WHO Classification of Undifferentiated Small Round Cell Sarcomas: *Context, Challenges and Molecular Tools*

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WHO Soft Tissue and Bone Tumours, 5th Ed. (2020)

Undifferentiated Small Round Cell Sarcomas of Bone and Soft Tissue

- Ewing sarcoma
- Round cell sarcoma with *EWSR1* - non-*ETS* fusions
- *CIC*-rearranged sarcoma
- Sarcomas with *BCOR* genetic alterations

WHO Soft Tissue and Bone Tumours, 5th Ed. (2020)
Undifferentiated Small Round Cell Sarcomas of Bone and Soft Tissue

Objectives

Review the clinical behavior, morphologic, immunophenotypic and molecular features that distinguish these entities.

Discuss challenges in molecular/testing and provide case examples where NGS testing resolved the diagnostic uncertainty.

Ewing sarcoma is strictly defined by specific *EWSR1* or *FUS* fusions to *ETS*-family genes

- **CD99+, FLI1+ IHC**
  - **sensitive but *very nonspecific***
- **NKX2.2+ IHC**
  - **sensitive but *nonspecific***
    - **URCS with *EWSR1*-non*ETS***
    - **Mesenchymal chondrosarcoma**
    - **Olfactory neuroblastoma**

- t(11;22)(q24;q12) *EWSR1-FLI1 (85%)*
- t(21;22)(q22;q12) *EWSR1-ERG (10%)*
- t(7;22)(p22;q12) *EWSR1-ETV1*
- t(2;22)(q33;q12) *EWSR1-FEV*
- t(17;22)(q12;q12) *EWSR1-E1AF*
- inv(22)(q21;12) *EWSR1-ZSG*
- t(16;21)(p11;q22) *FUS- ERG*

Reviewed in Kallen, ME and Hornick, JL 2021 Am J Surg Pathol 45:e1–e23.

Ewing sarcoma is strictly defined by specific *EWSR1* or *FUS* fusions to *ETS*-family genes

- **CD99+ membranous (essential for dx), FLI1+ IHC**
  - **sensitive but *very nonspecific***
- **NKX2.2+ IHC**
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- **WHO: Molecular detection** of a *EWSR1* or *FUS* gene rearrangement (DNA) or fusion (RNA) is **"desirable"** for diagnosis, "**often required**"

- NCCN guidelines (version 2.2022) – *consider comprehensive genomic profiling/fusion panel testing if conventional methods (FISH, cytogenetics, RT-PCR) are negative*

- SOC therapy (NCCN guidelines): specific, multimodality chemotherapy, ~80% 5 year survival
- 65-70% cure with localized disease
- <30% 5 year survival with early relapse or metastasis

Reviewed in Kallen, ME and Hornick, JL 2021 Am J Surg Pathol 45:e1–e23.

Ewing sarcoma is strictly defined by specific *EWSR1* or *FUS* fusions to *ETS*-family genes

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- **Molecular detection** of a *EWSR1* or *FUS* gene rearrangement (DNA) or fusion (RNA) is **essential** for diagnosis
  - NCCN guidelines – *consider comprehensive genomic profiling/fusion panel testing if conventional methods (FISH, cytogenetics, RT-PCR) are negative*

- Standard of care/NCCN guidelines: specific, multimodality chemotherapy, ~80% 5 year survival

Note! The term peripheral primitive neuroectodermal tumor is now obsolete

Undifferentiated RCS, Formerly "Ewing-like" Sarcomas, primitive high grade neoplasms

- ***CIC***-rearrangement*
  - ***CIC-DUX4* (95%)**
  - *CIC-FOXO4*, *LEUTX*, *NUTM1*, or *NUTM2*
- ***BCOR*** genetic alteration*
  - *BCOR-CCNB3*
  - *BCOR-MAML3*
  - *BCOR* ITD
  - *BCOR-ZC3H7B*
  - *BCOR*-altered high grade endometrial stromal sarcoma
    - *ZC3H7B-BCOR, BCOR-ZC3H7B*
    - *BCOR* exon 15 ITD
- ***EWSR1 rearrangement* with non-*ETS* gene family partner**
  - *EWSR1-PATZ1*
  - *EWSR1-NFATC2*

broad age range

bone, soft tissue or visceral*

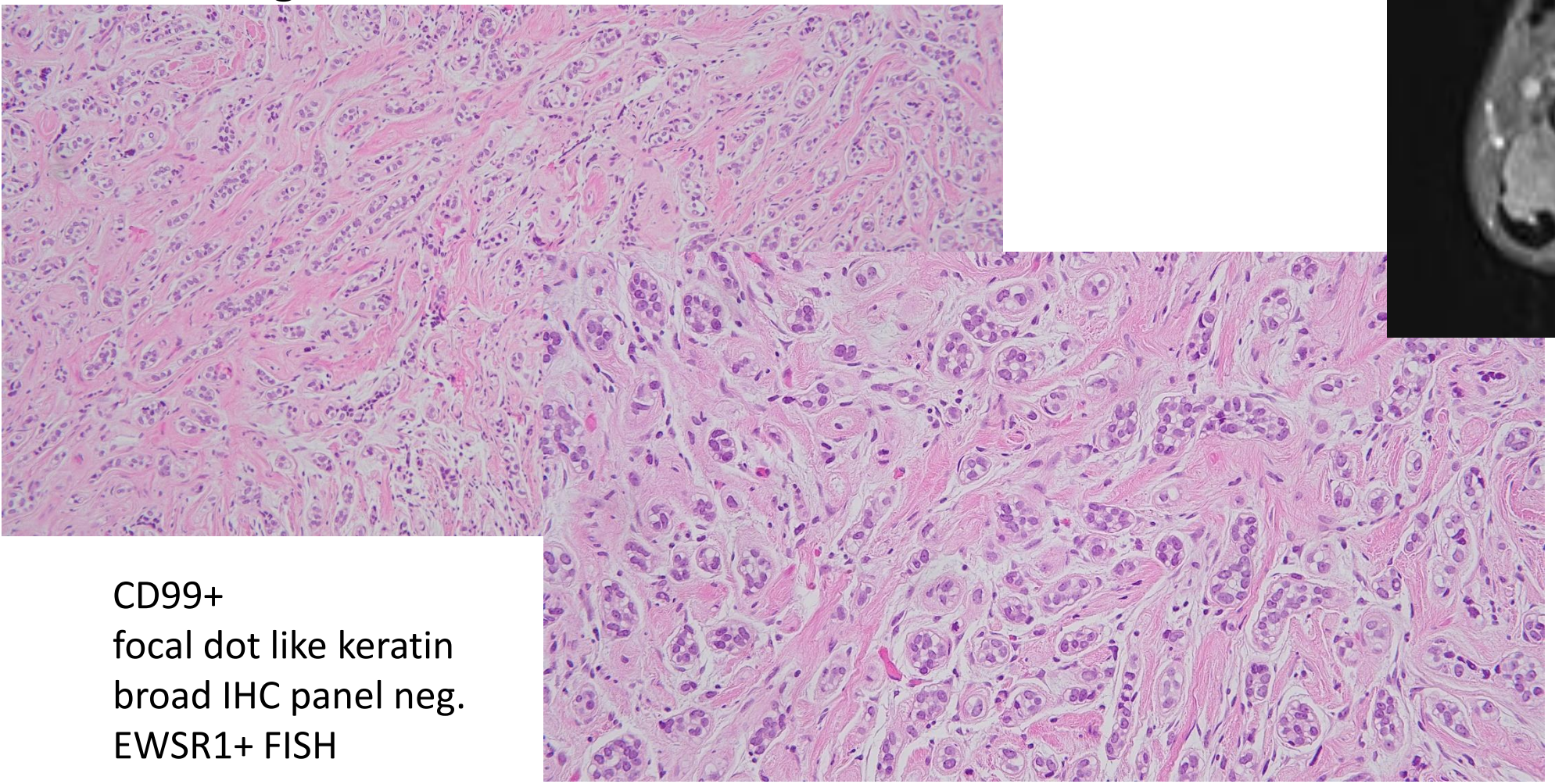
Broad morphologic overlap with other sarcomas defined by specific molecular alterations

- Synovial sarcoma — t(X;18)(p11;q11) *SS18-SSX1, SSX2, SSX4*
- Round cell/myxoid liposarcoma — t(12;16)(q13;p11) ***FUS -DD1T3*** (*TLS-CHOP*)
  t(12;22)(q13;q12) ***EWSR1-DD1T3*** (*EWSR1-CHOP*)
- Pulmonary myxoid sarcoma — t(2;22)(q34;q12) ***EWSR1****-CREB1*
- Mesenchymal chondrosarcoma — t(8;8)(q13;q21) ***HEY1- NCOA2***
- Alveolar rhabdomyosarcoma — t(2;13)(q35;q14) ***PAX3****-FKHR*
  t(1;13)(p36;q14) ***PAX7****-FKHR*
  t(X;2)(q13;q35) ***PAX3****-AFX*
- Sclerosing/spindle cell rhabdomyosarcoma — *MYOD1* mutation
- Embryonal rhabdomyosarcoma — *MYOD1*, *PIK3CA* mutations
- Sclerosing epithelioid fibrosarcoma — t(7;16)(p22;q24) ***FUS****-CREB3L2*
- Desmoplastic small round cell tumor — t(11;22)(p13;q12) ***EWSR1*** *-WT1*
- Neuroblastoma — *N-Myc* amplification
- Wilms Tumor
- Small cell variant of osteosarcoma

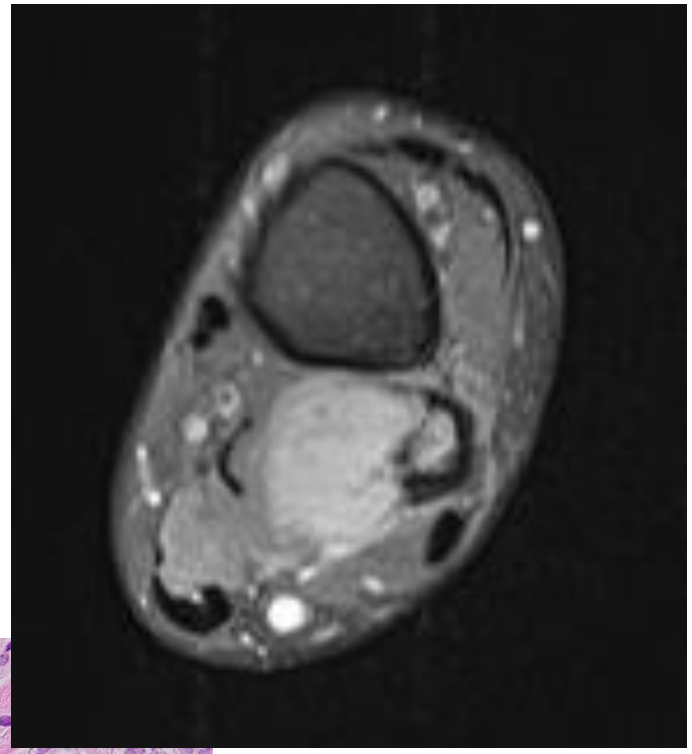
CIC-, *BCOR-*, *EWSR1-NFATC2*, *EWSR1-PATZ1* rearranged Round Cell Sarcomas

- Predominantly monomorphic, round -or- short spindled cells
- Intermediate-sized, no cytoplasm or little cytoplasm
- Primitive appearance, often finely disbursed chromatin
- Some with mild - moderate - severe atypia
- Variable cellularity
- Architecture
  - Solid sheets -or-
  - Nodules -or-
  - haphazard fascicles or bundles with dense collagenous or myxoid matrix
- ± CD99 IHC staining

case study
31yo male, ankle pain 6-8 mos,
aggressive distal fibular metaphyseal
enhancing mass with a soft tissue extension

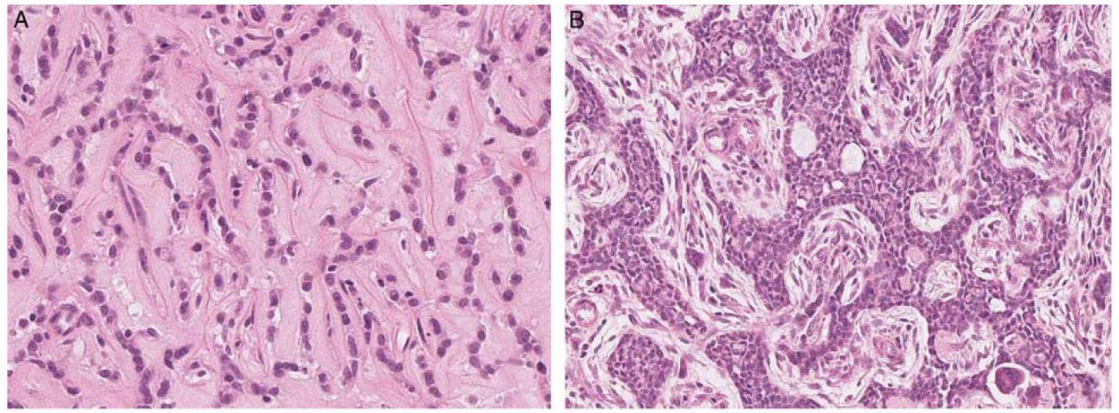


CD99+
focal dot like keratin
broad IHC panel neg.
EWSR1+ FISH



MRI

Round cell sarcoma with *EWSR1-NFATC2 or FUS-NFATC2* fusion



Am J Surg Pathol 2019;43:1112–1122

- Extremely RARE!!!
- male predilection (5:1)
- children and adults, 12-67 yrs
- Bone & soft tissue, generally occur in the metaphysis or diaphysis of long bones
- round, epithelioid, and/or spindle cells forming cords, nests, and trabeculae, with hyalinized or myxohyaline stroma.
- CD99 expression in ≥50% of cases
- Variable NKX2-2 and PAX7, and focal, often dot-like keratin expression.

WHO Classification of Tumours, 5th Ed. *Soft Tissue and Bone Tumours*.

EWSR1-NFATC2 Translocation-associated Sarcoma Clinicopathologic Findings in a Rare Aggressive Primary Bone or Soft Tissue Tumor

TABLE 2. Demographic and Clinical Outcome Data for 26 Reported Cases of *EWSR1-NFATC2* Sarcoma

Case	Age (y)/ Sex	Primary Location	Local Recurrence (mo)	Metastasis (mo)	Outcome	Follow-up (mo)
1	67/M	Left radius	No	No	AWOD	14
2	32/M	Periclavicular soft tissue	No	No	AWOD	24
3	42/M	Right radius	No	Lung (10)	AWD	16
4	24/F	Gastrocnemius muscle	No	No	AWOD	23
5	42/M	Right radius	No	Soft tissue and bone (35)	DOD	93
6	59/M	Left periclavicular soft tissue	Yes (4)	No	AWOD	144
Szuhai	39/M	Right humerus	NR	NR	NR	NR
Szuhai	16/M	Right femur	NR	NR	NR	NR
Szuhai	21/M	Right thigh soft tissue	NR	NR	NR	NR
Szuhai	25/M	Right femur	NR	NR	NR	NR
Romeo	32/M	Lower extremity bone	NR	No	AWOD	64
Sadri	30/M	Left femur	Yes	No	AWOD	4
Antonescu	42/M	Right femur	NR	NR	NR	NR
Kinkor	12/M	Left humerus	Yes	Suspicious lung	AWD	53
Kinkor	28/M	Left femur	No	No	AWOD	11
Machado	NR	NR	NR	NR	AWOD	NR
Toki	NR	NR	NR	NR	NR	NR
Wang/Lazar (2 cases)	NR	NR	NR	NR	NR	NR
Charville/Lazar (6 cases)*	NR	NR	NR	NR	NR	NR
Watson	32/M	Humerus	NR	NR	NR	NR
Watson	12/F	Tibia	NR	NR	NR	NR
Watson	61/M	Calf	NR	NR	NR	NR
Watson	23/M	Femur	NR	NR	NR	NR
Yau	42/M	Left femur	Yes (7)	No	AWOD	12

Cases 1 to 6 are from current study.
*Probably includes the 2 cases reported by Wang/Lazar above.
AWOD indicates alive without disease; F, female; M, male; mo, months postdiagnosis; NR, not reported.

