EXTENDED EWING FAMILY TUMORS (EEFTs)

STEPWISE APPROACH

TO

DIAGNOSIS

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EEFTs: Grateful Acknowledgement

- Thanks for providing articles, data, photos and for sharing cases to : Drs S Smith, L Wang, S Ranganathan, V Kumar, S Mandavilli, F Balarezo, B Bagci, S Moradi, Jayalakshmy and to
- Dr Nat Pernick and his colleagues at Pathology Outlines and to
- All the authors of review and focused articles on EEFTs and of other online materials.

- AIPNA FOR SPONSORING THE WEBINAR

PLAN OF PRESENTATION: Part B

.Brief review of Part A

.ICC profiles & genetic markers of EEFTs

.Request ICC stains in the profile matching the working Dx made on the basis of histologic profile

.Request targeted FISH/RT-PCR/NGS for molecular genetic marker of working Dx strengthened by targeted ICC stains

PLAN OF PRESENTATION: Part B

.Brief review of

Merits of FISH

NGS as a diagnostic & research method

Clinico-biologic relevance of molecular genetic markers

Summary

Next Step after working Dx based on the histologic profile of the case being signed out: Part B

. Request set of ICC markers suitable for strengthening the working Diagnosis

.Choose the set based on personal experience/literature review

.Total panel for EEFTs: >25 ICC markers

.Panel for EEFTs in practice: Conserving resources & reducing cost according to departmental/personal choice

Established & Evolving entities (NGS) (Davis 2020)-Genetic markers of EEFTs

- 1.ES(EWS-FLI1/other):(2-60+, Med 15yrs)
- 2.EWS-NAFTC2(12-70,32);PATZ1(1-81,42)
- 3.D-SRCT(EWS-WT1): (2-60+, 22)
- 4.BCOR Reargd: CCNB3 fusion sarcomas (2-44,15), BCOR-ITD (0-15,1);CCSK in children
- 5.CIC-DUX4/other sarcomas(5-80,32)
  (6.Undiff sarc: no distinctive gene marker)

Sets of SRCTs- DD:Established Entities with evidence of differentiation vs EEFTs

- EEFTs BASICALLY BELONG TO THE BROAD GROUP OF SRCTs IN CHILDREN (& ADULTS)
- Two sets in this broad group of SRCTs:

- A. Established SRCTs with demonstrable line of differentiation by histology/EM/ICC/Flow cytometry (Three subsets)
- B. EEFTs: Undifferentiated SRCTs (Exception: Subtype of ES showing neural differentiation)

Sets of SRCTs- DD:Established Entities with evidence of differentiation vs EEFTs

- Subsets of Established SRCTs
- 1. Conventional spectrum: NB, RMS, NHL;
- 2. SRC variants of tumors: Osteosarcoma,Syn Sarc, MPNST, Blastemal-WT, Hepatoblastoma;
- 3. Biphasic sarcomas with SRC component (only SRC component may be seen in core Bx) (e.g. Mesenchymal & Myxoid chondrosarcoma, Myxoid Liposarcoma)
- (Rarely : Germinoma, Granulocytic sarcoma, Nasopharyngeal carcinoma, Inflamm myofibr tumor)

DIAGNOSIS OF EEFTs: FIRST STEP

- Main established entities in DD of ES:

.NB (UD) (viscera),

.Embryonal RMS, dense pattern variant (Soft Tissue),

.Alveolar RMS, solid variant (ST),

.Lymphoblastic CD45-ve,CD99+ve NHL of bone

.SRC variant of osteosarcoma (bone)

EEFTs: Approach to Dx –First Step – Assumption for this presentation

- All aforementioned entities in subsets of Established Pediatric/Adult SRCTs

Ruled out by stepwise approach

EEFTs:Dx (Clues from routine Tumor Cell cytology/histology)

- Two groups on the basis of clues from basic cellular features:
- ES (uniform evenly spaced RCs without atypia)
- Non-ES (unevenly spaced RCs + Spindle cells, variable atypia)

Non-ES divided into two subsets

EEFTs:ICC profiles:Introduction

ICC = Morphologic Proteomics (Dr R Cartun)

.Important considerations:

a.Type of Antibody: N or C-terminus (e.g. only C-terminus WT1 Ab +ve in D-SRCT)

b.Pattern: nucl/cytopl/membrane

c.Extent: diffuse:>5/50%, focal

d. Degree:weak,moderate,strong

d.Overlap of ICC makers: e.g. CD99 +ve in ES & variable proportion of other EEFTs

EESFT: ICC Criteria for strengthening working Dx of each Entity

Individual marker by itself of limited value in EEFTs

PRINTOUTS OF PROFILES NECESSARY FOR

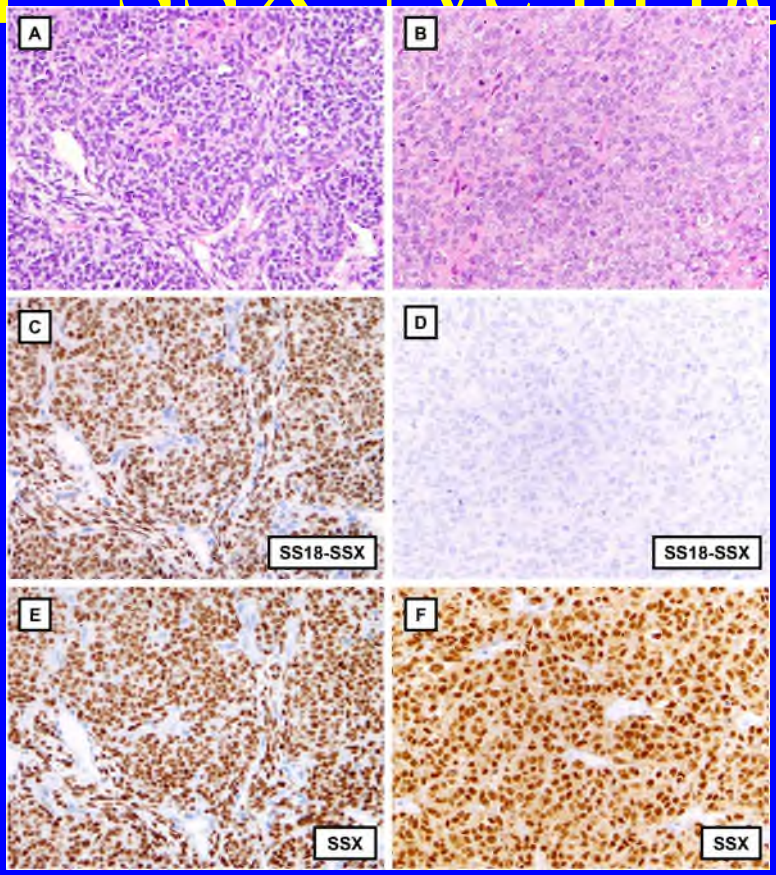
STRENGTHENING THE HISTOLOGIC WORKING DX

EESFT: Criteria for Definitive Dx of each Entity

- Gold standard: Gene fusion by RT-PCR/FISH/NGS
- ICC for FUSION protein likely to be most specific e.g. Ab for SS18-SSX fusion protein in Syn Sarc (100% specificity, 95% sensitivity – Baranov E AJSP 2020;44:492) (Confirmed by Perret R AJSP 2021;45:582)

