<td>Diffuse type giant cell tumor</td>
<td>t(1;2)(p13;q37) subset with +5 and/or +7 only</td>
<td>CSF1-COL6A3</td>
</tr>
<tr>
<td>Glomus tumour</td>
<td>t(1;5)(p11–13;?) t(5;19)(p13;?)</td>
<td>NOTCH2, NOTCH3</td>
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<tr>
<td>Hibernoma</td>
<td>11q13–21 rearrangements</td>
<td>Unknown</td>
</tr>
<tr>
<td>Inflammatory myofibroblastic tumor</td>
<td>t(1;2)(q22;p23) t(2;19)(p23;p13) t(2;17)(p23;q23) t(2;2)(p23;q13) t(2;2)(p23;q35) t(2;11)(p23;q15) t(2;4)(p23;q21) inv(2)(p23q35) t(3;8)(q22;q12.2) t(6;17)(q22;p13.3) t(5;12)(q33;q13.3) t(12;15)(p13;q26)</td>
<td>TPM3-ALK, TPM4-ALK, CLTC-ALK, RANBP2-ALK, ATIC-ALK, CAR5-ALK, SEC31A-ALK, ATIC-ALK, TGF-RO5, YWHAER-ROS, NAB2-PDGF-RB, ETV6-NTRK3</td>
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<tr>
<td>Intramuscular myxoma</td>
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<td>GNAS1 mutation</td>
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<tr>
<td>Leiomyoma</td>
<td></td>
<td>MEDI2 mutation</td>
</tr>
<tr>
<td>Lipoblastoma</td>
<td>8q11–13 rearrangements</td>
<td>PLAG1</td>
</tr>
<tr>
<td>Lipofibromatosis-like neural tumor</td>
<td>t(1;1)(q25;q22-22) t(1;1)(q21-22)</td>
<td>TPM3-NTRK1, HMGIC-PPAP2B</td>
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<tr>
<td>Lipoma</td>
<td>t(1;12)(p32;q14.3) t(3;12)(q28;q14.3) t(1;6)(p32–p21)</td>
<td>HMGIC-LLP, HMGIC-PPAP2B</td>
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<tr>
<td>Mammary (type) myofibroblastoma</td>
<td>del 13q14</td>
<td>RB1, FOXO1 loss</td>
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<tr>
<td>Myopericytoma</td>
<td>t(1;15)(p21–22;q13–15)</td>
<td>ACTB-GLI</td>
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<tr>
<td>Nodular fasciitis</td>
<td>t(17;22)(p13;q13.1)</td>
<td>USP6-MYH9</td>
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<tr>
<td>Ossifying fibromyxoid tumor</td>
<td>t(6;12)(p21;q24.3) t(X;16)(q26.1;p13.3) t(3;11)(q23–24;q13.2)</td>
<td>EPP40-PHF1, CREBBP-BCORL1, KDM2A-WWTR1</td>
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<tr>
<td>Palisaded myofibroblastoma</td>
<td></td>
<td>CTNNB1 mutations</td>
</tr>
<tr>
<td>Pleomorphic fibroblastoma</td>
<td>t(11;12)(q11;q13)</td>
<td>MALAT1-GLI1</td>
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<tr>
<td>Schwannoma</td>
<td>22q12 loss</td>
<td>NF2 loss</td>
</tr>
<tr>
<td>Solitary fibrous tumor</td>
<td>12q13</td>
<td>NAB2-STAT6</td>
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<tr>
<td>Spindle cell hemangioima</td>
<td></td>
<td>IDH1 R132C mutation</td>
</tr>
<tr>
<td>Spindle cell/pleomorphic lipoma</td>
<td>16q13-qter rearrangement/loss, monosomy 13 or partial del 13q</td>
<td>RB1 loss</td>
</tr>
</tbody>
</table>
Spindle Cell Tumors