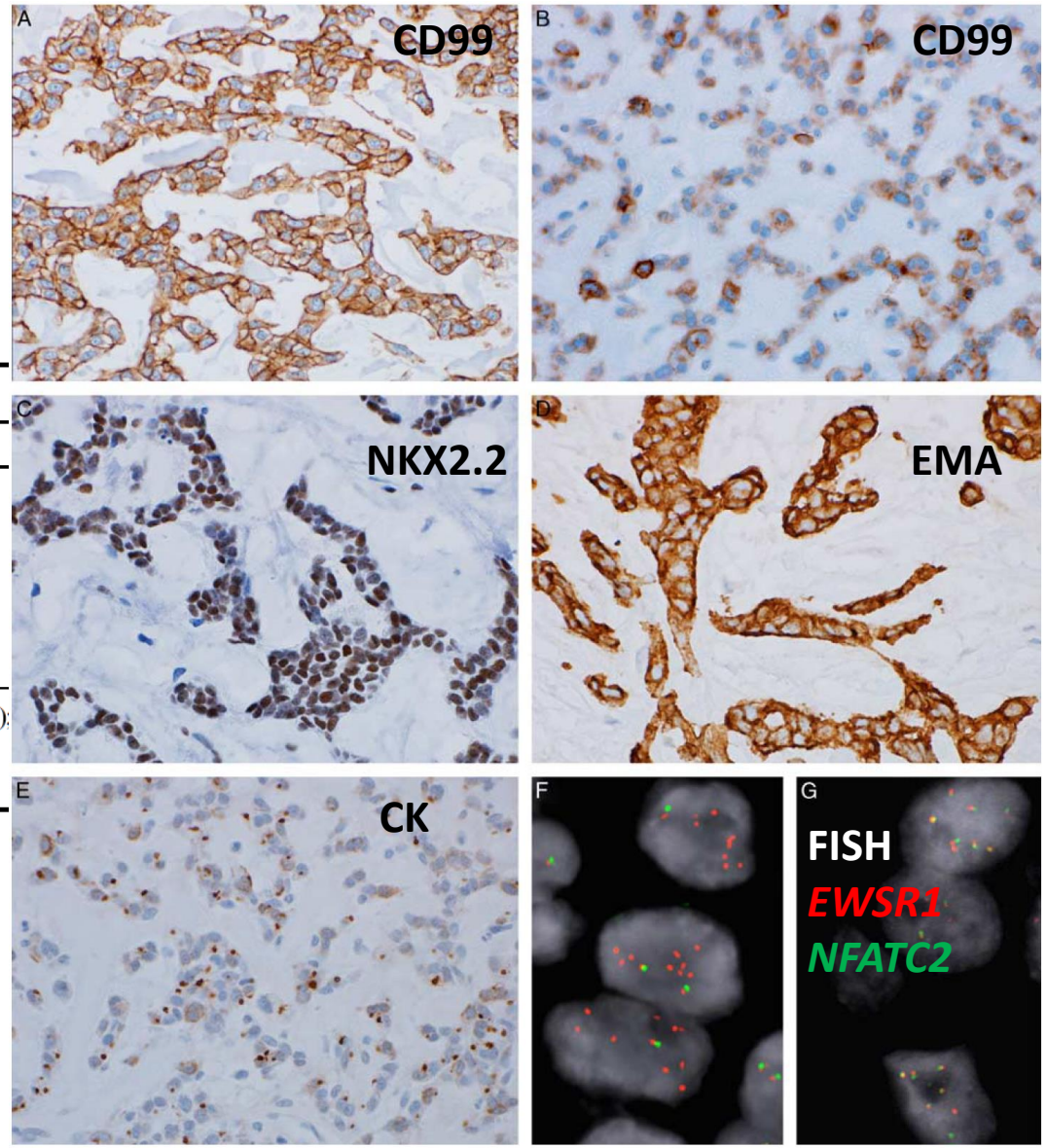
Variable CD99, NKX2.2, focal and dot-like keratin

TABLE 4. Immunohistochemical Results in *EWSR1-NFATC2* Sarcomas

Case	CD99 (% Cells)	NKX 2.2	WT1	EMA	SMA	CK
1	2+ (100%) mem, cyto, dot	2+ diff nuc	—	2+ focal cyto	–	2+ focal dot
2	1-2+ (80%) mem, dot	2+ diff nuc	2+ diff nuc	2+ diff mem	1+ focal cyto	—
3	1-2+ (70%) mem, cyto	2+ diff nuc	2+ diff nuc	2+ focal cyto	1+ Focal cyto	—
4	2+ (90%) mem, cyto	2+ diff nuc	2+ focal nuc	—	—	2+ focal dot
5	2+ (80%) mem, cyto	2+ diff nuc	1+ focal nuc	2+ diff cyto	—	2+ diff dot
6	2+ (100%) mem, cyto	2+ diff nuc	—	2+ diff cyto	1+ focal cyto	—

Intensity (0 = none, 1+ = weak, 2+ = strong); diffuseness of stain (0 = none, <50% of cells = focal, ≥ 50% of cells = diffuse [diff]); dot = perinuclear dot like).

CGA indicates chromogranin-A; CK, cytokeratin; DES, desmin; SYN, synaptophysin.



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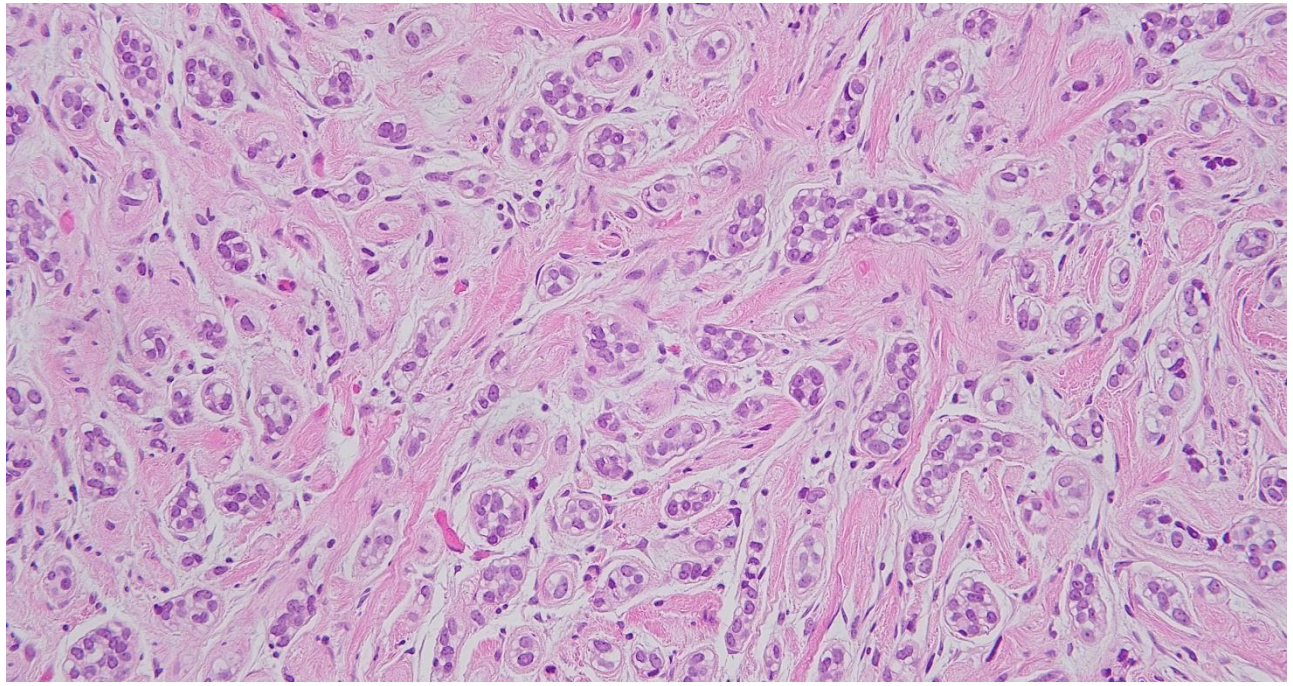
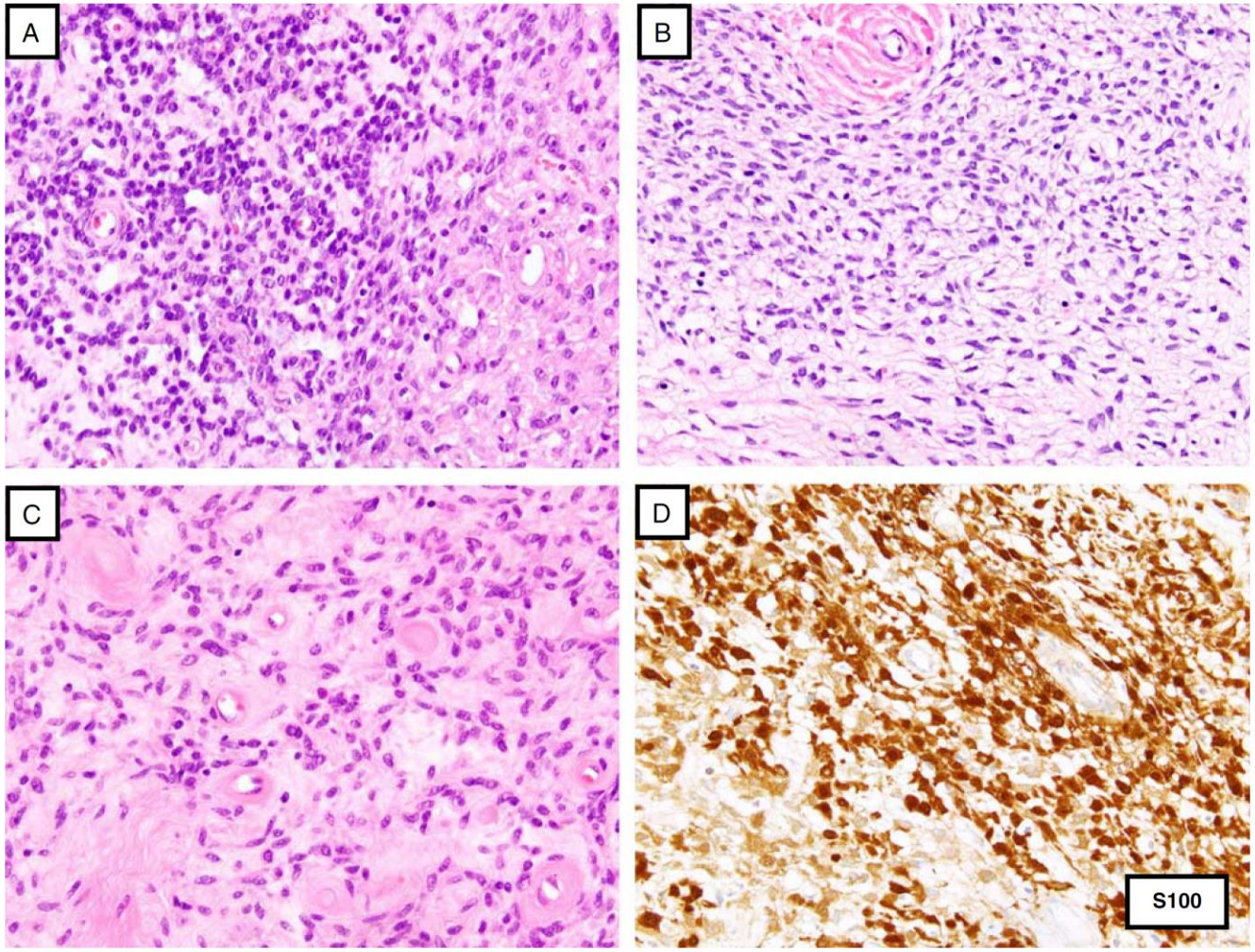


<u>Treatment follow up</u>

2 cycles VAI

below knee amputation

Disease free at 2 years



EWSR1 with NON-*ETS* gene family fusion

EWSR1-PATZ1 *(extremely rare)*

equal sex distribution

broader age range, 1-81 yrs

deep **soft tissues** of the chest wall, abdomen, and limbs

potentially aggressive behavior

rounded to spindle cells forming sheets and nests, with a dense fibrous or myxohyaline stroma

sometimes mimic myoepithelial or nerve sheath tumors

±S100 protein and SOX10 expression

±desmin, myogenin, and myoD1 positivity

"polyphenotypic sarcomas")

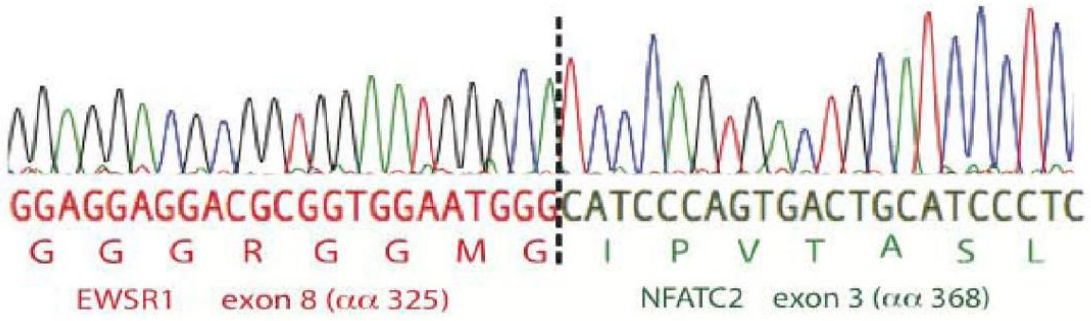
variable CD99 expression

Keratin negative (distinguishes from myoepithelial tumors)

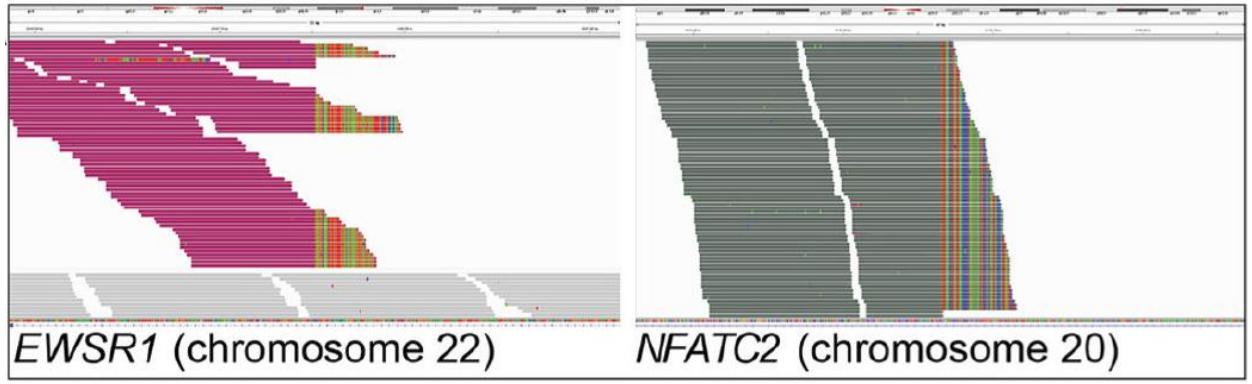
FIGURE 10. Round cell sarcomas with *EWSR1-PATZ1* fusions. A, This tumor contains an admixture of round cells and epithelioid cells. Note the bland nuclei. B, Some tumors show a reticular growth pattern with short spindle cells in a more myxoid stroma. C, The histology of these tumors is often reminiscent of myoepithelial neoplasms. Note the perivascular hyalinization. D, IHC for S100 protein is often positive, further mimicking a myoepithelial tumor.