Next Generation ICC

Baranov 2020-SS18-SSX: PD SS C:Case1+ve,D:Case2-ve (E,F: SSX +ve in both)



EEFTs:Dx (Dxstic value of INDIVIDUAL ICC marker)

.Such antibodies (e.g. against fusion proteins in EEFTs) could replace molecular genetic testing for fusion transcripts or cytogenetic teasing for translocations (Baranov 2020)

Next Generation ICC Stains

(Such antibodies for EEFTs ? Under development)

EEFTs:Dx (Clues from ICC profile)

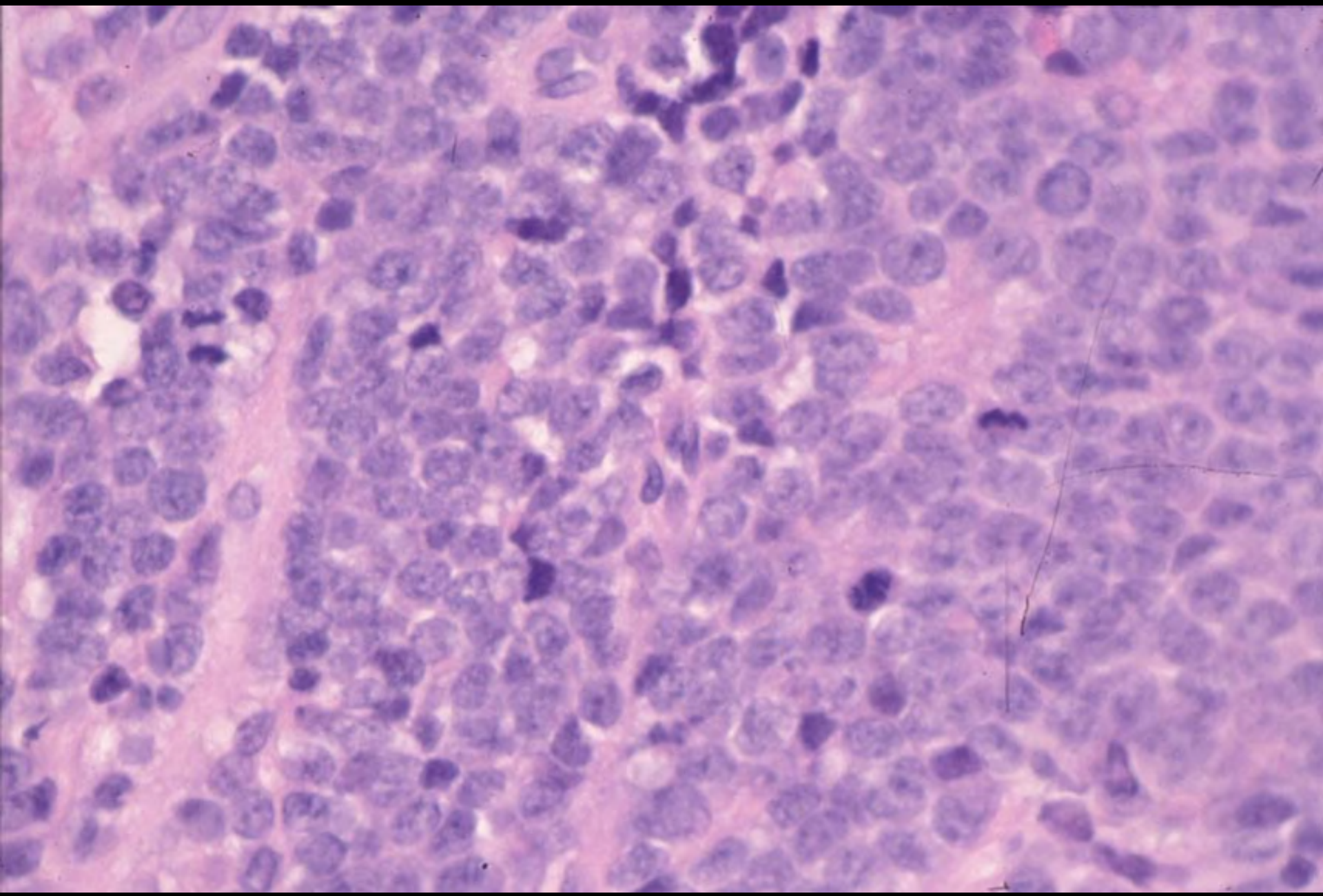
Knowledge of ICC profile for a particular histologic working Dx enables requesting targeted ICC stains for conserving resources and reducing cost.