- Synovial Sarcoma
- Malignant Peripheral Nerve Sheath Tumor
- Fibrosarcoma
- Myofibroblastic tumors
Fig. 2 — Même technique. — Grossissement : 210 diamètres. Des tubes de type épithélial, à parois formées de une ou plusieurs assises de cellules cylindriques ou cubiques indifférenciées, sont sectionnées en tous sens, à la manière de ceux d’un épithéliome tubulé. Mais le stroma de cette prolifération d’aspect épithélial affecte d’une manière non moins nette le type du sarcome fasciculé à cellules fusiformes.
A Consistent Chromosome Translocation in Synovial Sarcoma

Recently, a specific chromosome change t(12;18) has been described in myxoid liposarcoma [1]. Here we present evidence that a second category of human soft tissue sarcoma—synovial sarcoma—may also be characterized by a specific chromosome abnormality.

Results from three patients are reported. Histologically, each case showed characteristic features of synovial sarcoma [2, 3]. One patient (case 1) had a recurrent tumor of biphasic morphology although she had originally presented with a monophasic tumor. Two patients (cases 2 and 3) had monophasic tumors. Cultures were initiated from the three tumor biopsies and cells harvested for chromosome analysis after 4–15 days. In all three cases the translocation t(X;18)(p11.2;q11.2) was present as a consistent abnormality. G-banded partial karyotypes are presented (Fig. 1).

![G-banded partial karyotypes of three synovial sarcomas showing t(X;18) (p11.2;q11.2): (a) case 1: a cell with the full karyotype 46,XX,t(X;18)(p11.2;q11.2),t[dic(10;11)(p1.2;q11.2)],+18,(11p23),-14q,-14p; (b) case 2: a cell with the full karyotype 46,XX,t(X;18)(p11.2;q11.2),t(4;7)(p12;q32),-10,-10q32; t(9;11)(p22;q22),+16; (c) case 3: a cell with the full karyotype 46,XX,t(X;18)(p11.2;q11.2),t[dic(11;1)(q22;p12)],+18,18p12.](image)

There are few reports of the involvement of either the X chromosome or chromosome #18 in structural karyotypic changes in solid tumors [4]. We know of two previous reports of the chromosome constitution of synovial sarcomas. One concerns the U-4SS cell line, where four markers were described none of which involved the X or #18. The origin of a fifth marker was unknown [5]. The second report described hypodiploid tumor cells from a short-term culture with the missing chromosomes being mostly X, Y, #10, or #19. However, structural abnormalities of either X or #18 were not noted [6].

The presence of an identical translocation t(X;18)(p11.2;q11.2) in every cell analyzed from each of the three tumors we examined suggests that this is a primary karyotypic change. hA-rof-1, a member of the rof oncogene group, has been mapped to Xp11-Xp13 [7], a region that includes the X chromosome breakpoint in the translocation reported here. We are currently investigating whether or not the structural or regulation of the hA-rof-1 gene is altered in synovial sarcoma.

The authors thank Prof. G. Westbury for providing the biopsy material and for his interest in this study.

SANDRA SMITH
BRIAN R. REFUEZ


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Synovial Sarcoma

- **SS18** (18q11.2)
- **SSX** (Xp11.21-23)
  - types 1, 2, 4 (of 5)
- **SS18/SSX** fusion genes
  - 60% SSX1
  - 40% SSX2
  - <1% SSX4
- Fusion protein competes with wild type SS18
  > SOX2 activation > proliferation

Smith 1987; Ladanyi 2002; Guillou 2004; Baird 2005; Storlazzi 2007; Amary 2007; Nakayama 2007; Kadoch 2013
Synovial Sarcoma

%
TLE1

- Synovial sarcoma 90-100%
- Sometimes positive in
  - MPNST
  - fibrosarcoma
  - solitary fibrous tumor
  - mesothelioma
  - \textit{BCOR-CCNB3} sarcomas
  - sarcoma NOS
- Most useful when negative

Kosemehmetoglu 2009; Jagdis 2009; Knosel 2009; Foo 2011; Li 2016
Malignant Peripheral NST

- 50% in NF-1 (3-15% incidence)
- Usually origin from nerve, trunk or neurofibroma
- Origin in schwannoma v rare
- Mass ± pain, paresthesia
Malignant Peripheral Nerve Sheath Tumor