Diagnosis of Round Cell Sarcomas with *EWSR1-NFATC2*, *FUS-NFATC2*, *EWSR1-PATZ1*

- Essential (WHO)
  - Minimum - break-apart FISH with appropriate morphology and IHC profile
  - Gold-standard = detection of specific fusion (RT-PCR or NGS)



Virchows Arch. 2014 August ; 465(2): 233–239

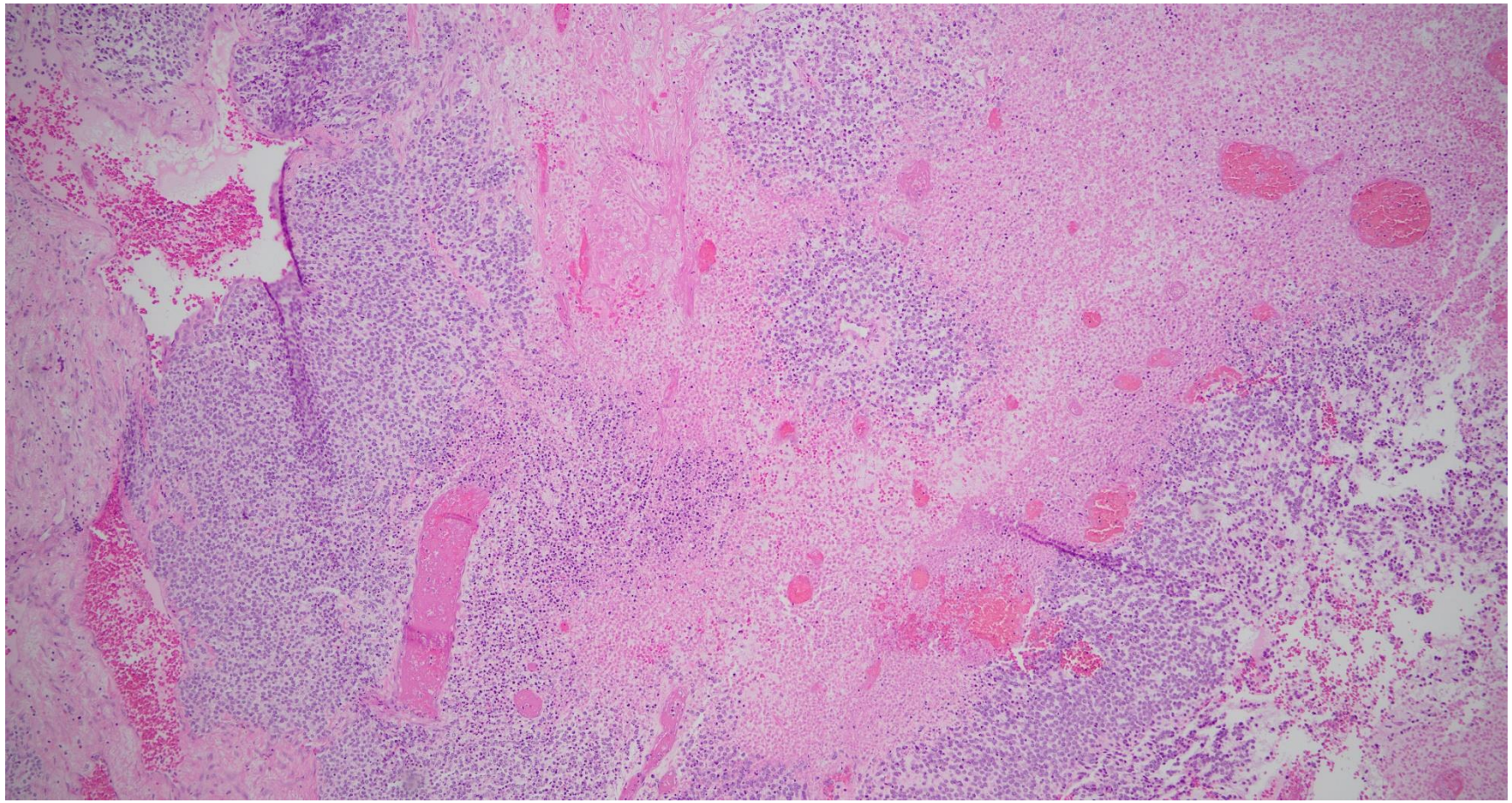


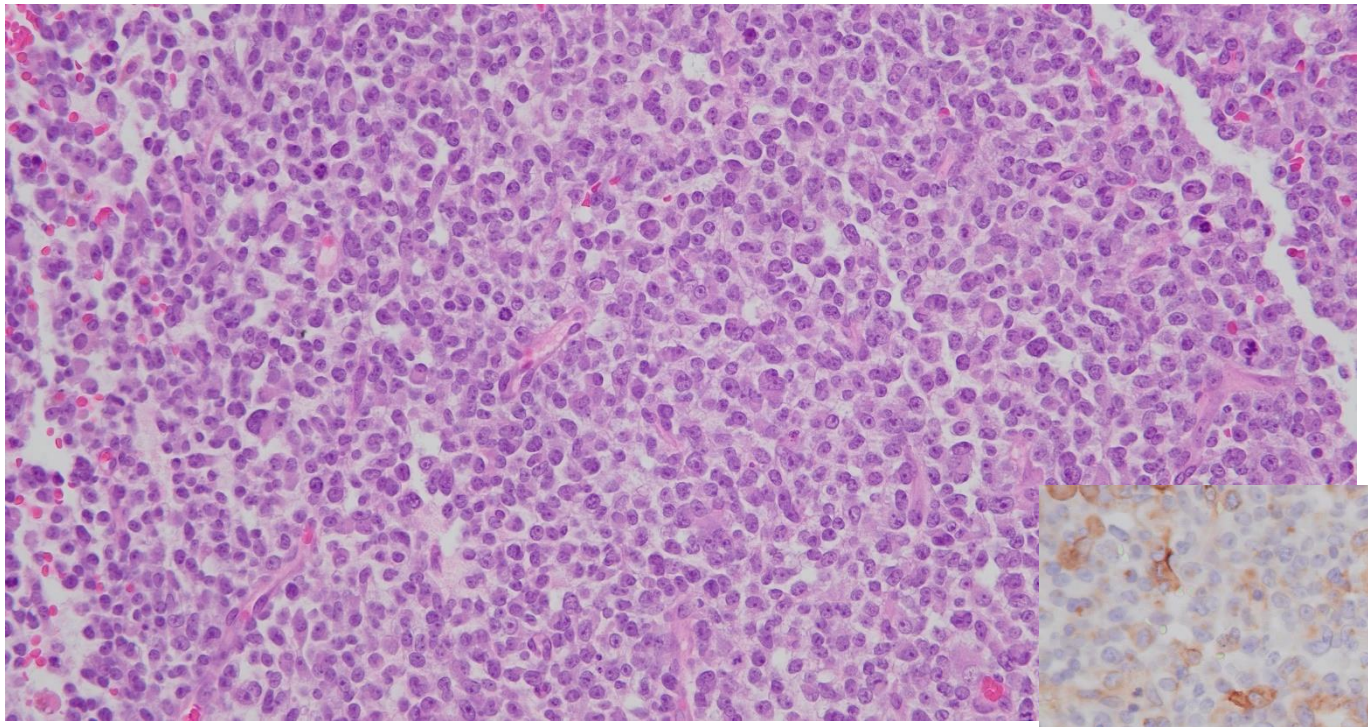
Hum Pathol. 2018 November ; 81: 281–290

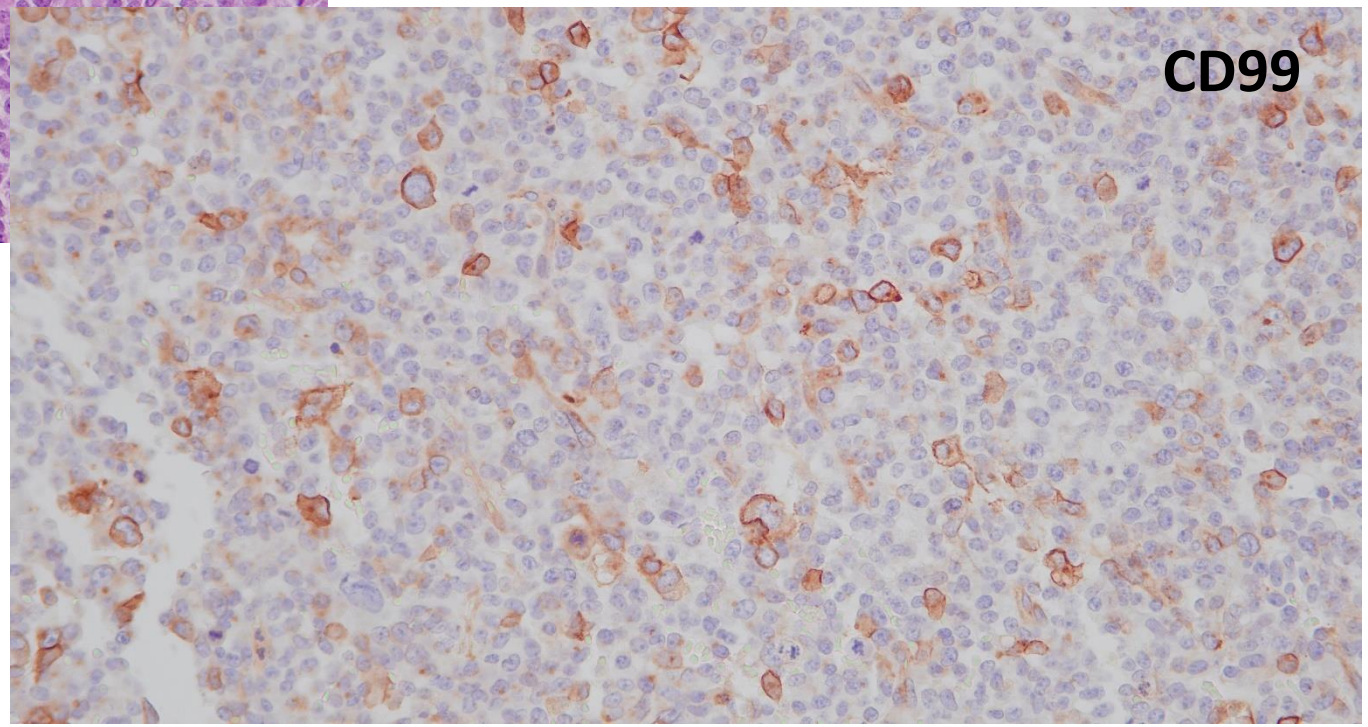
Case study: palliative lung and chest wall resection

42yo female admitted with a “Ewings-like sarcoma” after rapid progression during systemic chemotherapy (s/p resection of primary groin tumor 6 mos prior)

deceased within 2 weeks of hospital admission

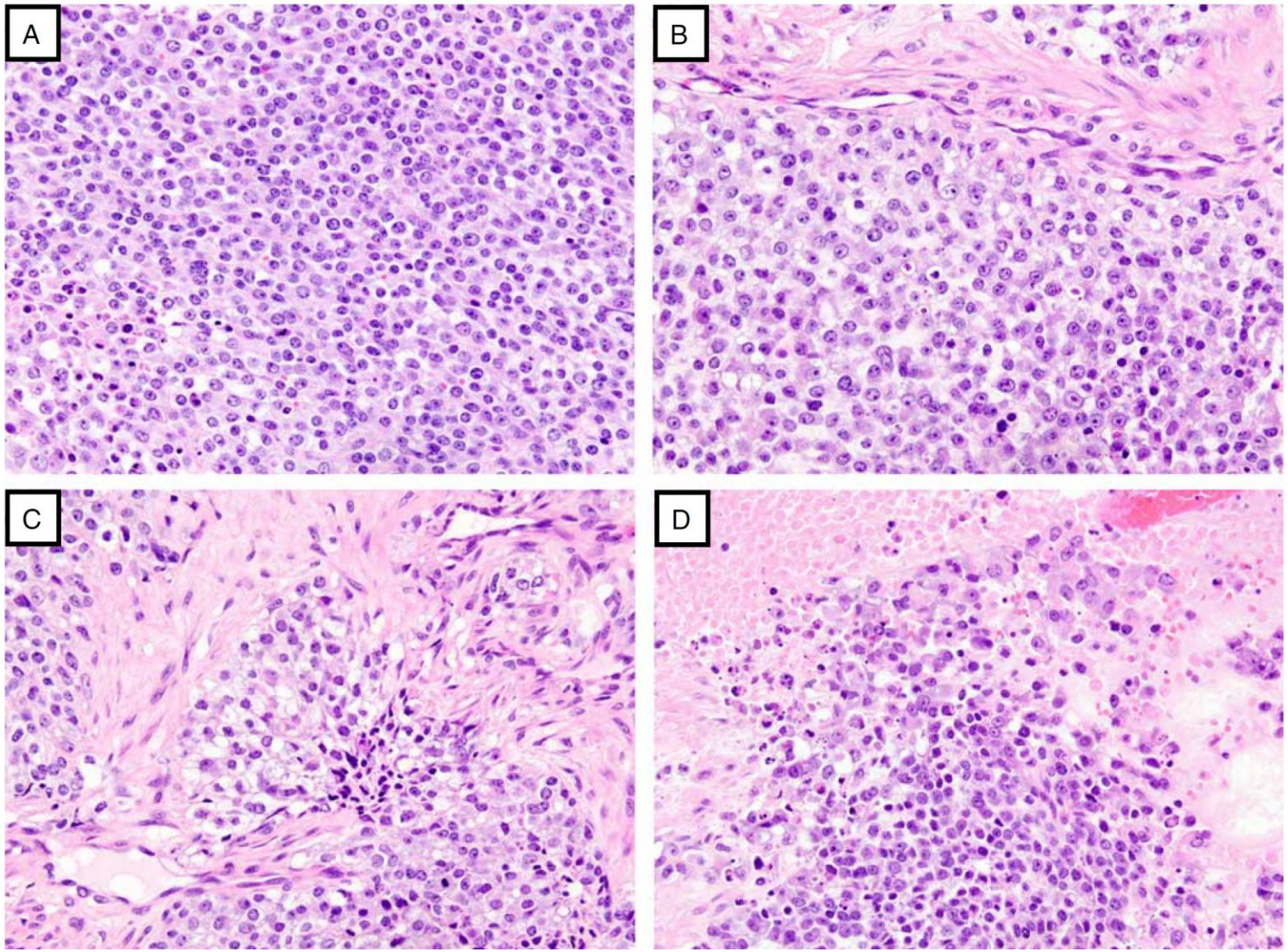





CD99

CIC-rearranged sarcomas

~70% of the undifferentiated round cell sarcomas that lack *EWSR1* or *FUS* fusions



Sarcomas with CIC-rearrangements are a distinct pathologic entity with aggressive outcome: A clinicopathologic and molecular study of 115 cases

Cristina R. Antonescu[1], **Adepitan A. Owosho**[2], **Lei Zhang**[1], **Sonja Chen**[1], **Kemal Deniz**[3], **Joseph M. Huryn**[2], **Yu-Chien Kao**[1,4], **Shih-Chiang Huang**[1,5], **Samuel Singer**[2], **William Tap**[6], **Inga-Marie Schaefer**[7], and **Christopher D Fletcher**[7]

Am J Surg Pathol. 2017 July ; 41(7): 941–949

CIC = capicua transcriptional repressor

CIC-DUX4

t(4;19)(q35;q13)

t(10;19)(q26;q13)

age 6–81 years, mean 32 years

22% <18 years of age

Anatomic location of CIC-rearranged sarcomas

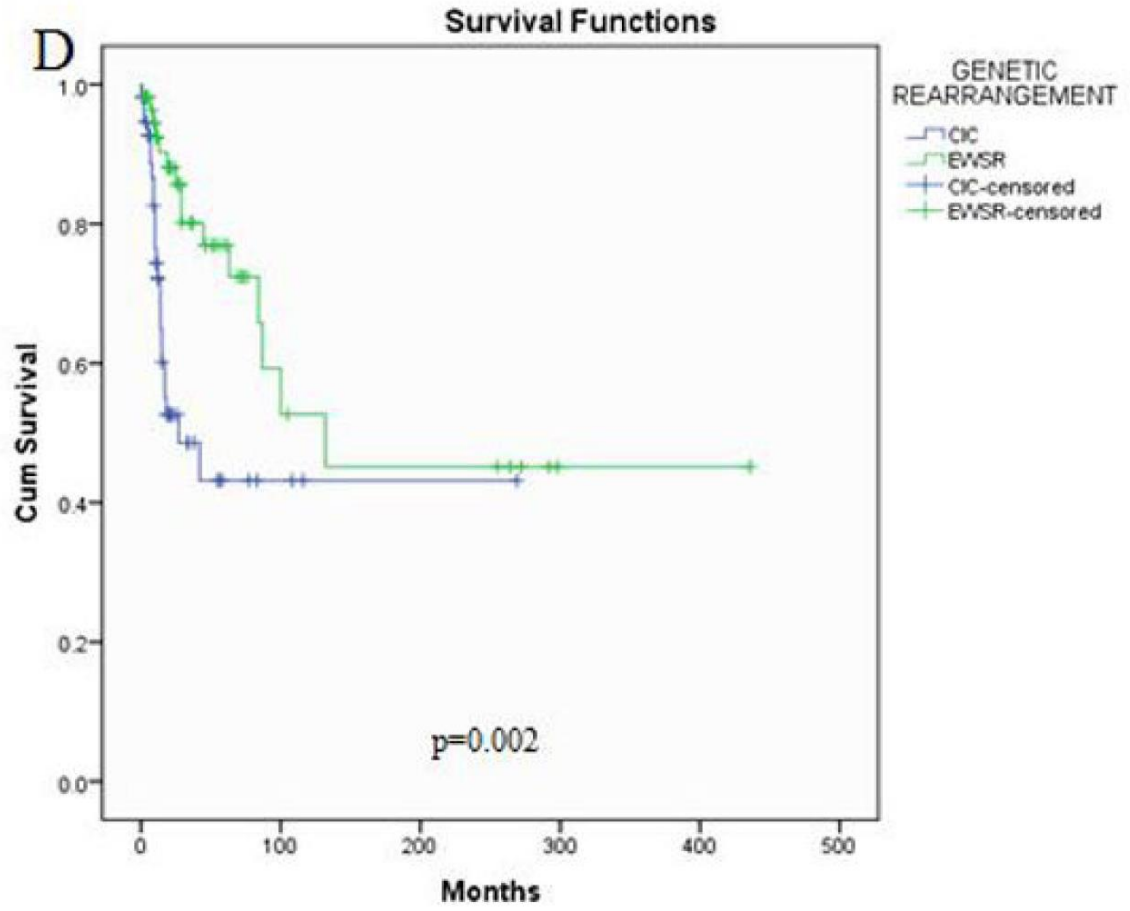
Location of the tumor	Number of cases (n=111)
Soft tissue	**95 (86%)**
Trunk	39
Lower extremity	31
Upper extremity	7
Head/neck	12
Retroperitoneum/perineum/pelvis	6
Viscera	**13 (12%)**
Stomach	1
Small/large intestine	5
Kidney/prostate	4
Tonsils/parapharyngeal	3
Bone	**3 (3%)**
Pelvic bones	3

Table 1 Clinical features of *CIC*-rearranged round-cell sarcomas

Case	*Age (years)*	*Gender*	*Location*	*Depth*	*Size (cm)*	*Necrosis*	*Number of mitoses/10 HPF*
1	14	M	Colon	Deep	14	No	25
2	19	M	Spine	Deep	8	Yes	46
3	47	M	Spine	Deep	4.5	No	22
4	42	F	Thigh	S	NA	Yes	20
5	12	F	Back	Deep	5	Yes	68
6	24	F	Stomach	Deep	5	No	NA
7	20	M	Head/neck	Deep	5.5	Yes	58
8	43	M	Chest wall	Deep	2.5	Yes	25
9	53	F	Lung	Deep	11	Yes	22
10	83	F	Kidney	Deep	14.5	Yes	20
11	20	F	Pleural	Deep	NA	Yes	20
12	18	M	Chest wall	Deep	15	Yes	11
13	26	M	Thigh	Deep	NA	No	NA
14	47	M	IVC	Deep	5.5	No	125
15	18	M	Calf	Deep	NA	Yes	43
16	17	M	Axillary	Deep	3.2	Yes	30
17	57	M	Retro peritoneal	Deep	NA	Yes	11

Abbreviations: F, female; IVC, inferior vena cava; M, male; NA, not available; S, superficial.