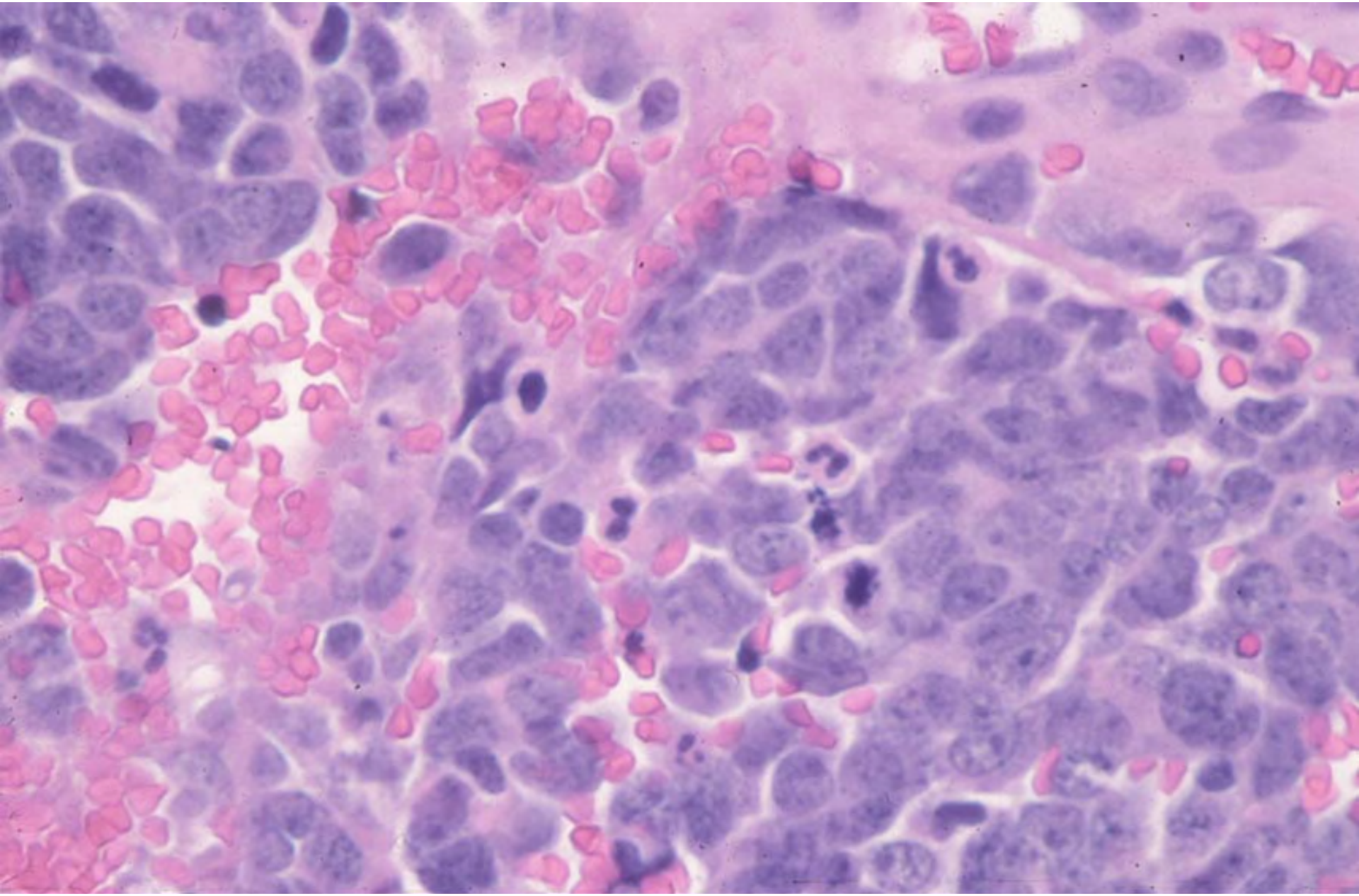
EEFTs:Dx (Clues from routine Tumor Cell cytology/histology)

- Two groups on the basis of clues from basic cellular features: ES & Non-ES

1.EEFT with SINGLE cell type - uniform evenly spaced RCs, generally low mitotic activity : ES, typical(Atypical large cell ES)

2.EEFTs generally with TWO cell types - unevenly spaced RCs & spindle cells, variable but generally atypia of one/both cell types, generally moderate mitotic activity: Non-ES





EEFT:ICC profile supporting histologic working Dx of ES

Positive results

A.CD99 Diffuse, strong, crisp membranous

NKX2.2 Strong nuclear

FLI1+ PAX7+

Neural marker+/- NSE, Synaptophysin etc

Supporting Typical ES, with/out neural differentiation & Atypical large cell ES (Histologic profile)

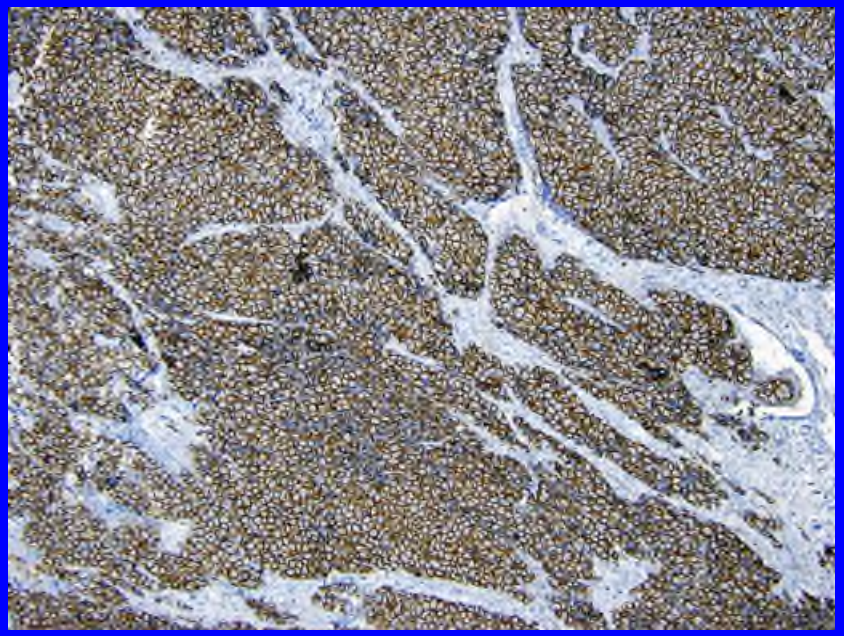
B. All of above + focal CK, HMWCK:

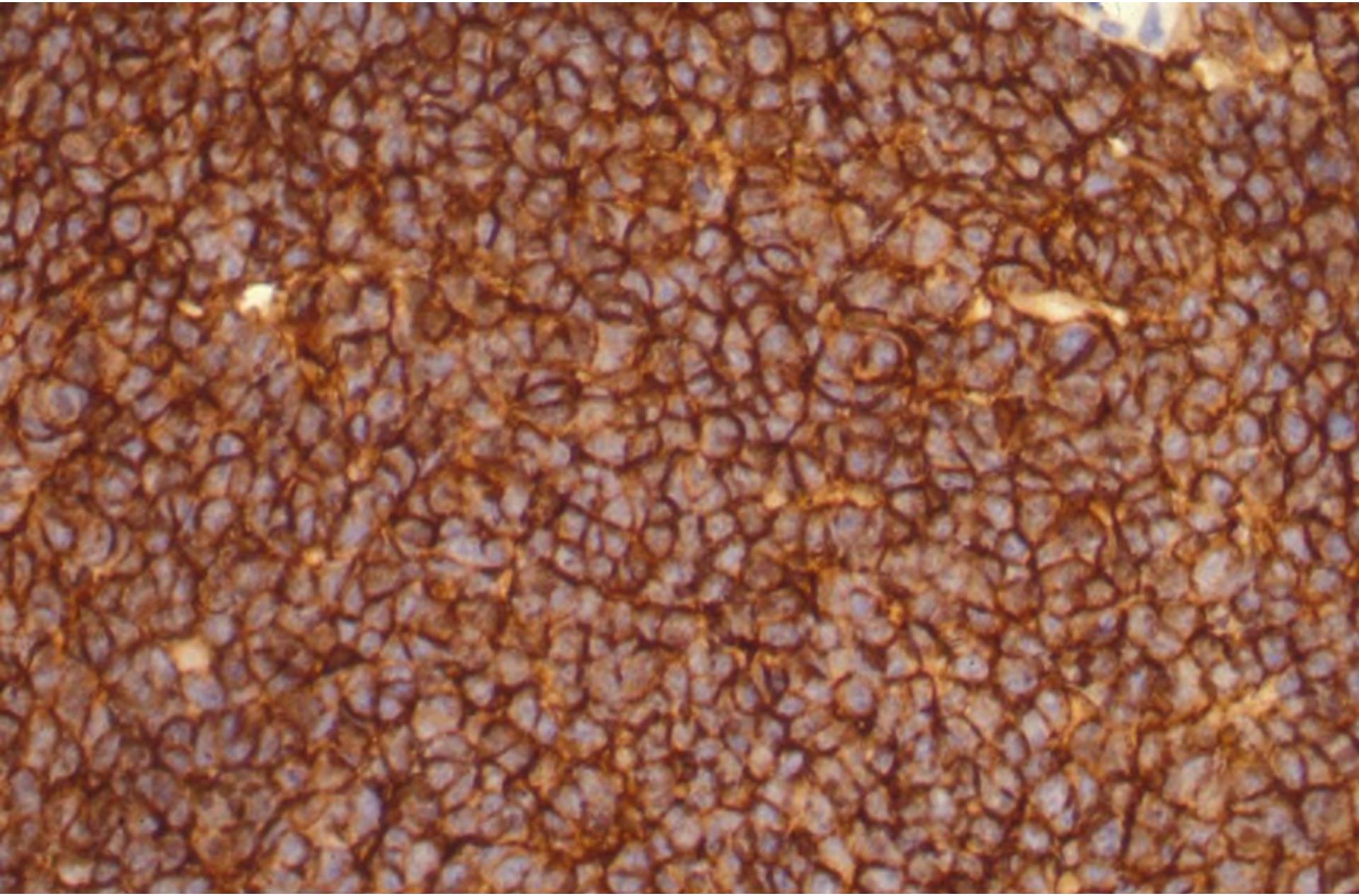
Supporting ES, typical with epithelial differentiation or adamantinoma-like pattern(Antonescu 2014,Folpe 2005)

Negative results:

WT1,TLE1,Desmin,Myogenin,ETV4,BCOR,& in most cases CCNB3,(PHOX2B +ve in NB, -ve in ES)

ES, CD99 diffuse, strong,crisp membranous staining (Parham D 2010)

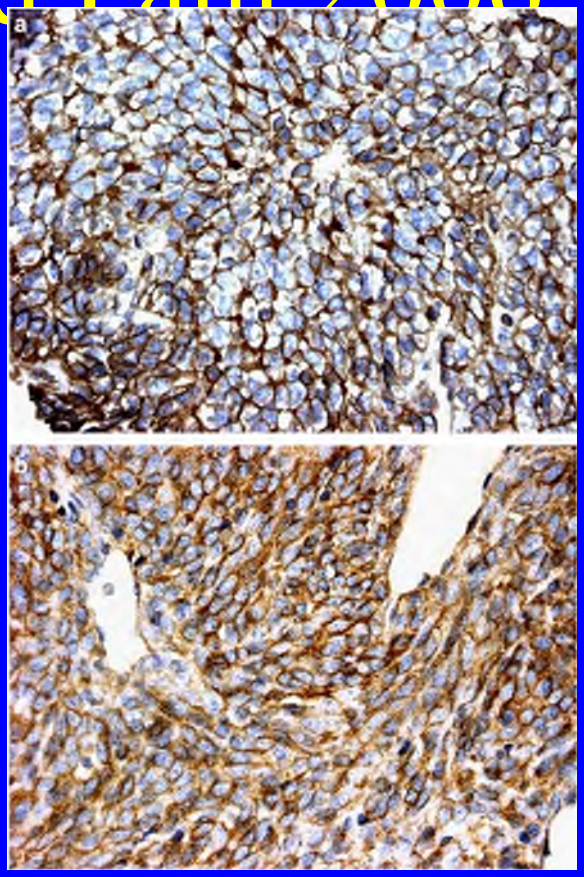




EWS/FUS-NFATC2 fusion:7 cases sarcoma (Perret R Mod Path 2020; 33:1930)

- a,b:Aggrecan strong, diffuse,cytoplasm in all cases;
- c,d:CD99 cytoplasmic dot-like (c) or membranous (d) (all cases)
- e:Pan-keratin,cytopl, in 3 cases