SOX10

Graph showing the percentage of staining for various markers:

- S100
- CD34
- bcl2
- GFAP
- PGP
- Des
- SMA
- CK
- EMA
- βcat
- calret
- nestin
- TLE1
- SOX10
SOX10

- SRY-related HMG Box 10 (22q13.1)
- Melanocytes, schwann cells, myoepithelial cells, mast cells
- Schwannoma, neurofibroma, granular cell tumor
- Malignant peripheral nerve sheath tumor  49% (SS 3%)
- Clear cell sarcoma of soft tissue
- Myoepithelial tumors
- Salivary gland tumors

Nonaka 2008; Ordonez 2013; Miettinen 2015
Malignant Peripheral Nerve Sheath Tumor

- 17p, 17q abnormalities
- \textit{NF1} (17q11.2) inactivation
  - sporadic and in NF-1
    - neurofibromin
      - Ras inactivation
- \textit{TP53} (17p13)
  - mutations
- \textit{CDKN2A} (9p21) (\textit{p16}^{\text{INK4A}})
  - mutations, deletion
- \textit{EGFR} (7p12)
  - Overexpression
- Loss of \textit{H3K27me3} expression (1q42)
  (sporadic and postradiation)

Carroll 2012; Prieto-Grenada 2015
Malignant Peripheral Nerve Sheath Tumor
Loss of H3K27 trimethylation

> rabbit polyclonal* or monoclonal (C36B11**) anti-H3K27me3 antibody,

- Complete or partial loss of H3K27me3
  - 34-68% of all MPNST (>grade)
  - 100% of post-radiation MPNST
- No or mosaic loss
  - neurofibroma (mosaic)
  - myxofibrosarcoma (mosaic**)
  - epithelioid MPNST
  - melanoma
  - synovial sarcoma (weak +*)
  - gastrointestinal stromal tumor
  - ossifying fibromyxoid tumor
  - myoepithelial carcinoma

Lee 2014; Prieto-Grenada 2015; Schaefer 2016; *Cleven 2016; **Asano 2017
Fibroblastic Sarcomas

- Fibrosarcoma in DFS
- Low grade fibromyxoid sarcoma
- Sclerosing epithelioid sarcoma

**Infantile fibrosarcoma**
- t(17;22)(q22;q13)
  - COL1A1-PDGFB

**Myxoinflammatory fibroblastic sarcoma**
- t(7;16)(q34;p11)
  - FUS-CREB3L2
  - FUS-CREB3L2/1

**Myxofibrosarcoma**
- t(11;22(p13;q12)
  - EWSR1-CREB3L1

**Fibrosarcoma in DFS**
- t(1;10)(p22;q24)
  - ?TGFB3-MGEA5
  - t(2;6)(q31;p21.3)

**Low grade fibromyxoid sarcoma**
- t(7;16)(q34;p11)
  - FUS-CREB3L2
  - FUS-CREB3L2/1

**Sclerosing epithelioid sarcoma**
- TGFBR3
  - MGEA5
  - various, complex
Low-grade Fibromyxoid Sarcoma
MUC4

Mucin 4, cell-surface associated, 3q29

- Low grade fibromyxoid sarcoma 100%
- Sclerosing epithelioid sarcoma 78%
- Synovial sarcoma 90%
- Ossifying fibromyxoid tumor 33%
- GIST (epithelioid) 10%

- Carcinomas

Graham 2011; Doyle 2012
Low-Grade Fibromyxoid Sarcoma and Hyalinizing Spindle Cell Tumor of Viscera: A Synonymous Diagnosis

Robin Reid, MD, MS, and Michelle Zhen, MD

- t(7;16) (q32-34;p11); FUS-CREB3L2
- t(11;16)(p13;p11); FUS-CREB3L1 (rare)
- t(11;22(p13;q12); EWSR1-CREB3L1 (rare)