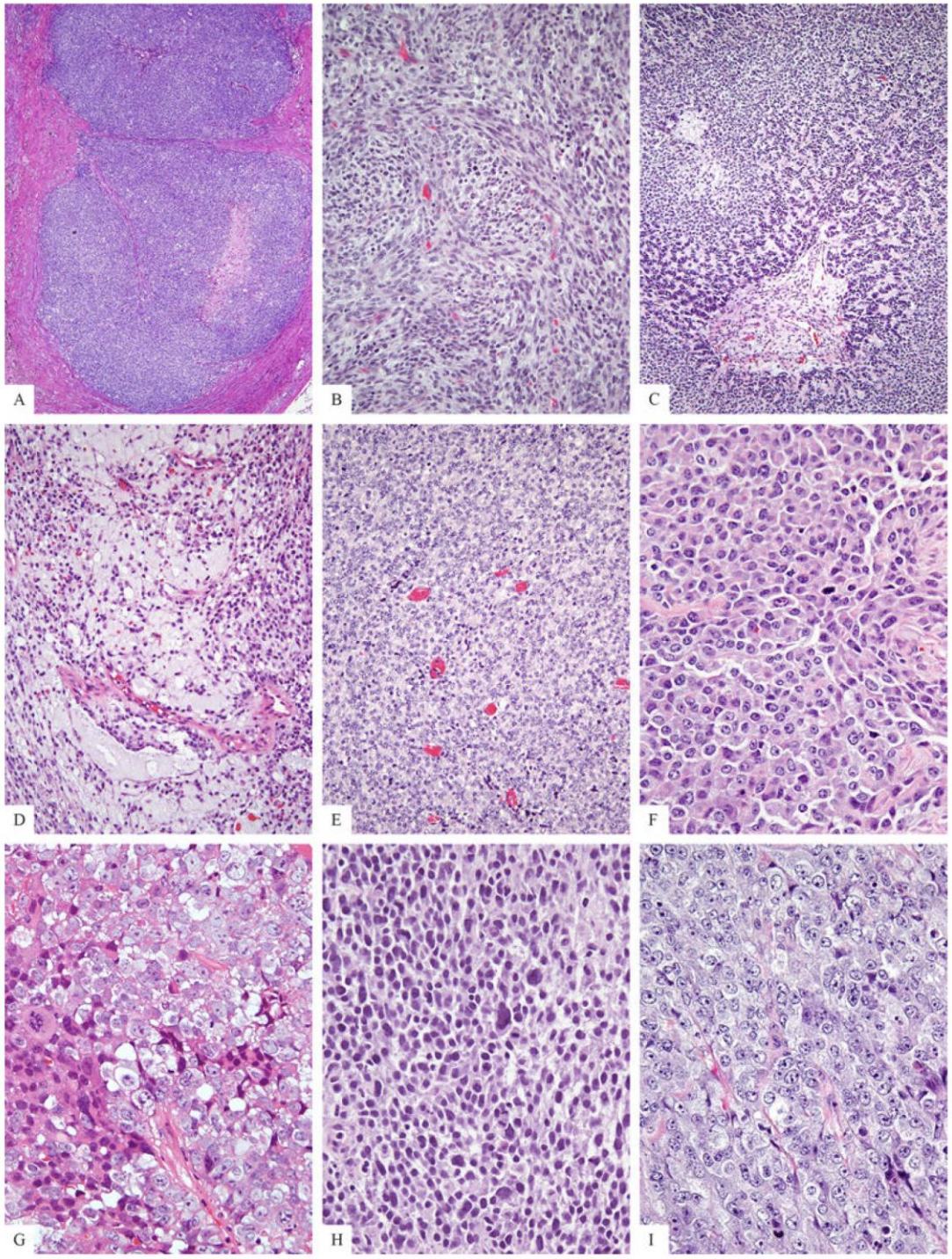
CIC-rearranged sarcomas confer inferior survival compared to Ewing sarcoma



	2 yr	5yr
CIC	**53%**	**43%**
Ewing		**77%**

Response to Ewing sarcoma chemotherapy has been "dismal"
(WHO, Soft Tissue and Bone Tumours, 5th Ed.)

Am J Surg Pathol. 2017 July ; 41(7): 941–949.



Variable cytomorphology

Most round to ovoid

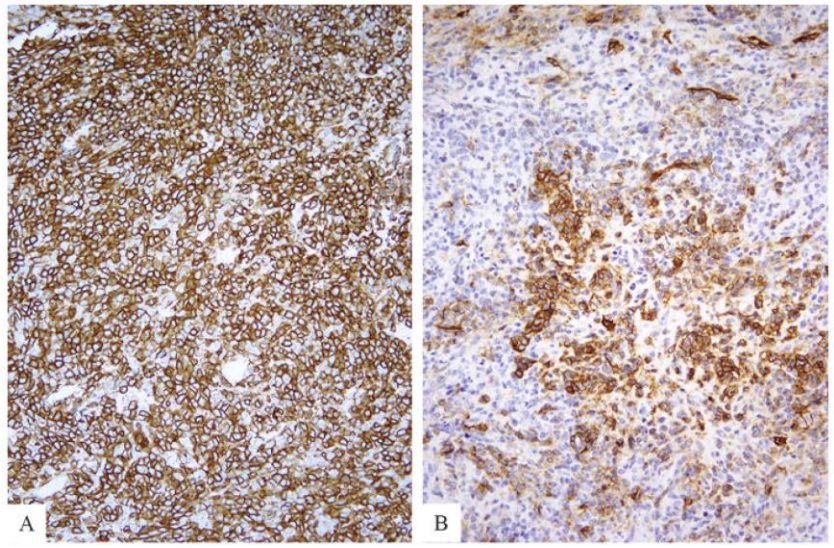
Focal spindled, epithelioid, plasmacytoid or rhabdoid

nuclear features = variable chromatin

fine (G), dark, hyperchromatic (H) or vesicular (I)

HIGH mitotic counts

Frequently necrotic

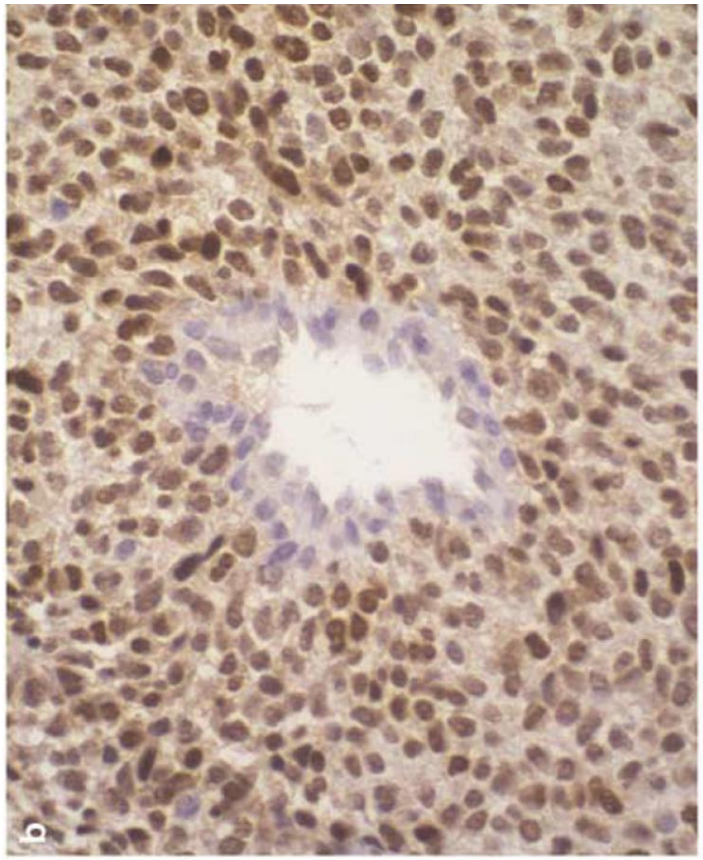


+/-CD99

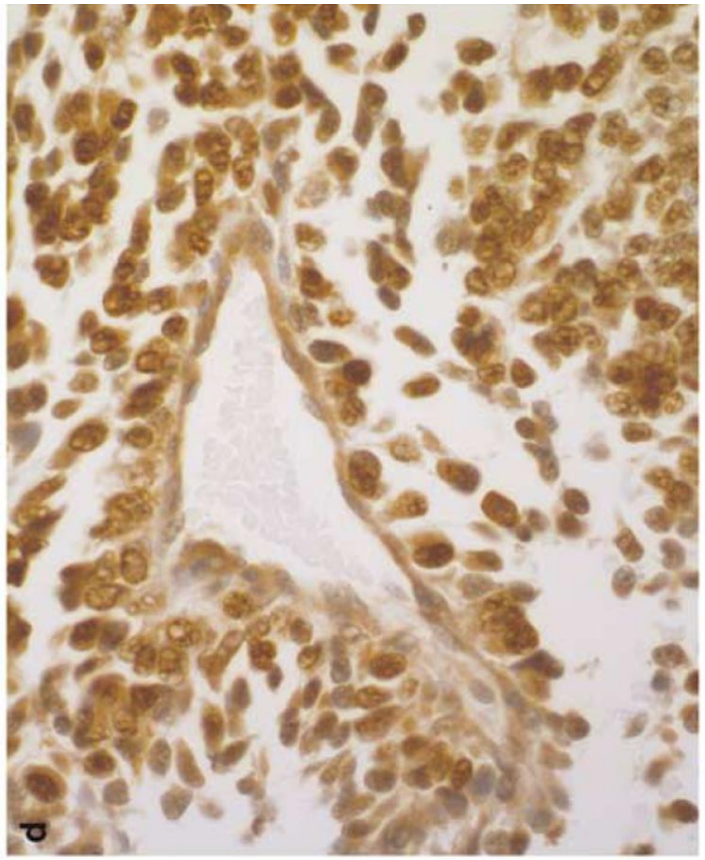
Am J Surg Pathol. 2017 July ; 41(7): 941–949.

Diagnostic Workup

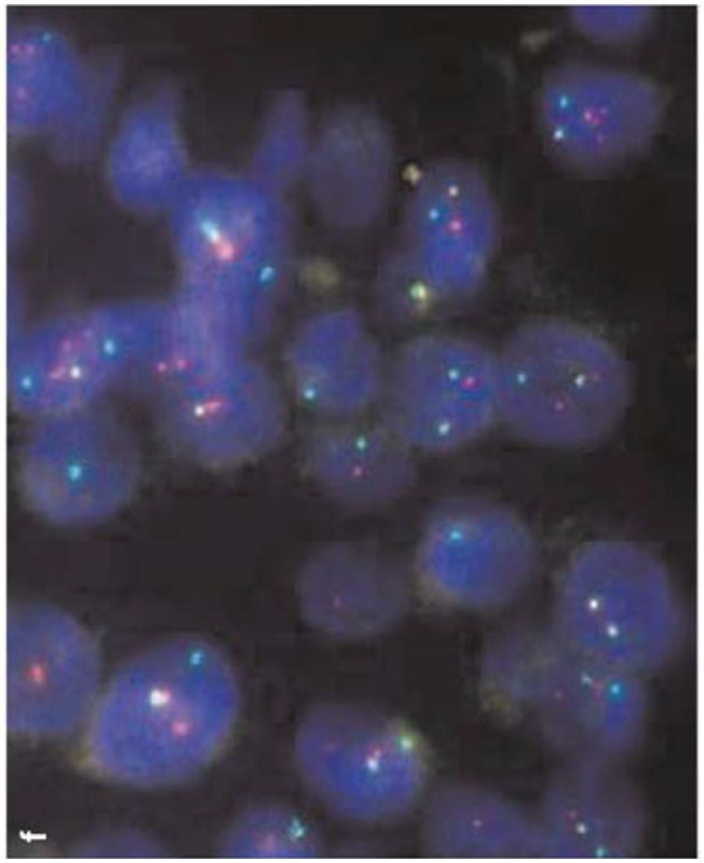
ETV4



WT1



CIC



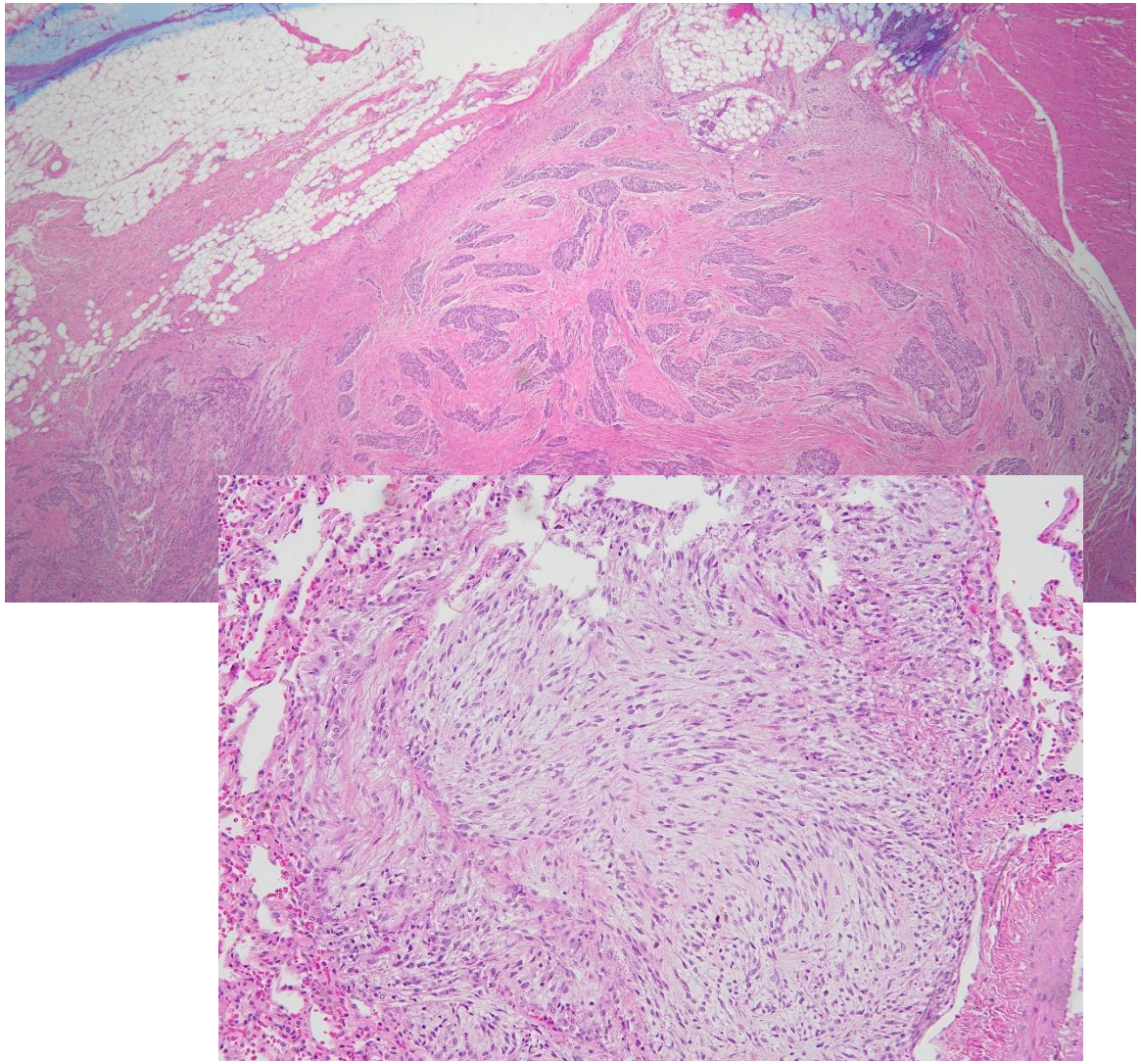
WHO: *CIC* Testing is "Desirable" for Diagnosis