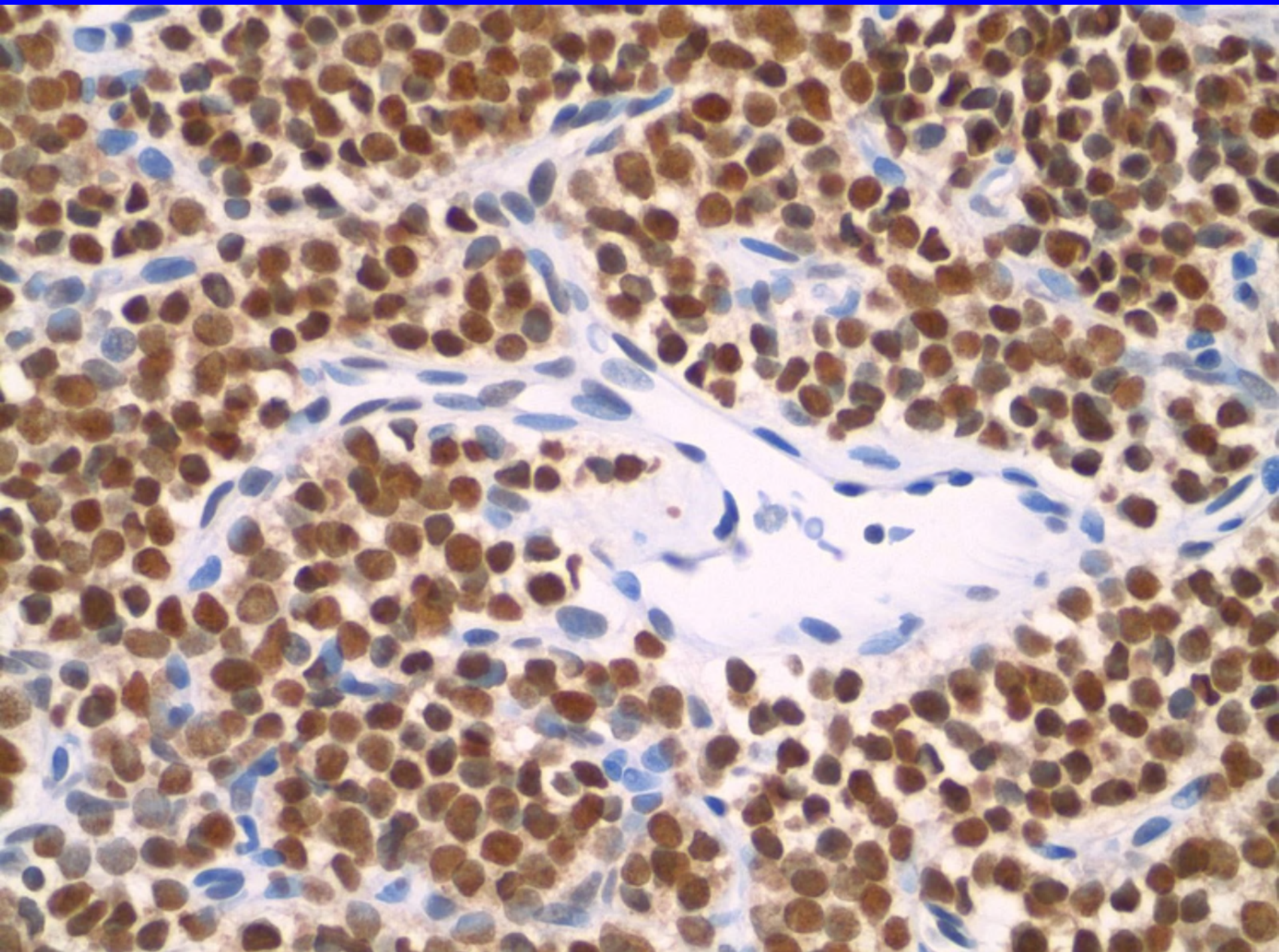
CD99 in ES & 70% Syn Sarc(diffuse membr 23%) (Olsen SH Mod Path 2006;19:659)



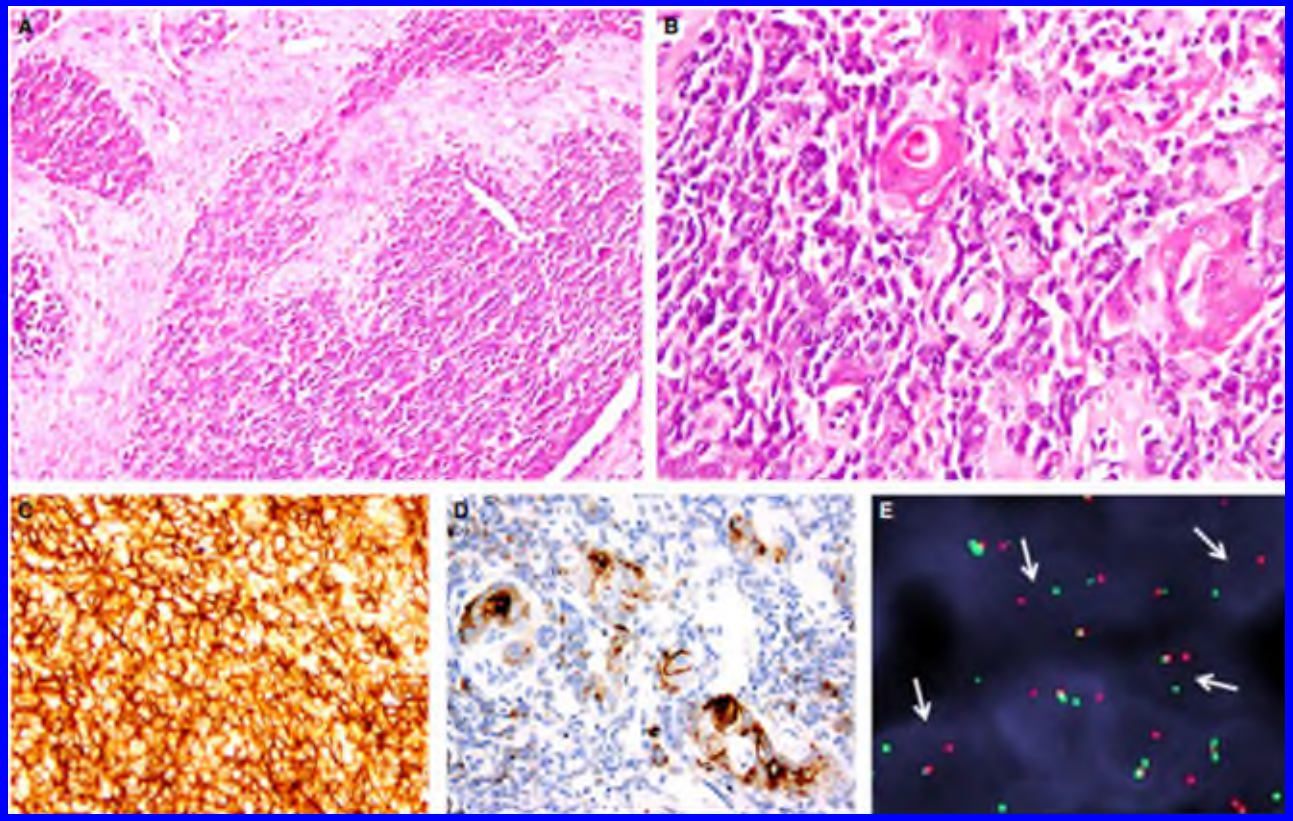
EEFTs:Dx(ICC profile:Markers of particular import:NKX2.2)

.An adjunct in the work-up of ES

(Can be -ve in occasional ES cases and +ve in other EEFTs and Non-EEFTs)



Antonescu 2014:ES-A:basaloid,B sq,C:CD99,D-Epith Keratin+



EEFT:ICC profile supporting histologic working Dx of ES

ICC profile found to correlate with working histologic Dx:

Request targeted test (FISH/RT-PCR) for EWS-FLI1 gene fusion

NGS may be needed in rare genetic variants of ES.