FUS at 16p11

Cancer Center, New York, NY, USA; *Department of Pathology, Institut Bergonie and University Victor Segalen, Bordeaux, France; †Department of Pathology, Istituto Nazionale Tumori, Milan, Italy; ‡Department of Pathology and Cytology, Lund University Hospital, Lund, Sweden; §Department of Anatomic Pathology, L25, The Cleveland Clinic Foundation, Cleveland, OH, USA; ¶Department of Pathology, Royal Marsden Hospital, London, UK; #Department of Pathology, Lausanne University Hospital, Lausanne, Switzerland; **Department of Pathology, Western Infirmary, Glasgow, UK and ***Department of Pathology, University of Leuven, Leuven, Belgium
Myofibroblastic Tumors

- Nodular fasciitis: Reactive/‘transient neoplasia’
- Fibromatosis: Intermediate locally recurrent
- Inflamm myofibroblastic tumor: Intermediate rarely metastasizing
  - epi inflamm myofibro sa: Malignant
- LG myofibrosarcoma: Intermediate rarely metastasizing
- Pleo myofibrosarcoma: Malignant

SMA +, Calponin +, Desmin +/-, h-caldesmon -
Nodular fasciitis

**USP6-MYH9** fusion

Inflammatory myofibroblastic tumor

**ALK and other** rearrangements

Fibromatosis

**APC** loss

**CTNNB1** mutations in 85%
exon 3, codons 41, 44, 34, 33

Low grade myofibrosarcoma

Multiple ring chromosomes
12p11, 12q13-q22 amplif.
Inflammatory Myofibroblastic Tumor

- F>M 2 mos -74 yrs (8)
- Lung, intra-abdominal
- HN, urinary tract
- Systemic symptoms
- Multinodular, infiltrative
- 29% recur 4% met
- 12.5% DOD

Meis 1991; Coffin 1995
Inflammatory Myofibroblastic Tumor

IgG4/IgG ratio increased in some
## ALK Protein Expression in IMT

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Site</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Yousem</td>
<td>Lung</td>
<td>33%</td>
</tr>
<tr>
<td>2001</td>
<td>Coffin</td>
<td>All sites</td>
<td>36%</td>
</tr>
<tr>
<td>2002</td>
<td>Cessna</td>
<td>All sites</td>
<td>40%</td>
</tr>
<tr>
<td>2006</td>
<td>Montgomery</td>
<td>GU</td>
<td>57%</td>
</tr>
<tr>
<td>2007</td>
<td>Coffin</td>
<td>All sites</td>
<td>55%</td>
</tr>
<tr>
<td>2015</td>
<td>Antonescu</td>
<td>All sites</td>
<td>56%</td>
</tr>
</tbody>
</table>

- Childhood, abdominal/pulmonary IMT
- Also some of: MPNST, RMS, leiomyosa.
Fusions & IHC in IMT

- **TPM3-ALK**
  - \( t(1;2)(q22;p23) \)
  - Cytoplasmic

- **TPM4-ALK**
  - \( t(2;19)(p23;p13) \)
  - Cytoplasmic

- **PPFIBP1-ALK**
  - \( t(2;12)(p23;p11) \)
  - Cytoplasmic

- **ATIC-ALK**
  - \( t(2;2)(p23;q35) \)
  - Cytoplasmic

- **SEC31A-ALK**
  - \( t(2;4)(p23;q21) \)
  - Cytoplasmic

- **CARS-ALK**
  - \( t(2;11)(p23;p15) \)
  - Cytoplasmic

- **CLTC-ALK**
  - \( t(2;17)(p23;q23) \)
  - Granular

- **RANBP2-ALK**
  - \( t(2;2)(p23;q13) \)
  - Nuclear membrane

- **EML4-ALK**
  - \( t(2;2)(p23;p21) \)

- **TFG-ROS1**
  - \( t(3;6)(q22;q12.2) \)
  - Cytoplasmic dot

- **YWHAE-ROS1**
  - \( t(6;17)(q22;p13.3) \)
  - Nuclear, cytoplasmic

- **NAB2-PDGFRβ**
  - \( t(5;12;)(q33;q13.3) \)

- **ETV6-NTRK3**
  - \( t(12;15)(p13;q26) \)

References:
- Takeuchi 2011; Lovly 2014; Hornick 2015; Antonescu 2015; Alassiri 2015
Small Round Cell Tumors