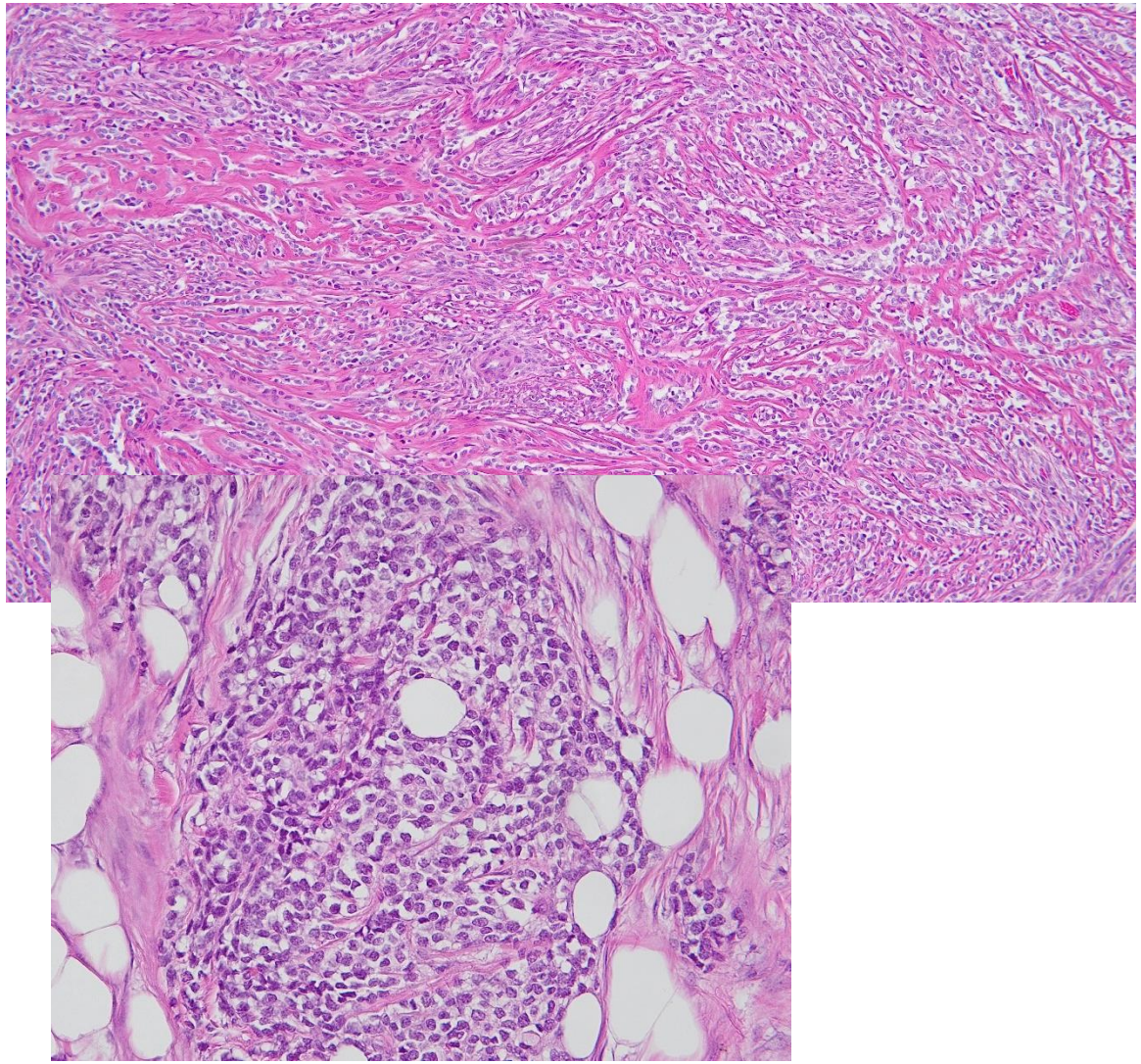
- CD99 IHC is variable and obviously not specific
- ETV4 IHC can be a helpful but *not* specific (Guellec, S. et al. 2016 Mod Pathol 29:1523-31)
  - Focal and/or weak staining reported in
    - 4/43 Ewing sarcomas
    - 1/25 alveolar rhabdomyosarcomas
    - 1/10 desmoplastic small round cell tumors
    - 0/20 poorly differentiated (round cell) synovial sarcoma
- WT1 is variable and nonspecific (especially for new workup from lung bx!)
- FISH or NGS testing is available for detection of *CIC* rearrangements(DNA) or fusions (RNA) from various labs and is listed as "desirable" by the WHO

  (can be definitive!)

Case Study

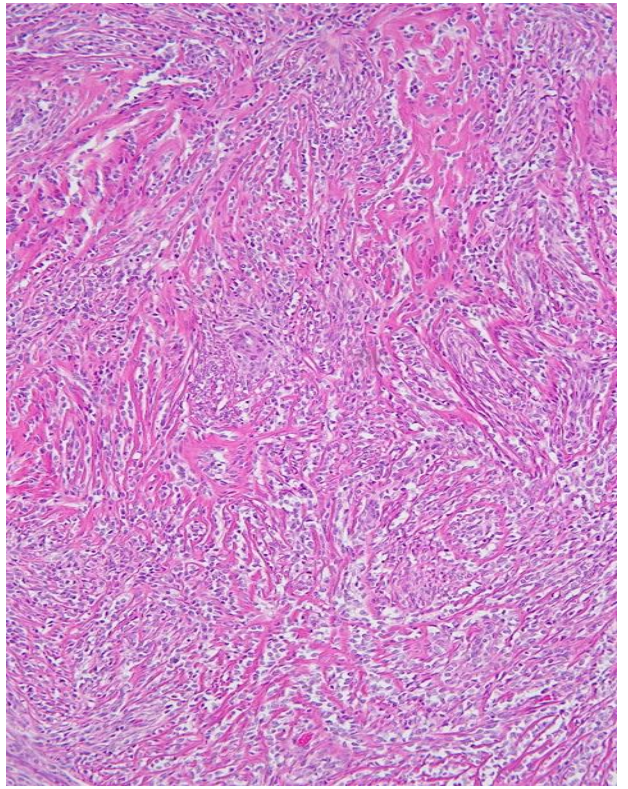
51yo female, h/o multicentric invasive ductal CA breast (mixed ER+, PR+, HER2+, TN) now with 2 yr growing **gluteal mass**, "*atypical spindle cell neoplasm, treat as low grade sarcoma*", relapsed within 11 mos of resection with lung and parotid metastasis





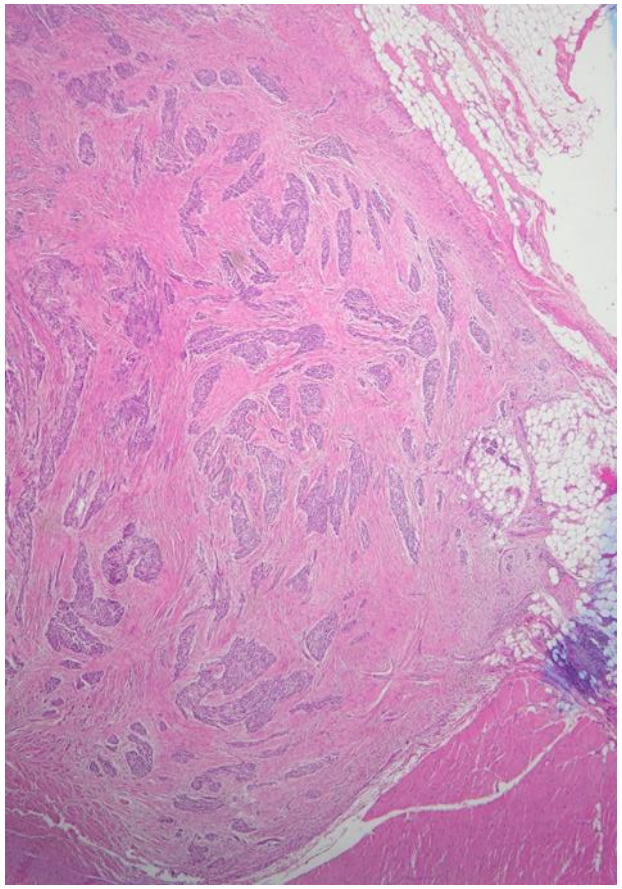
IHC & Molecular Workup Inconclusive

- Gluteal mass resection
  - Weakly MUC4+
  - Negative S100, EMA, CK5/6, p63, Cam5.2, AE1/3, GATA3, ER, PR
  - Negative EWSR1, FUS, SYT FISH
- Original gluteal mass biopsy (outside report)
  - Patchy SMA+, negative for CD34, CD68, CD117, desmin, EMA, ER, PR, MART-1, S100
- Lung mass
  - Negative CK5/6, p63, Cam5.2, AE1/3, GATA3



Final Diagnosis: Recurrent/residual spindle and epithelioid sarcoma,
Comments: favor translocation-associated sarcoma, can not exclude sclerosing epithelioid fibrosarcoma
Recommend NGS

<1 year later…relapse in lungs



***ZC3H7B-BCOR* fusion detected by NGS**

→metastatic high grade endometrial stromal sarcoma?

CD10 and cyclinD1 IHC strongly positive

→uterine mass identified on imaging (not biopsied)

9/19-12/19: pazopanib with progressive disease (PD)

1/20-2/20: Doxorubicin x 2 cycles -->PD

3/20-6/20 t: Gemcitabine/Dacarbazine --> mixed response

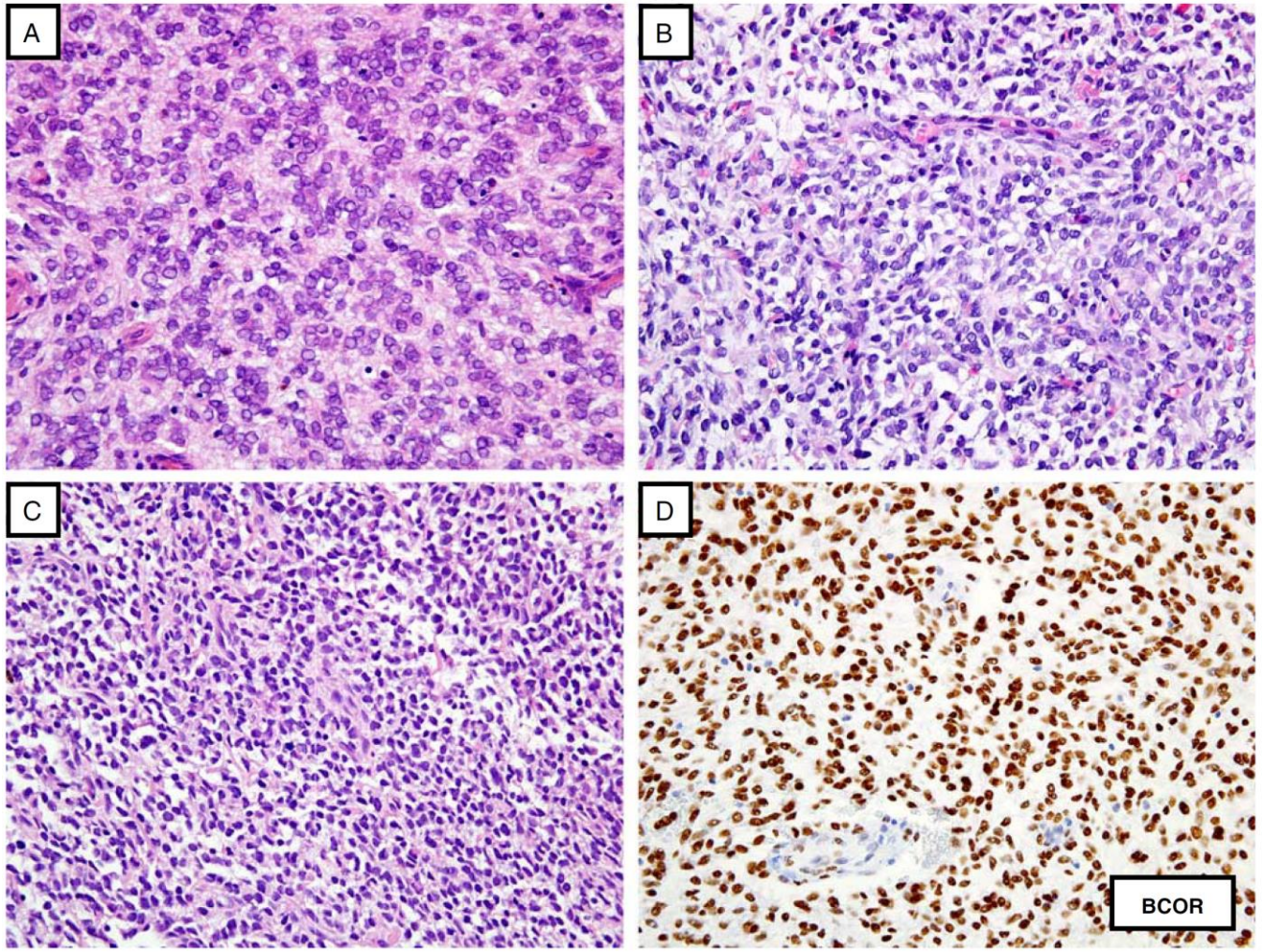
7/20-11/20: treatment break

11/9/20 significant progression of right lung tumor

2/18/21 continued progression, considering *hospice*

Sarcomas with *BCOR* genetic alterations

~5% of non-EWSR1 or FUS rearranged "Ewing-like" sarcomas



Bcl6 Co-repressor gene

"*BCOR*-rearranged sarcoma"

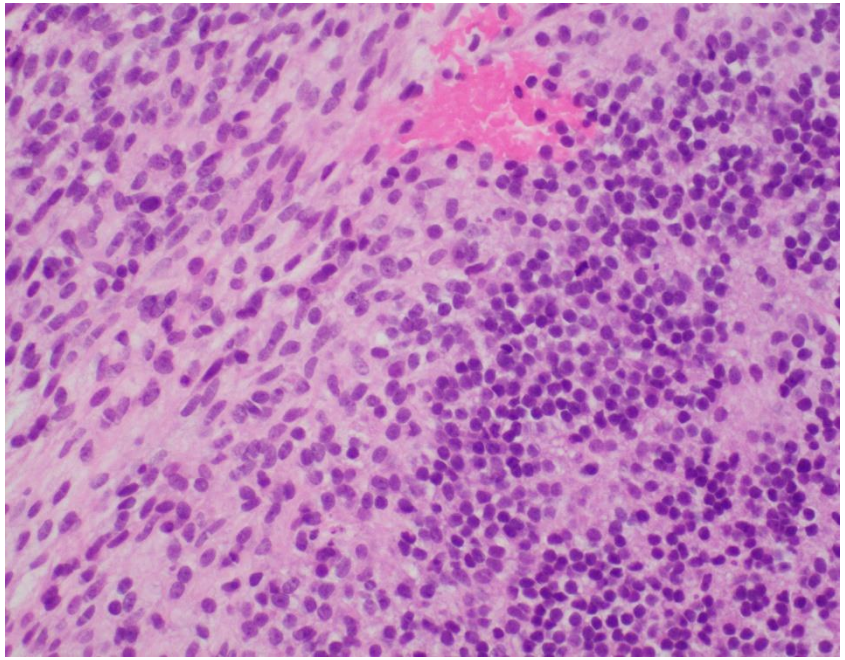
BCOR-CCNB3
BCOR-MAML3
BCOR-ZC3H7B
ZC3H7B-BCOR
BCOR exon 15 ITD

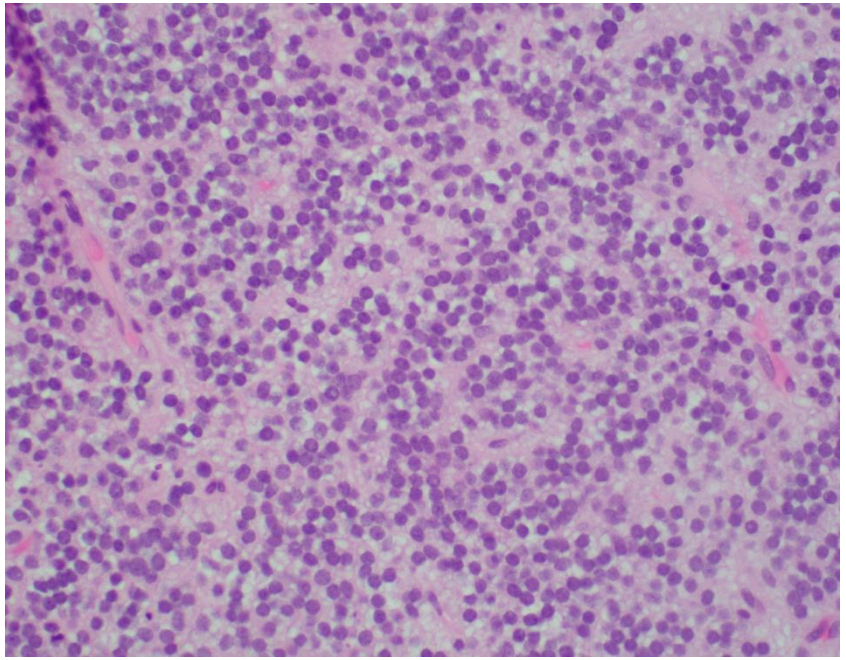
includes

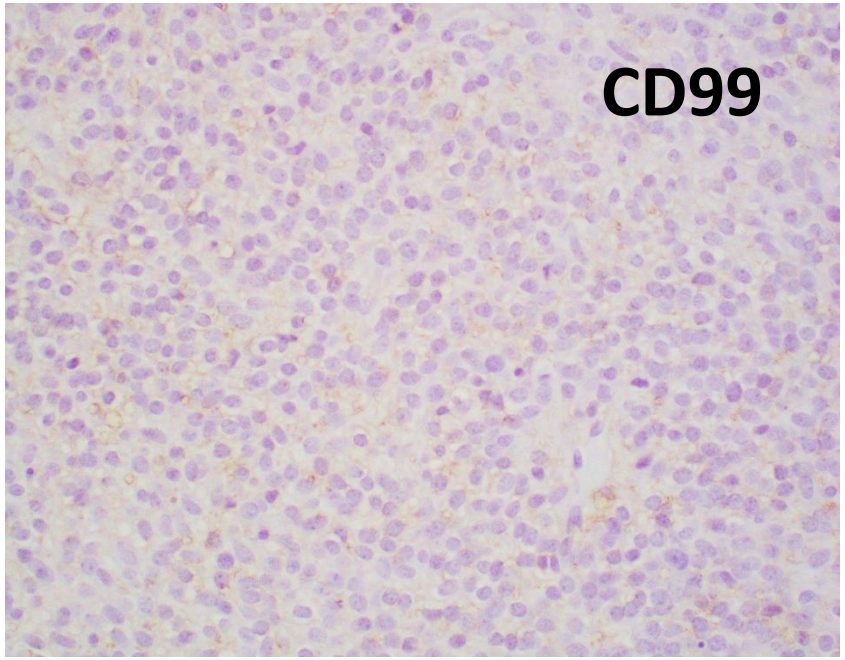
- Some high grade endometrial stromal sarcomas
- Primitive mixed mesenchymal tumor of infancy