Details of gene fusions of all EEFTs to be discussed

EEFTs:Dx (Clues from routine Tumor Cell cytology/histology)

- Non-ES divided into two subsets
- BCOR Rearrgd sarcomas (minimal atypia, four elements and classic pattern with chicken wire capillaries)
- Non-BCOR Rearrgd sarc (moderate atypia,brisk mitosis,patterns-desmoplasia):
- EWS-NFATC2 EWS-PATZ1
- D-SRCT(EWS-WT1) CIC Rearrgd Sarc

EEFTs: Histologic components and patterns in BCOR rearrg sarcomas

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Classic pattern and apparent variation in subsets:

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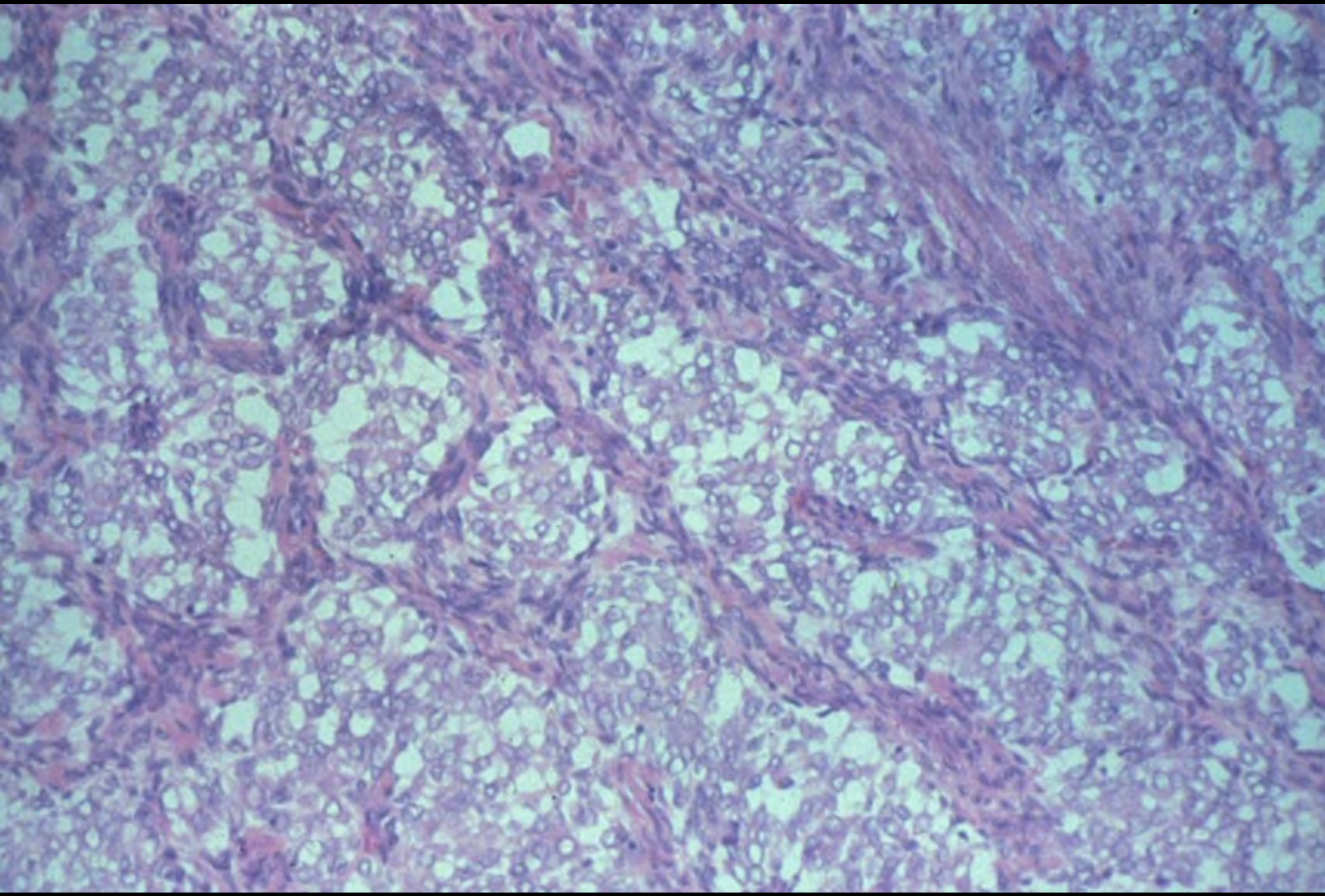
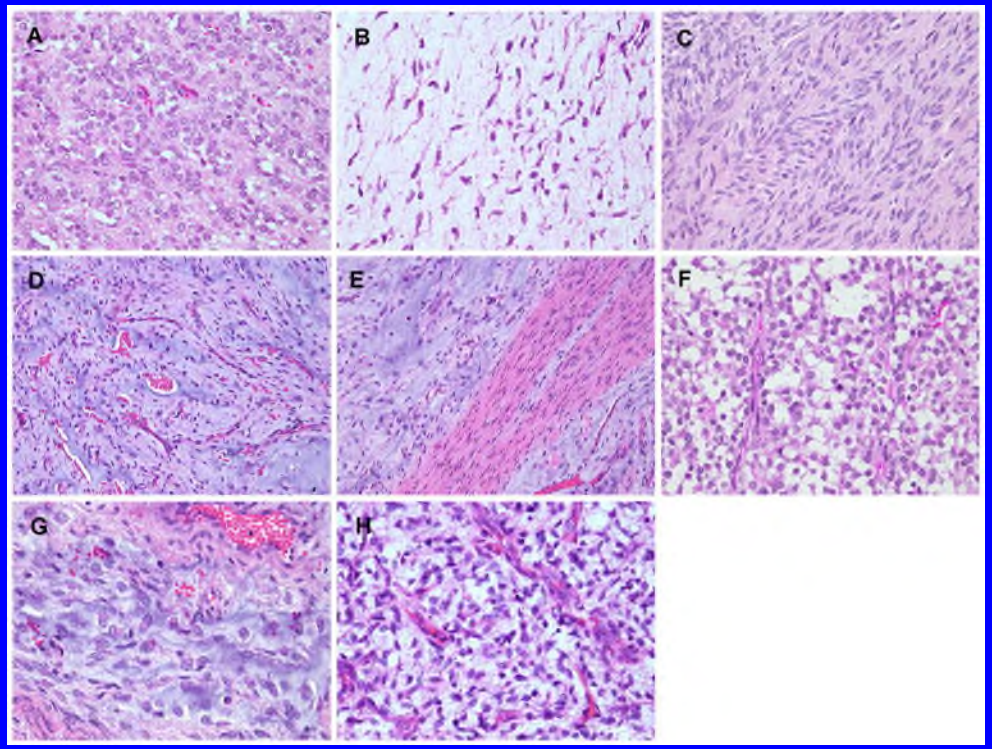
Four elements (RCs in cords/nests, spindle cells of septa, chicken wire cap network, myxoid stroma - classic pattern), best described/seen in CCSK