- Ewing sarcoma & Ewing sarcoma-like tumors
- Alveolar rhabdomyosarcoma
- Desmoplastic small round cell tumor
- Mesenchymal chondrosarcoma
- Poorly differentiated synovial sarcoma
- Small round cell variant of MPNST
- Small round cell variant of myoepithelial tumor
- Small round cell variant of malignant rhabdoid tumor
- Carcinoma, lymphoma, melanoma
Ewing Sarcoma

- Young adults
- Bone or soft tissue
- Thorax, paravertebral
- Abdomen
- Lung/chest wall
- Skin
- Aggressive
Ewing Sarcoma

- Small round cell
- Atypical
  - large cell
- Adamantinoma-like
- Clear cell
- Sclerosing

Folpe 2005; Llombart-Bosch 2009
Ewing Sarcoma

- CD99 +
- CK ±
- S100 pr ±
- CD56 ±
- FLI1 ±
- ERG ±
- NKX2.2 +
- Cyclin D1 +
NKX2.2

NK2 homeobox 2, at 20p11.22 (target of EWSR1-FLI1)

- Ewing sarcoma  >90%
- Olfactory neuroblastoma  80-100%
- Small cell carcinoma  25-30%
- Mesenchymal chondrosarcoma  33-75%
- PD synovial sarcoma  13%
- Desmoplastic SRCT  0%
- Merkel cell carcinoma  0%

*1 of CIC-DUX4 sarcoma, DSRCT, neuroblastoma, myoepithelial ca

Yoshida 2012;* Hung 2016
Cyclin D1

Cell cycle regulation 11q13.3

- Ewing sarcoma 100%
- Neuroblastoma 100%
- Ganglioneuroblastoma 100%
- Ganglioneuroma 0%
- Desmoplastic SRCT 50% (wf)
- Rhabdomyosarcoma 0%
- Lymphoblastic lymphoma 0%

Magro 2016
Ewing Sarcoma and ES-like Tumors

- t(11;22)(q24;q12)  \textit{EWSR1-FLI1} 85%
- t(21;22)(q22;q12)  \textit{EWSR1-ERG} 10\%
### Ewing Sarcoma and ES-like Tumors

- t(11;22)(q24;q12) \( \textit{EWSR1-FLI1} \ 85\% 
- t(21;22)(q22;q12) \( \textit{EWSR1-ERG} \ 10\% 
- t(2;22)(q35;q12) \( \textit{EWSR1-FEV} 
- t(7;22)(p22;q12) \( \textit{EWSR1-ETV1} 
- t(17;22)(q12;q12) \( \textit{EWSR1-E1AF} 
- \text{inv}(22)t(1;22)(q12;q12) \( \textit{EWSR1-ZSG} 
- t(2;22)(q31;q12) \( \textit{EWSR1-SP3} 
- t(20;22)(q13;q12) \( \textit{EWSR1-NFATC2} 
- t(4;22)(q31;q12) \( \textit{EWSR1-SMARCA5} 
- t(16;21)(p11;q22) \( \textit{FUS-ERG} 
- t(2;16)(q35;p11) \( \textit{FUS-FEV} 
- t(4;19)(q35;q13) \( \textit{CIC-DUX4} 
- \text{inv}(X)(p11.4p11.22) \( \textit{BCOR-CCNB3} 
- t(X;19)(q13;q13) \( \textit{CIC-FOXO4} 

Tumors with CIC-DUX4 fusion

- $t(4;19)(q35;q13.1)$
- $t(10;19)(q26.3;q13.1)$
- Adult or pediatric
- Trunk, extremities
- Mild pleomorphism, nucleoli
- Highly aggressive
  - lung mets at presentation
  - 5 year survival 43%

Graham 2012; Italiano 2012; Choi 2013; Machado 2013; Specht 2014; Yoshida 2015; Smith 2015; Gambarotti 2016; Antonescu 2017
CIC-DUX4

- CD99+ (67% diffuse)
- CK
- S100pr
- MUC4
- desmin
- WT1
- Calretinin
- FLI1±
- ERG
- c-MYC
- ETV4