Variable CD99 (~50% positive)

BCOR-rearranged sarcoma, maxillary mucosa, 15yo female







BCOR-CCNB3-Fusion Positive Sarcomas: A Clinicopathologic and Molecular Analysis of 36 cases with Comparison to Morphologic Spectrum and Clinical Behavior of other Round Cell Sarcomas

Yu-Chien Kao, MD[1,2], Adepitan A. Owosho, DDS[3,4], Yun-Shao Sung, MSc[1], Lei Zhang, MD[1], Yumi Fujisawa, MS[1], Jen-Chieh Lee, MD, PhD[5], Leonard Wexler, MD[6], Pedram Argani, MD[7], David Swanson, BSc[8], Brendan C Dickson, MD[8], Christopher D.M. Fletcher, MD, FRCPath[9], and **Cristina R Antonescu, MD[1,*]**

Am J Surg Pathol. 2018 May ; 42(5): 604–615

- Broad age range
  - *BCOR-CCND3,* 90% <20yo, M:F 4.5:1
- Varied anatomic Locations
  - Bone or Soft tissue
  - pelvis
  - Lower > upper extremities
  - Spine, paraspinal
  - Chest wall
  - H&N
  - Visceral cavities

Case	Age/Sex	Location
1	13/F	Soft palate
2	15/M	Femur
3	15/F	Pelvic cavity
4	9/M	Sacrum
5	13/M	Femur
6	14/M	Iliac bone
7	2/M	RP/paraspinal
8	17/M	Pubic ramus
9	14/M	Foot
10	15/M	Femur
11	17/M	Calcaneus
12	12/M	Pubic ramus
13	5/M	Calcaneus
14	18/M	Shoulder
15	18/F	Paraspinal C2–C6
16	10/M	Femur
17	18/M	Sacrum
18	44/M	Thigh
19	18/F	Sacrum
20	14/M	Foot
21	12/M	Kidney
22	2/M	Posterior neck
23	15/M	Chest wall
24	19/M	Pelvic cavity

Case	Age/Sex	Location
25	21/M	Chest wall
26	24/M	Tibia
27	11/M	Kidney
28	13/M	Tibia
29	15/M	Leg
30	15/M	Elbow(bone)
31	16/M	Tibia
32	10/M	Femur
33	13/M	Tibia
34	15/M	Iliac bone
35	16/M	Calcaneus
36	13/F	Back/paraspinal

BCOR-CCNB3-Fusion Positive Sarcomas

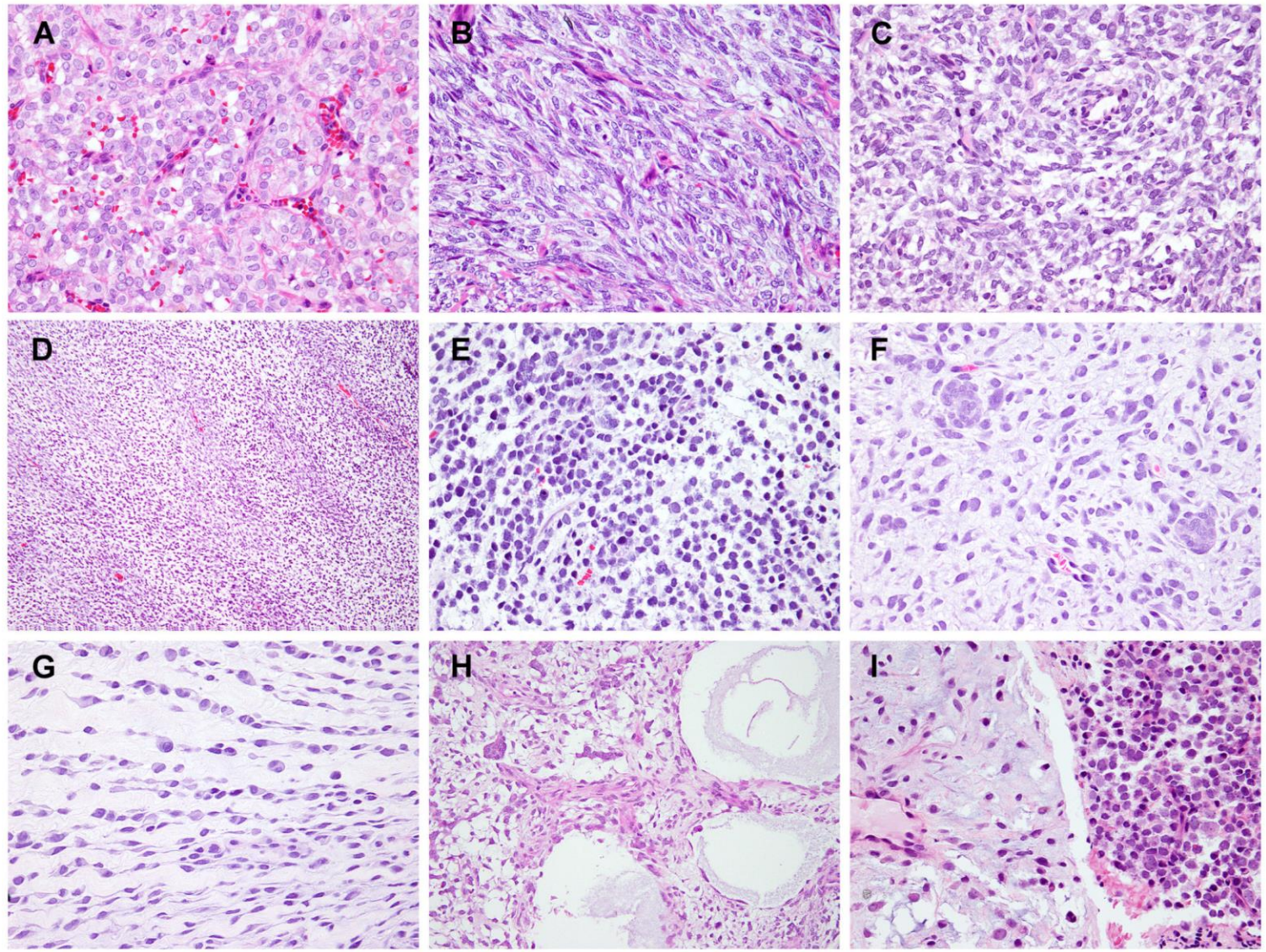


Figure 1. Histologic spectrum of BCS with round to spindle cells and occasional myxoid stroma

Am J Surg Pathol. 2018 May ; 42(5): 604–615

On biopsy, can show deceptively low grade morphology

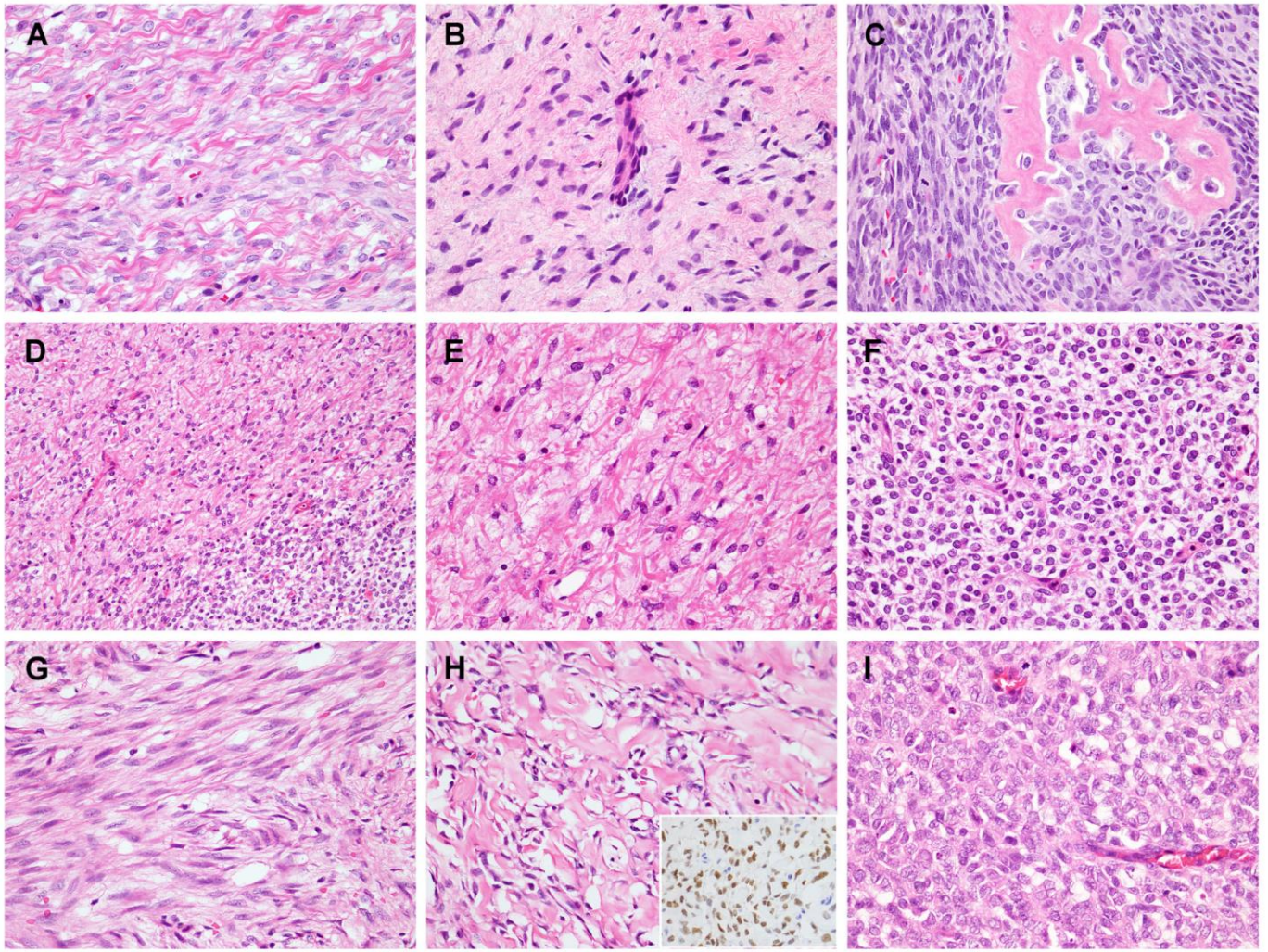


Figure 2. Infrequent morphologic patterns of BCS

Am J Surg Pathol. 2018 May ; 42(5): 604–615

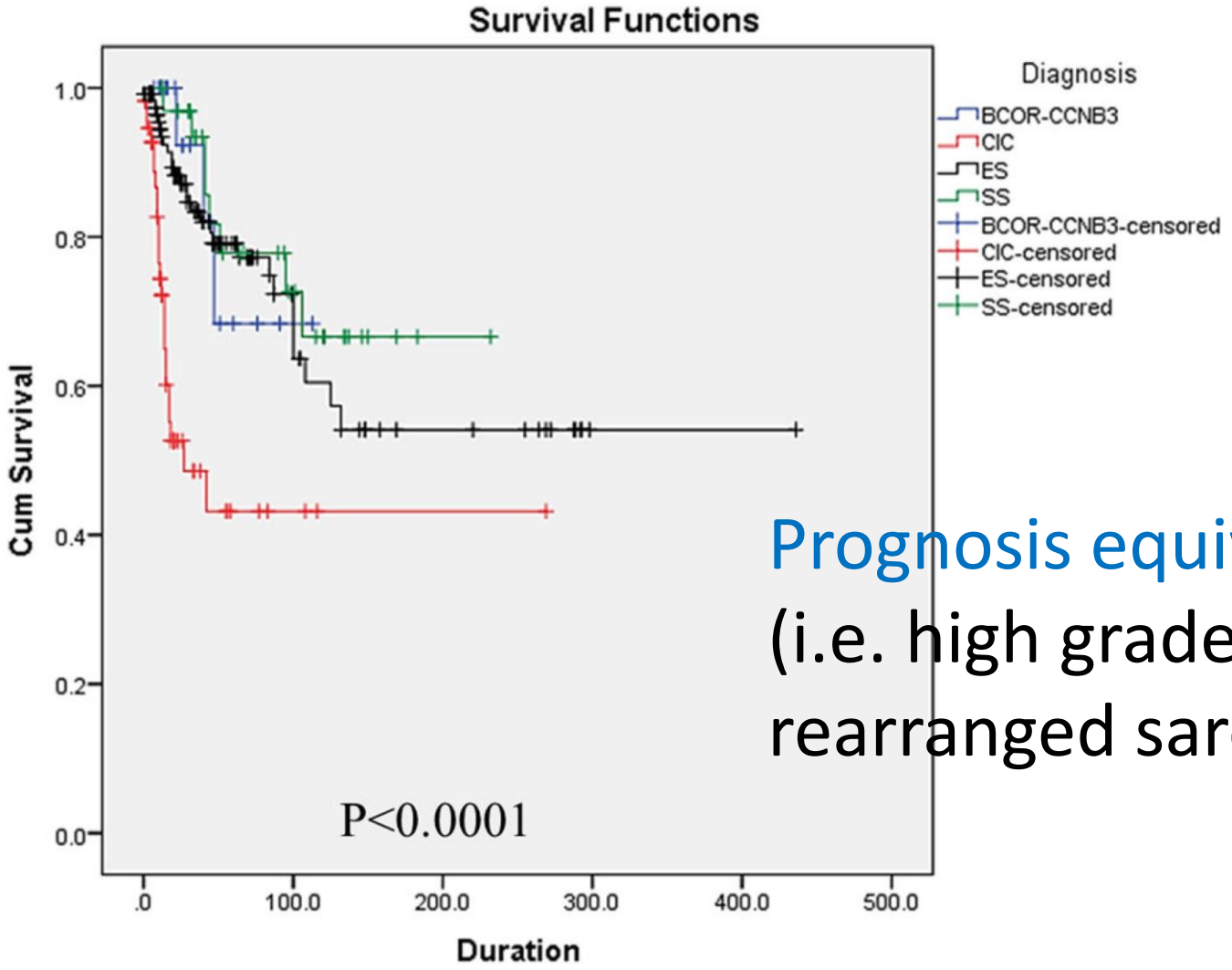


Figure 7.
Overall survival of 22 BCS (blue), 121 ES (black), 34 SS (green), and 57 *CIC*-rearranged sarcomas (red). BCS was associated with a more favorable outcome compared to *CIC*-rearranged sarcoma (p=0.005), while no significant survival difference was noted between BCS and ES (p=0.738) or BCS and SS (p=0.802). Duration is shown in months.