Kao 2016:PMMTI/URCS:A-H:RCs,spindle cells,cap,cellular septa,rhabd cells,myx str(cytopl)



EEFT:Clues from NEXT ICC profile (13 +ve/-ve markers reported) supporting Dx of BCOR Rearrngd sarcoma

Positive results

.CD99 +ve rarely membr. (40% cases of BCOR rearrg sarc)

.BCOR +ve (94%) CCNB3 +ve (95%) TLE1 +ve (75%)

.SATB2 +ve (83%) .NKX2.2 +ve (29%)

Negative results

WT1, Desmin, Cytoker, S100

(CCNB3 apparently -ve in BCOR ITD Sarcomas)

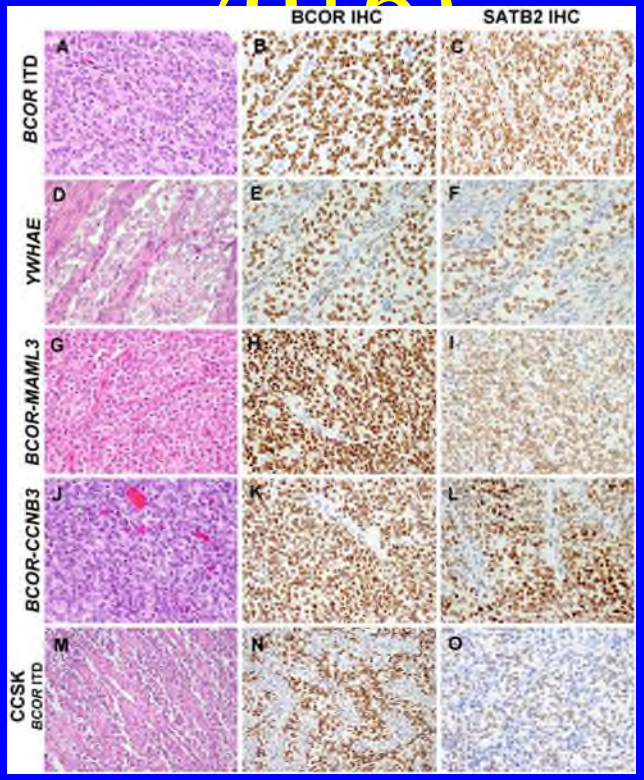
(Above data given by Carter 2019)

SS18-SSX -ve (Baranov E AJSP 2020;4:922)

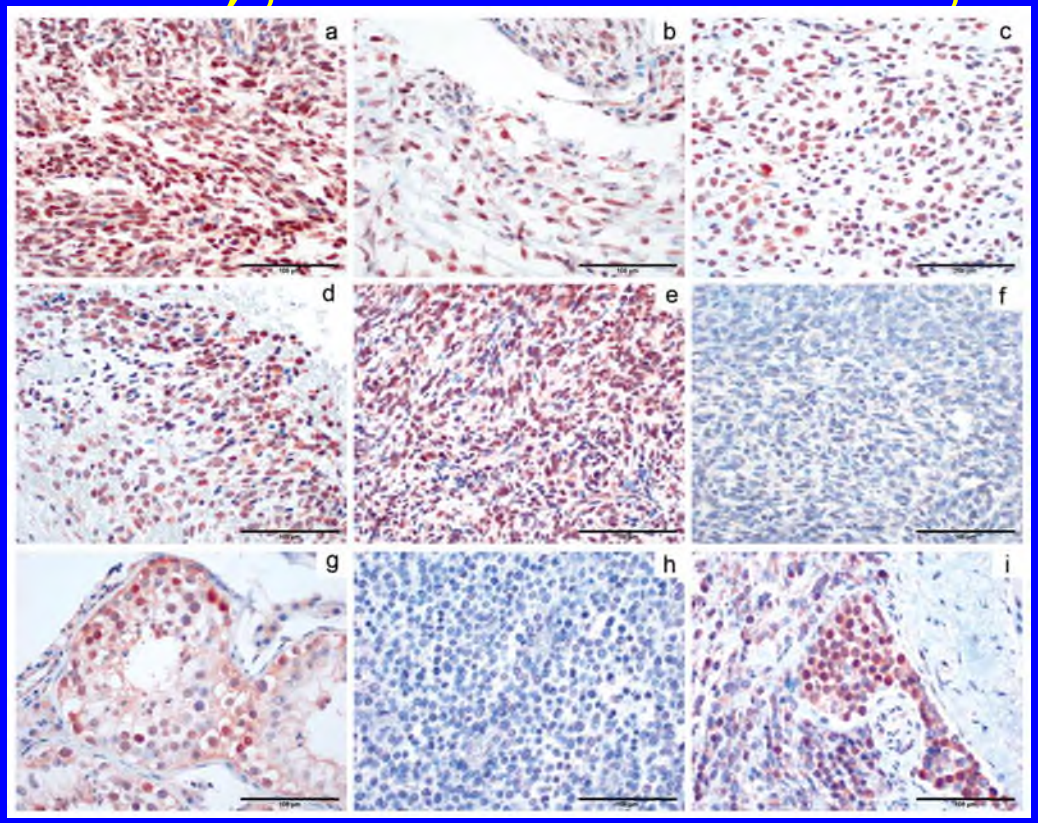
(PAX8 +ve - Ludwig CK Ped Devel Path 2017;20:327,

CyclinD1 +ve - Argani P AJSP 2017;41:1702)

BCOR&SATB2 ICC+ve in BCOR-ITD,BCOR & YWHAE sarc;weak:SATB2 in CCSK(Kao 2016)



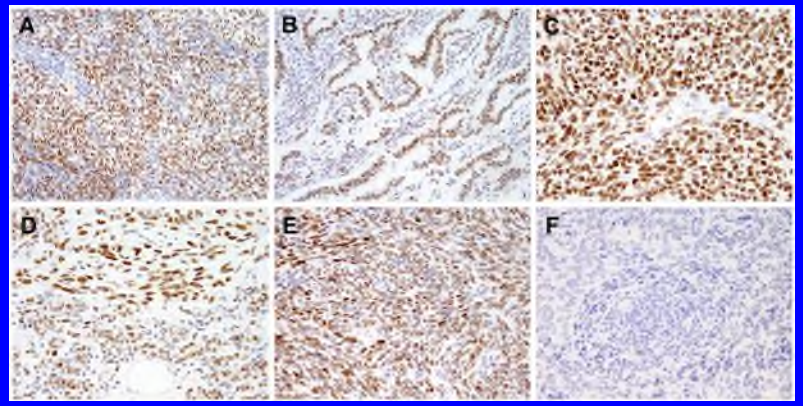
(Kumar 2015)CCNB3 ICC:Ped BCOR rearrg:a-e:nucl++/+,f-ve: g+(testis),h:CIC sarc-ve,i:ES+