ETV4

ETS variant 17q21.31

- CIC-DUX4 90-100%
- Ewing sarcoma <10%
- Desmoplastic SRCT <10%
- Alveolar RMS <5%
- BCOR-CCNB3 0%
- Mesenchymal chondrosarcoma 0%
- PD synovial sarcoma 0%
- Neuroblastoma 0%

Le Guellec 2016; Hung 2016
Tumors with *BCOR-CCNB3* Fusion

- t(X;X)(p11;p11)
- M, 7-44 years, trunk, extremities
- Bone or soft tissue
- 5 year survival 75%, 10 year 56%
- Also
  - *BCOR-MAML3*
  - *BCOR-ZC3H7B*

Pierron 2012; Cohen-Gogo, 2014; Puls 2014; Peters 2015; Li 2016
- BCOR
- CCNB3
- CD99+ (60%)
- bcl-2 (90%)
- CD117 (60%)
- TLE1 (75%)
- SATB (71%)
Small Round Cell Tumors

- Alveolar rhabdomyosarcoma
desmin, **myogenin**

- Desmoplastic round cell tumor
  **WT1**, EMA, CK, desmin, NSE, NF, **CD56**

- Ewing like sarcomas
  **CD99, FLI1, ERG, CK, desmin**

- PD synovial sarcoma
  **TLE1**, EMA, CK, CD99, CD56, bcl-2

- Mesenchymal chondrosarcoma
  **CD99**

- Small cell neuroendocrine carcinoma
  **TTF1**, CK, CD56, CG, brachyury (41%)
Small Round Cell Tumors

- Alveolar rhabdomyosarcoma
desmin, myogenin FOXO1 (+PCR)
- Desmoplastic round cell tumor
  WT1, EMA, CK, desmin, NSE, CD56 EWSR1 (+PCR)
- Ewing like sarcomas
  CD99, FLI1, ERG, CK, desmin EWSR1 (+PCR),
  FUS, CIC-DUX, BCOR-CCNB3
- PD synovial sarcoma
  TLE1, EMA, CK, CD99, CD56, bcl-2 SS18
- Mesenchymal chondrosarcoma
  CD99 HEY-NCOA2
- Small cell neuroendocrine carcinoma
  TTF1, CK, CD56, CG, brachyury (41%)
STT with **EWSR1 Rearrangement**

*(Ewing sarcoma RNA-binding protein 1, 22q12.2)*

- **EWSR1-FLI1, EWSR1-ERG, EWSR1-8 others**
  - Ewing & similar sarcomas
- **EWSR1-WT1**
  - desmoplastic SRCT
  - low grade myoid tumor
- **EWSR1-DDIT3**
  - myxoid liposarcoma
- **EWSR1-NR4A3**
  - e/s myxoid chondrosa
- **EWSR1-CREM**
  - myxoid mesenchymal tumor
- **EWSR1-CREB3L1**
  - sclerosing epithelioid fibrosa
- **EWSR1-CREB1, EWSR1-ATF1**
  - various
- **EWSR1-CREM**
  - myxoid mesenchymal tumor
- **EWSR1-POU5F1, EWSR1-PBX1, EWSR1-PBX3, EWSR1-ZNF44, EWSR1-KLF17**
  - myoepithelial tumors
- **EWSR1-YY1**
  - mesothelioma

Thway 2012; Flucke 2012; Panagopoulos 2013; Gru 2013; Bilodeau 2013; Ud Din 2015; Huang 2015; Antonescu 2017
Tumors with *EWSR1-ATF1* or *EWSR1-CREB1* fusions

- Angiomatoid fibrous histiocytoma
- Primary pulmonary myxoid sarcoma
- Intracranial myxoid mesenchymal tumor
- Clear cell sarcoma of soft tissue
- Clear cell sarcoma-like tumor of GIT