Prognosis equivalent to Ewing sarcoma (i.e. high grade), but better than CIC-rearranged sarcoma

Am J Surg Pathol. 2018 May ; 42(5): 604–615

ZC3H7B-BCOR high-grade endometrial stromal sarcomas: a report of 17 cases of a newly defined entity

Natasha Lewis[1], Robert A Soslow[1], Deborah F Delair[1], Kay J Park[1], Rajmohan Murali[1], Travis J Hollmann[1], Ben Davidson[2,3], Francesca Micci[4], Ioannis Panagopoulos[4], Lien N Hoang[5], Javier A Arias-Stella III[1], Esther Oliva[6,7], Robert H Young[6,7], Martee L Hensley[8], Mario M Leitao Jr[9], Meera Hameed[1], Ryma Benayed[1], Marc Ladanyi[1], Denise Frosina[1], Achim A Jungbluth[1], Cristina R Antonescu[1] and Sarah Chiang[1]

Modern Pathology (2018) 31, 674–684

High-grade endometrial stromal sarcoma likely encompasses underrecognized tumors harboring genetic abnormalities besides *YWHAE–NUTM2* fusion. Triggered by three initial endometrial stromal sarcomas with *ZC3H7B–BCOR* fusion characterized by high-grade morphology and aggressive clinical behavior, we herein investigate the clinicopathologic features of this genetic subset by expanding the analysis to 17 such tumors. All of them occurred in adult women with a median age of 54 (range, 28–71) years. They were predominantly based in the endomyometrium and demonstrated tongue-like and/or pushing myometrial invasion. Most were uniformly cellular and displayed haphazard fascicles of spindle cells with mild to moderate nuclear atypia. Myxoid matrix was seen in 14 of 17 (82%) tumors, and collagen plaques were seen in 8 (47%). The mitotic index was ≥10 mitotic figures/10 high-power fields (HPFs) in 14 of 17 (82%) tumors with a median of 14.5 mitotic figures/10 HPFs. No foci of conventional or variant low-grade endometrial stromal sarcoma were seen. All tumors expressed CD10

Utility of BCOR Immunohistochemical Stain

bone & soft tissue
BCOR rearrangements

BCOR Overexpression is a Highly Sensitive Marker in Round Cell Sarcomas with *BCOR* Genetic Abnormalities

Yu-Chien Kao, MD[1,2], Yun-Shao Sung, MSc[2], Lei Zhang, MD[2], Achim A. Jungbluth, MD[2], Shih-Chiang Huang, MD[2,3], Pedram Argani, MD[4], Narasimhan P Agaram, MBBS[2], Angelica Zin, PhD[5], Rita Alaggio, MD[6], and Cristina R. Antonescu, MD[2]

Am J Surg Pathol. 2016 December ; 40(12): 1670–1678.

high grade ESS
BCOR rearrangements
BCOR tandem dup.

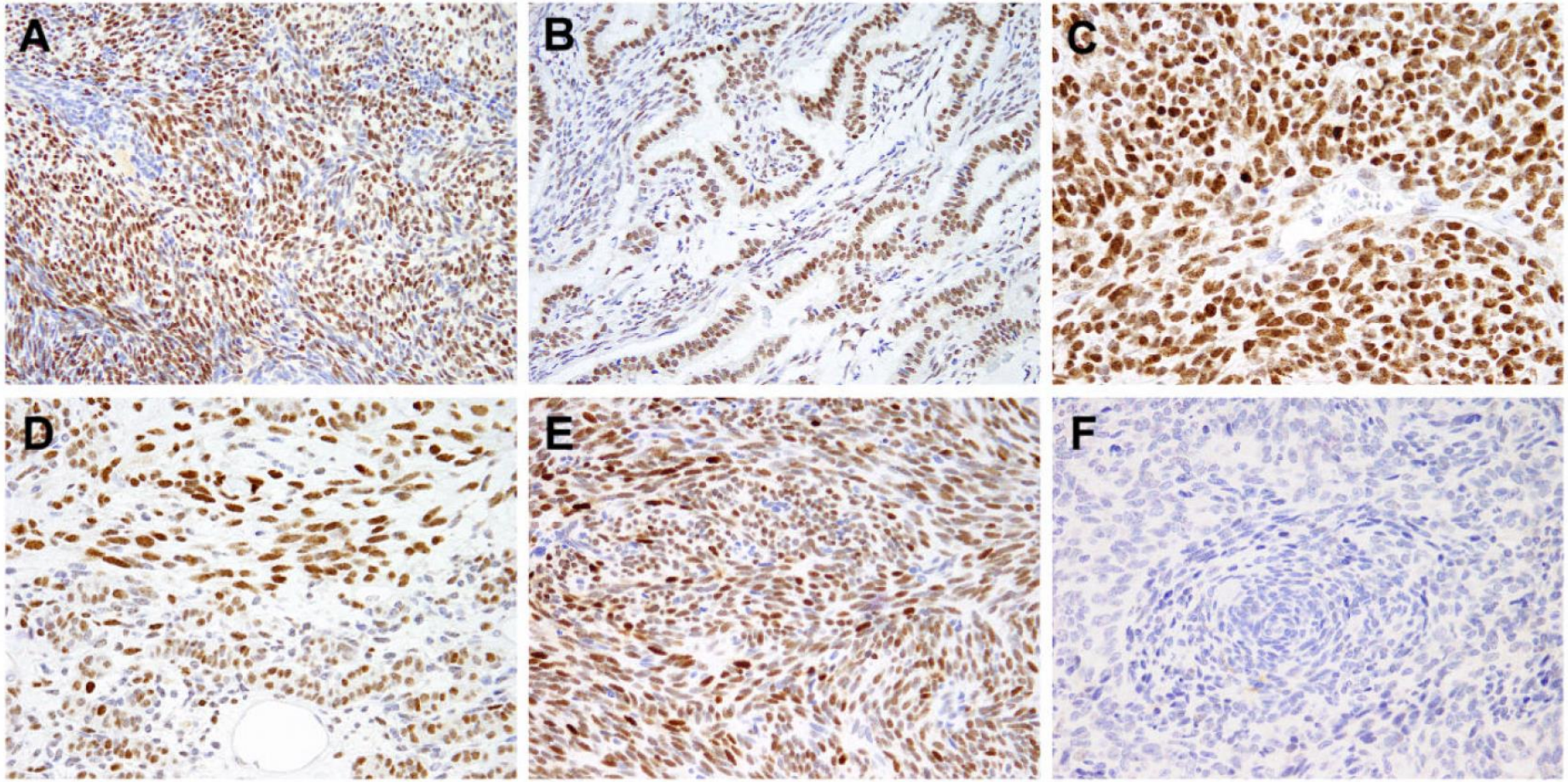
BCOR is a robust diagnostic immunohistochemical marker of genetically diverse high-grade endometrial stromal sarcoma, including tumors exhibiting variant morphology

Sarah Chiang[1], Cheng-Han Lee[2,3], Colin JR Stewart[4], Esther Oliva[5], Lien N Hoang[3], Rola H Ali[6], Martee L Hensley[7], Javier A Arias-Stella III[1], Denise Frosina[1], Achim A Jungbluth[1], Ryma Benayed[1], Marc Ladanyi[1], Meera Hameed[1], Lu Wang[1], Yu-Chien Kao[1,8], Cristina R Antonescu[1] and Robert A Soslow[1]

MODERN PATHOLOGY (2017) 30, 1251–1261

BCOR IHC is *not* specific

49% synovial sarcomas are BCOR+



n=74 tested

Am J Surg Pathol. 2016 December ; 40(12): 1670–1678.

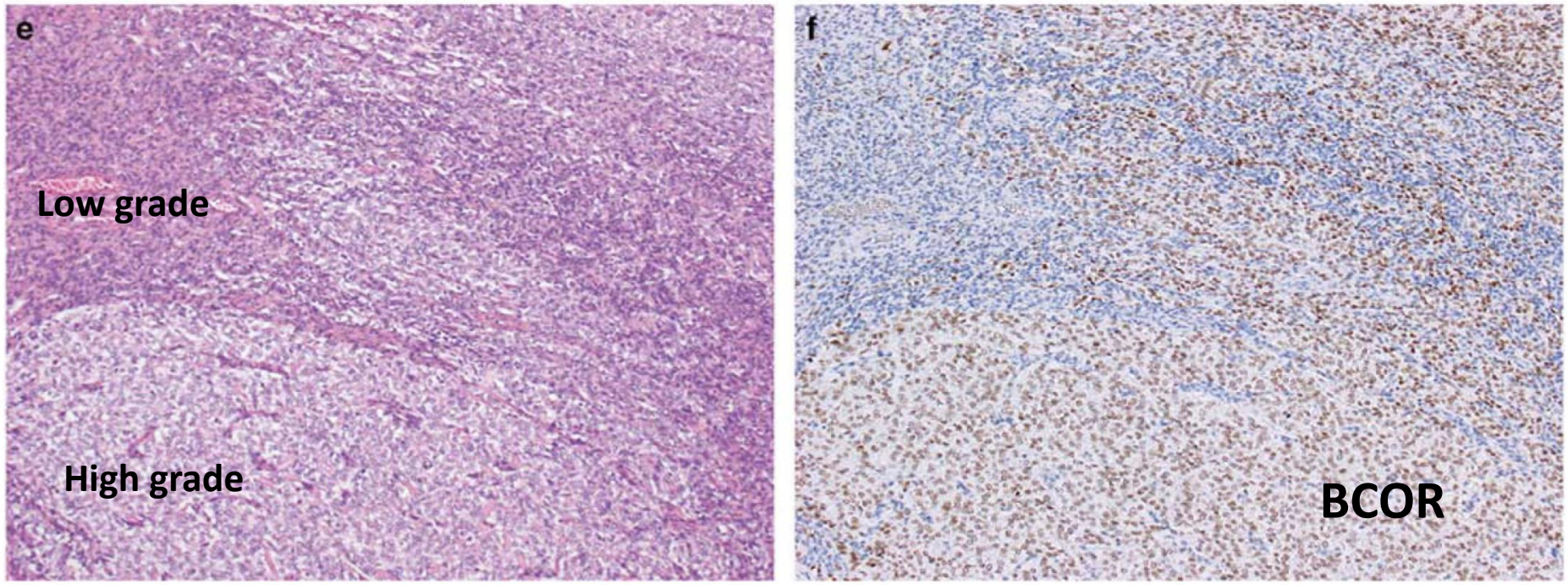
BCOR IHC stains *High Grade* Endometrial Stromal Sarcoma
irrespective of the driver translocation

Low grade

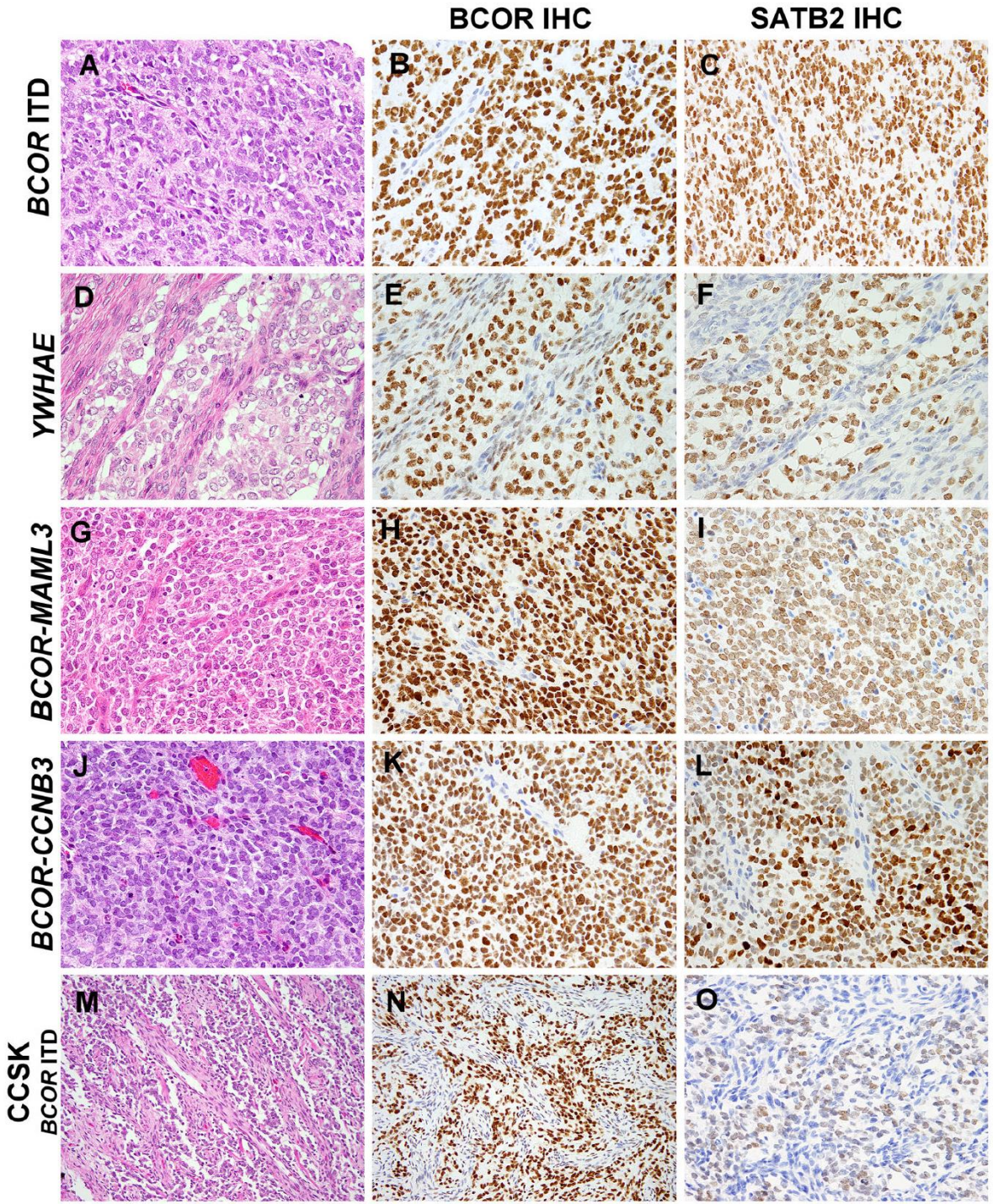
- *JAZF1* fusions
- *PHF1* fusions

High Grade

- (10;17)(q22;p13), ***YWHAE-NUTM2***
- t(X;22)(p11.4;q13.2) ***ZC3H7B-BCOR***



MODERN PATHOLOGY (2017) 30, 1251–1261



IHC panel for BCOR sarcoma

Variable CD99

BCOR+, SATB2+, cyclin D1+

Am J Surg Pathol. 2016 December ; 40(12): 1670–1678.

WHO Criteria for Diagnosis
Sarcoma with *BCOR* genetic alteration

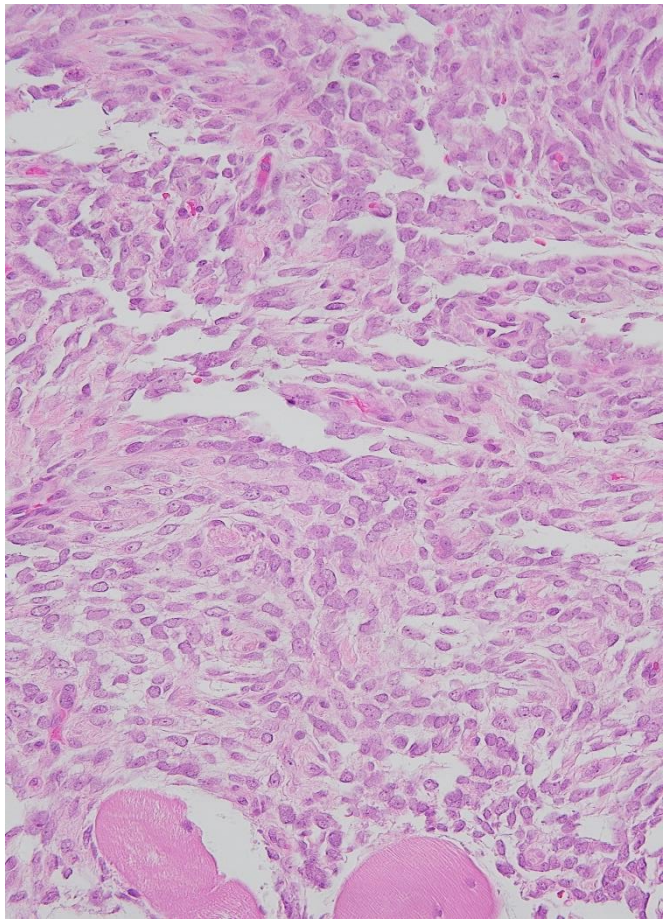
Essential

- Primitive round to spindle cells
- arranged in nests, sheets or fascicles
- variable myxoid stroma, delicate vessels
- IHC + BCOR, SATB2, cyclin D1

Desirable

- *BCOR* fusion or *BCOR* ITD

(can be definitive!)