- Myoepithelial tumor of soft tissue
- Angiosarcoma of salivary gland

- Hyalinizing clear cell salivary carcinoma
- Clear cell odontogenic carcinoma

Thway 2011; Gru 2013; Bilodeau 2013; Kao 2017
Angiomatoid Fibrous Histiocytoma

Desmin

Clear Cell Sarcoma Soft Tissue

S100pr
HMB45
Melan A

Primary Pulmonary Myxoid Sa

IHC negative

CCS-like Tumor of GIT

S100pr
Primary Pulmonary Myxoid Sarcoma With EWSR1-CREB1 Fusion: A New Tumor Entity

Khin Thway, FRCPath,* Andrew G. Nicholson, DM, FRCPath,† Kay Lawson, MBBS,†
David Gonzalez, PhD,‡ Alexandra Rice, FRCPath,‡ Bonnie Balzer, MD,§ John Swansbury,
FRCPath,|| Toon Min, PhD,|| Lisa Thompson, PhD,‡ Kwame Adu-Poku, FRCPath,¶
Anne Campbell, MD, FRCPath,# and Cyril Fisher, MD, DSc, FRCPath*

Primary Pulmonary Myxoid Sarcoma

- Young adults
- 70% in females
- Parenchymal
- Endobronchial
- ‘Low grade’
- 2/6 metastasised
  - kidney, brain
Primary Pulmonary Myxoid Sarcoma
Primary Pulmonary Myxoid Sarcoma

- S100 pr -
- HMB45 -, melan A -
- desmin -
- t(2;22)(q33;q12)

EWSR1-CREB1

t(2;22)(q33;q12)

EWSR1 exon 7 $\longrightarrow$ CREB1 exon 8

EWSR1 exon 7 $\longleftarrow$ CREB1 exon 7
Primary Pulmonary Myxoid Sarcoma

- 20 possible cases
- F 12:8, 26-80 years
- Endobronchial 15
  - \textit{EWSR1-CREB1} 14/17 (PCR or FISH)
  - \textit{EWSR1} only rearranged 2
  - 3/16 metastasised (2 with transcripts)
‘Promiscuous’ Fusions

- **EWSR1-ATF1**
  - angiomatoid FH, clear cell sarcoma, CCSLTGIT
  - myoepithelial tumor, angiosarcoma, salivary HCCC, CCOC

- **EWSR1-CREB1**
  - angiomatoid FH, clear cell sarcoma, CCSLTGIT, PPMS

- **ETV6-NTRK3**
  - infantile fibrosarcoma, inflammatory myofibroblastic tumor, mesoblastic nephroma, AML, secretory ca breast, mammary analogue secretory carcinoma of salivary glands, papillary thyroid ca,

- **ASPRC1-TFE3**
  - alveolar soft part sa, juvenile renal cell carcinoma

- **TMP3-ALK**
  - inflammatory myofibroblastic tumor, anaplastic large cell lymphoma

- **YWHAE-NUTM2A/B**
  - endometrial stromal sarcoma, clear cell sarcoma of kidney

- **FUS-ERG**
  - Ewing sarcoma, AML

- **BRD4-NUT**
  - Ewing-like sarcoma, thymic & other carcinomas
Myoepithelial Tumors of ST/Bone

- M > F, 0-83 years (38)
- Limbs, HN, trunk, lung
- Subcutaneous > deep
- Circumscribed
- 42% myoepithelioma
- 25% mixed tumors
- 32% ca or malignant mixed tumors

Stout, 1959; Kilpatrick, 1997; Fernandez-Figuera, 1998; Michal, 1999; Kutzner, 2001; Mentzel, 2003; Hornick, 2003; Flucke 2011
Malignant Myoepithelial Tumors
Myoepithelial Tumors of ST/Bone/Lung

- t(6;22)(p21;q12)  \textit{EWSR1-POU5F1} (4/6 malignant)
- t(1;22)(q23;q12)  \textit{EWSR1-PBX1} (3/7 mal)
- t(19;22(q13;q12)  \textit{EWSR1-ZNF444} (3/3 mal)
- t(12;22)(q13;q12)  \textit{EWSR1-ATF1} (0/1 mal)
- t(9;22)(q33;q12)  \textit{EWSR1-PBX3} (0/3 mal)
- t(6;16)(p21;p11)  \textit{FUS-POU5F1} (0/1 mal)
- t(1;22))(p34;q12)  \textit{EWSR1-KLF17} (0/1 mal)*
- t(1;16)(p34;p11)  \textit{FUS-KLF17} (1/5 mal)