BCOR-rearranged sarcoma of the spine

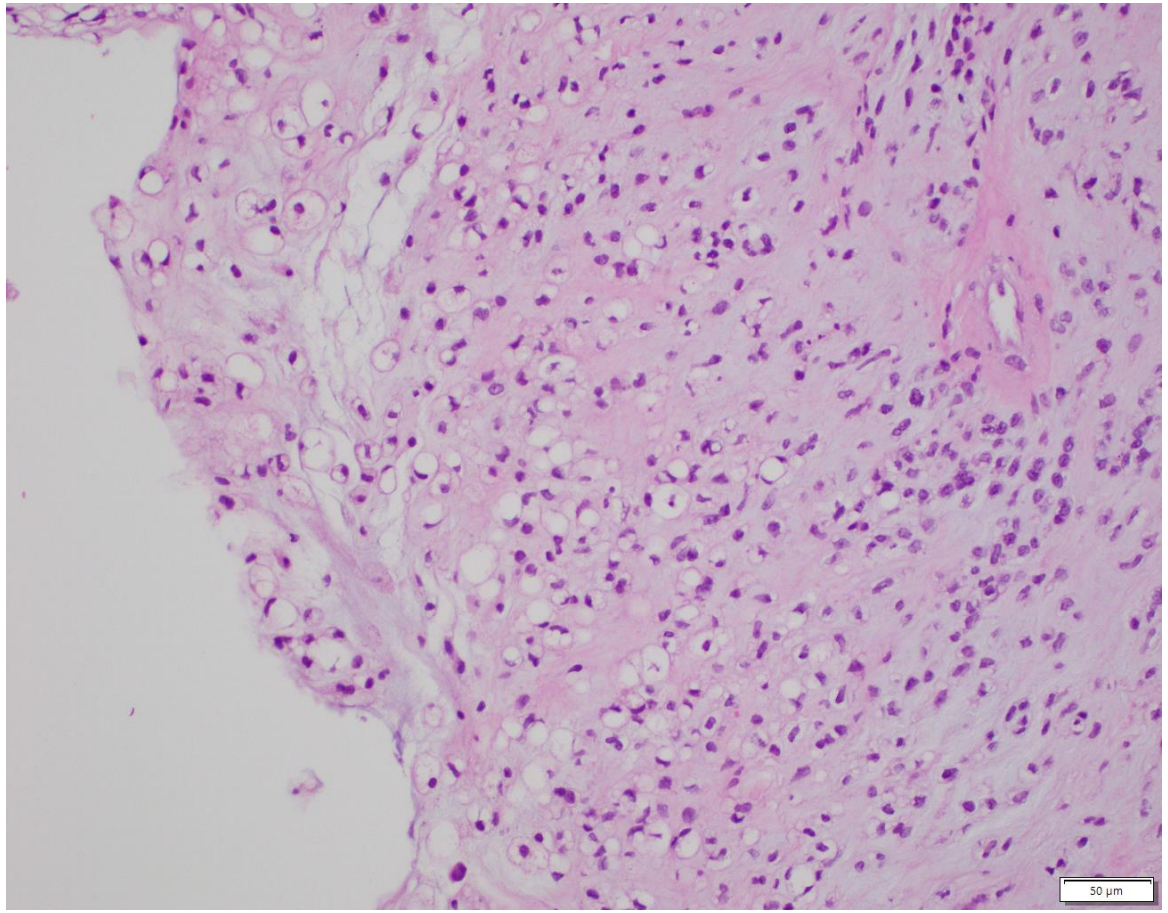
Continuous discovery of novel fusions and persistent low level false negative FISH indicate that comprehensive genomic testing is needed sometimes for accurate diagnosis

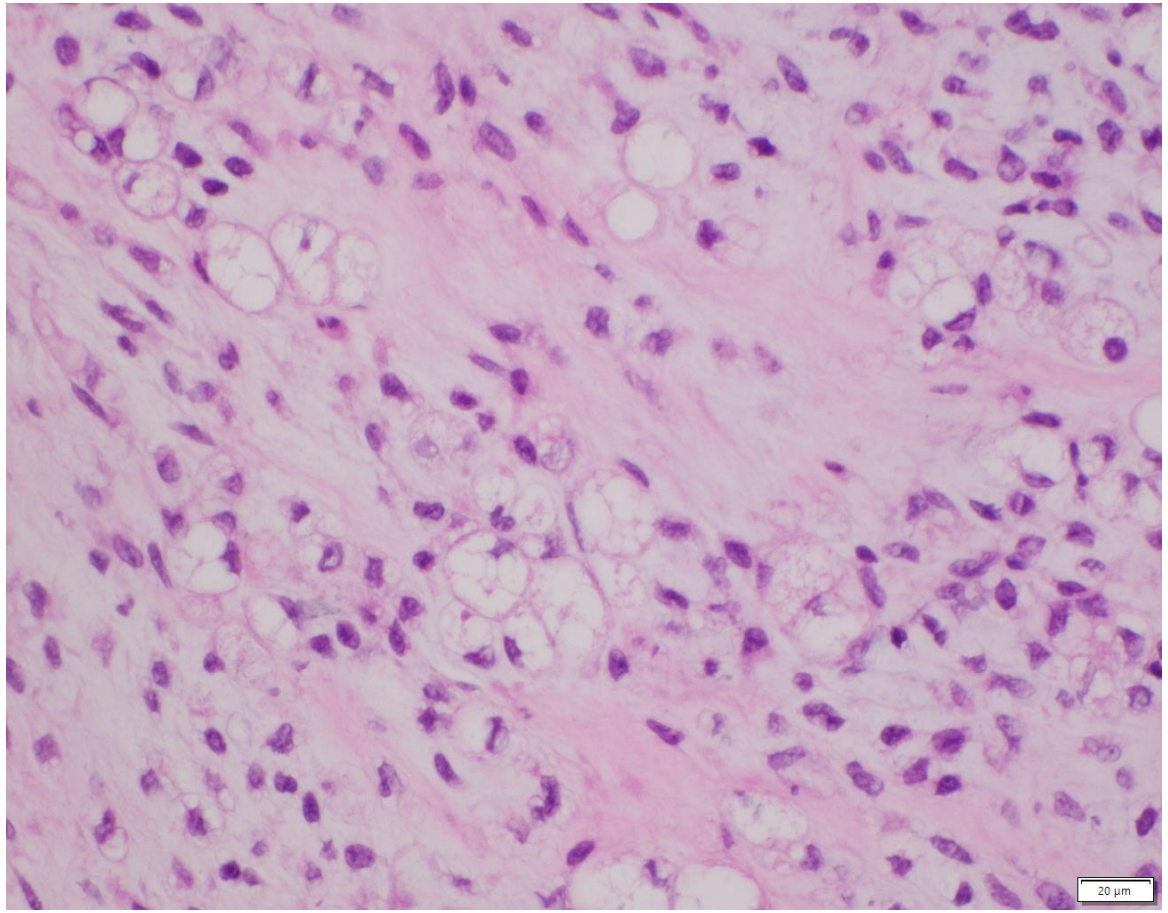
Antonescu, CR, Agaram, NP, Sung, Y-S, Zhang, L, Dickson, BC.
Undifferentiated round cell sarcomas with novel *SS18-POU5F1* fusions.
Genes Chromosomes Cancer. 2020; 59: 620– 626.

Yoshida, A et al.
CIC break-apart fluorescence in-situ hybridization misses a subset of CIC-DUX4 sarcomas: a clinicopathological and molecular study.
Histopathology. 2017 Sep;71(3):461-469

Inconclusive Molecular Results resolved with NGS

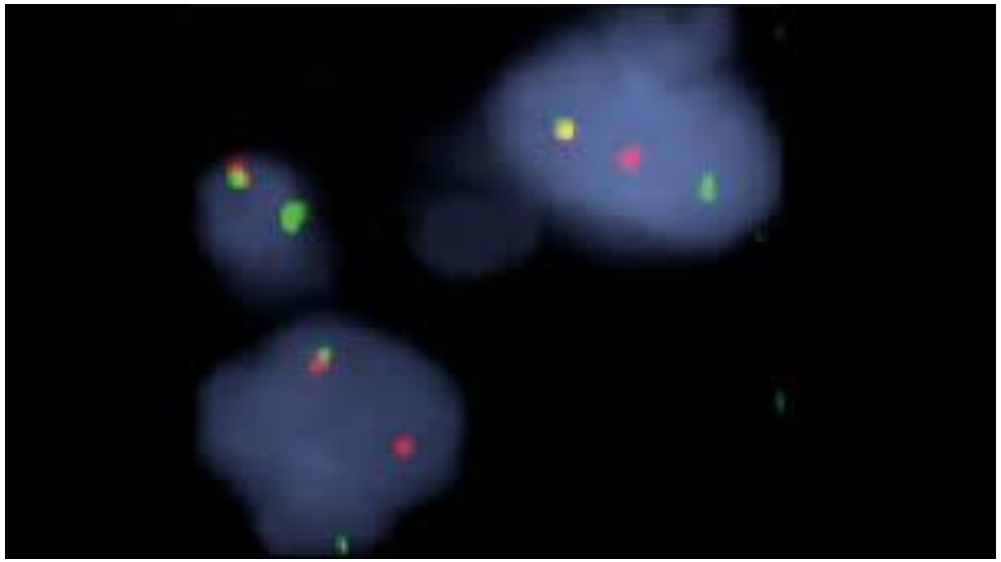
12cm distal thigh mass, 40yo male

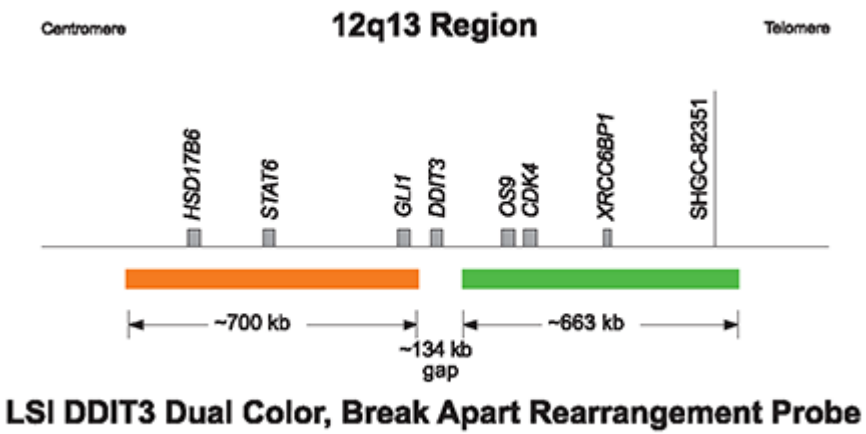




Molecular workup

- ***DDIT3 (CHOP)*** FISH
- Myxoid/Round Cell Liposarcoma
  - > 95% with **t(12;16)** ***FUS*-*DDIT3***,
  - Remaining cases **t(12;22)** ***EWSR1*-*DDIT3***





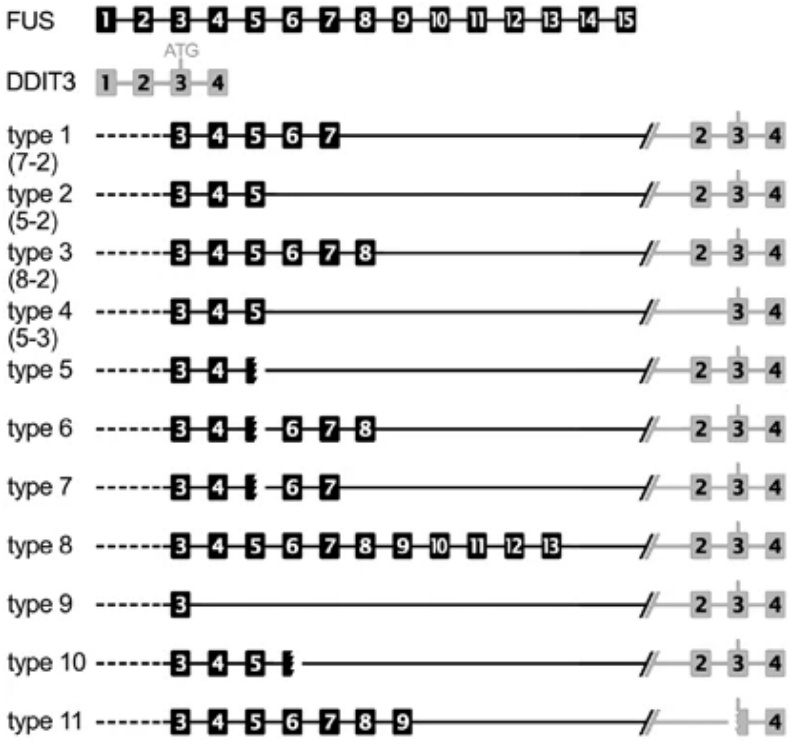
LSI DDIT3 Dual Color, Break Apart Rearrangement Probe

FISH results

- ***CHOP/DDIT3*** **NEGATIVE**
- ***EWSR1*** **INDETERMINATE** – loss of 3' probe precludes assessment

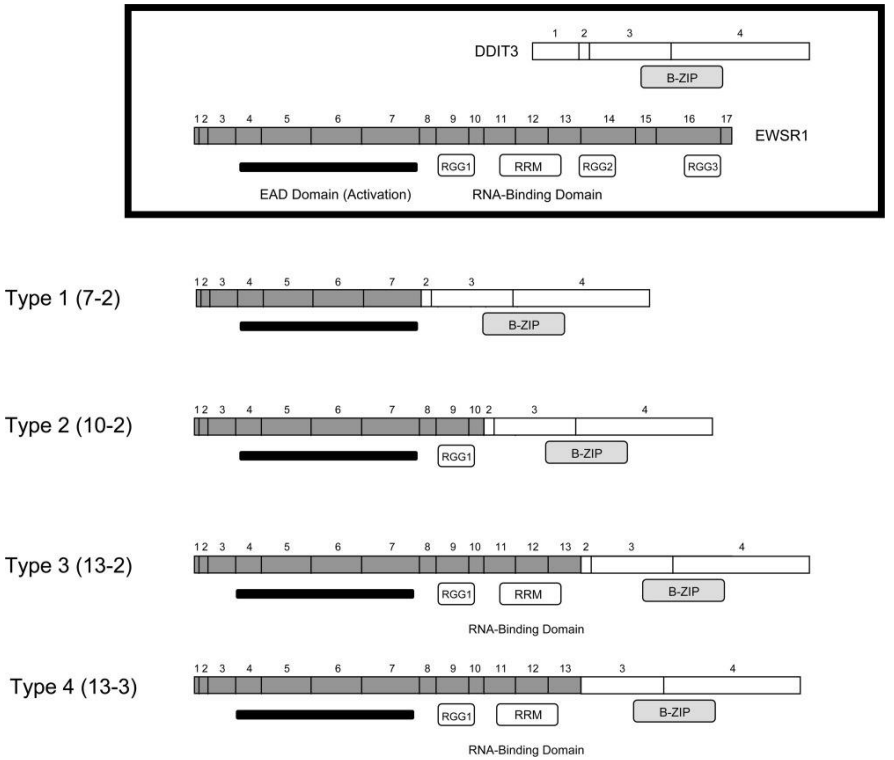
FISH results and molecular context

- ***CHOP/DDIT3*** **NEGATIVE**



Powers MP et al. 2010 *Mod Pathol* **23**:1307–1315

- ***EWSR1*** **INDETERMINATE** – loss of 3' probe precludes assessment



Bode-Lesniewska B. et al. 2007 Genes Chromos Cancer 46: 961-971

FISH testing results

- ARUP
  - *CHOP/DDIT3* negative
  - *EWSR1* indeterminate – loss of 3’ probe in 25% of the cells precludes assessment

- MSKCC, Cristina Antonescu consultation report
  - **Custom probe confirms *EWSR1* rearrangement**
  - NO abnormalities in *FUS, DDIT3, NR4A3*

Director, Soft Tissue & Bone Pathology, MSKCC

Myxoid Liposarcoma

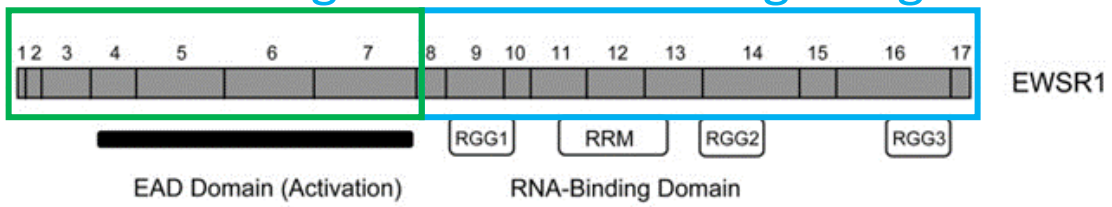
- ARUP
  - *CHOP/DDIT3* negative
  - *EWSR1* indeterminate – loss of 3' probe in 25% of the cells precludes assessment

- MSKCC, Cristina Antonescu consultation report
  - Custom BAC probe confirms *EWSR1* rearrangement
  - No abnormalities in *FUS, DDIT3, NR4A3*
  - **Two possible explanations for confusing molecular results**
    - **Cryptic rearrangement/unbalanced translocation undetectable by FISH**
    - **Novel fusion variant**

Resolution with NGS

- Archer Fusion Plex MSKCC
  - *EWSR1-DDIT3* fusion (mRNA) detected
  - Exon 2 of *DDIT3*
  - Exon 7 of *EWSR1*

- ARUP FISH
  - Loss of 3' *EWSR1* probe signal
  - Rearrangement deleted large fragment



Bode-Lesniewska B. et al. 2007 Genes Chromos Cancer 46: 961-971

- *CHOP/DDIT3* probes were normal (not split) because the 5' translocated fragment of *EWSR1* is not large enough to split the *DDIT3* probes

There is no perfect test!

- Variable and nonspecific IHC
- False negative FISH: cryptic translocations
  - SYT, EWSR1, CIC, etc.
- False negative RT-PCR: when the primers do not flank the breakpoint
  - DFSP- infamous for highly variable breakpoints
  - Rare breakpoints
- False negative/positive NGS: complex wet chemistry and bioinformatics

Acknowledgements

- Ting Liu, MD – Section Lead for Bone & Soft Tissue Pathology, Genitourinary Pathology, University of Utah, ARUP Laboratories

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