*\textit{kidney}

Brandal 2008; Brandal 2009; Antonescu 2010; Flucke 2012; Agaram 2014; Puls 2014; Huang 2015; Leduc 2016
Myoepithelial Tumors of ST/Bone/Lung

Aggregated Data
SMARC-deficient Tumors

- **SWI/SNF** (SWItching defective/Sucrose Non-Fermenting) related, **Matrix associated**, **Actin dependent** **Regulators of Chromatin**
- ATP-dependent chromatin remodelling complex
- **SMARCB1** = **INI1**$^*$ 22q11.2 (SNF5, BAF47)
- **SMARCA4** = **BRG1** 19p13.2
- **SMARCA2** = **BRM** 9p24.3
- Deleted/mutated in several tumor types
INI1 (SMARCB1)

**Negative:**
- epithelioid sarcoma (>90%)
- malignant rhabdoid tumor
INI1 (SMARCB1)

**Negative:**
- epithelioid sarcoma (>90%)
- malignant rhabdoid tumor
- epithelioid MPNST (50%)
- e/s myxoid chondrosa (some)
- myoepithelial tumor (some)
- atypical teratoid/rhabdoid tumor
- cribriform neuroepithelial tumor
- some carcinomas
  - SNUC (3%), GIT, pancreatic rhabdoid (28%), renal medullary, CDC (<15%)
- primary meningeal tumors
SMARCA4-deficient Thoracic ‘Sarcoma’
SMARCA4-deficient Thoracic ‘Sarcoma’

- M:F, Median 59
- Mediastinum, pleura, lung
- Poor outcome
- Ovoid cells, prominent nucleoli, rhabdoid
- Defined by sequencing
- BRG-1 and BRM (SMARCA2) negative
- CD34+, focal CK+, SOX2+, INI1+
  - Also small cell ovarian ca, endometrial adenoca, NSCLC (SOX2 -), GI, renal, urothelial ca

Le Loarer 2015; Strehl 2015; Agaimy 2016; Agaimy 2017; Yoshida 2017; Ramalingam 2017; Boland 2017
Limitations in Sarcoma Diagnosis

- Immunohistochemistry
  - increasing numbers of antibodies
  - increasing lack of specificity
- FISH for gene rearrangements/amplifications
  - Increasing numbers of probes
  - increasing lack of specificity
- PCR for fusion gene transcripts
  - less available, limited repertoire
  - not all rearrangements identified
- Poor cost-benefit of increasing panels for rare tumors
Next Generation Sequencing

• Identifies molecular events
  • Targetable mutations
    • single-nucleotide variants,
    • small insertions & deletions
    • copy number variation
    • complex structural variations
  • Fusion genes
CINSARC

- Complexity INdex in SARComas

Gene expression signature (67 genes)

- Frozen tissue in microarray
- NGS - RNA sequencing in paraffin

Can predict metastasis in:
- leiomyosarcoma
- UPS
- DDL
- synovial sarcoma

- CDCA2 or KLF14
  - Small molecule inhibitors

PDL1 expression is a poor-prognosis factor in soft-tissue sarcomas

François Bertucci, Pascal Finetti, Delphine Perrot, Agnès Leroux, Françoise Collin, Axel Le Cesne, ... show all

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Chibon 2010; Lagarde 2013; Lesluyes 2016
Conclusions
THE END
ENDEN
Liposarcoma

ALT/Well differentiated

Myxoid

t(12;16)(q13;p11) FUS-DDIT3
t(12;22)(q13;q12) EWSR1-DDIT3

Pleomorphic

complex karyotype

Dedifferentiated

Myxoid

t(12;16)(q13;p11) FUS-DDIT3
t(12;22)(q13;q12) EWSR1-DDIT3

Epithelioid pleomorphic

FUS-DDIT3 transcripts