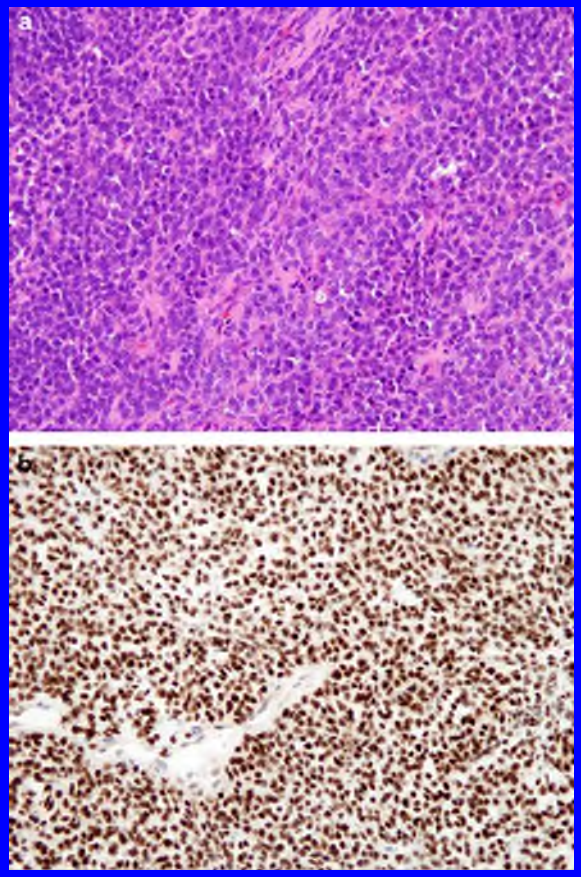
EEFTs:DD- BCOR Rearranged Sarcoma & Poorly Diff Syn Sarc

- Overlap in ICC: CD99, SATB2, CyclinD1, TLE1, BCOR in both (Kao VY AJSP 2016; 40: 1670; Argani P AJSP 2017; 41:1702)

BCOR ICC: +ve in all Syn Sarc subtypes (C-PDSS)(Kao 2016)



Synov Sarc, PD: TLE1



EEFTs:DD- BCOR Rearranged Sarcoma & Poorly Diff Syn Sarc

Overlap in ICC

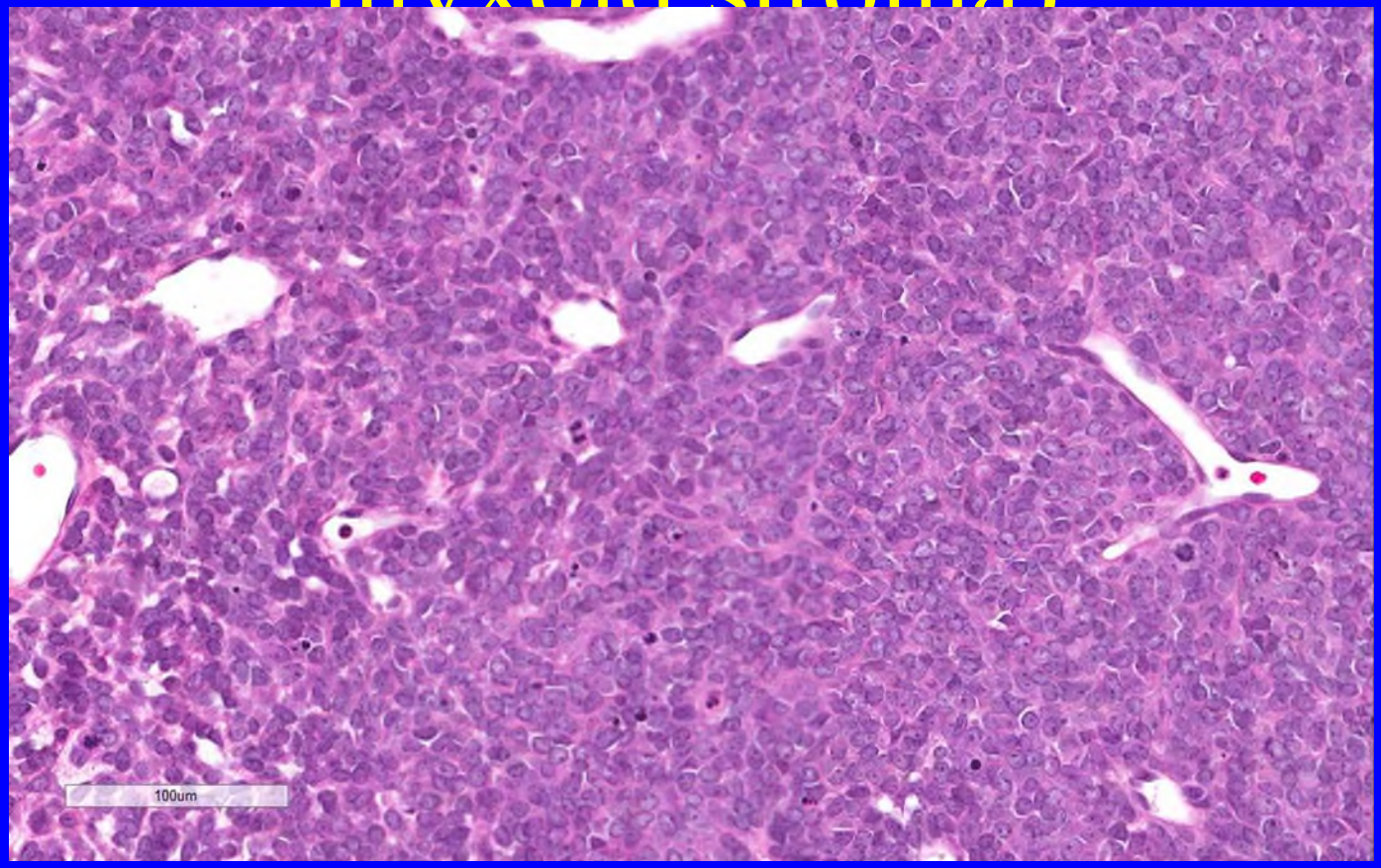
BUT

Little or no overlap in histology - four basic elements showing classic/variant patterns of BCOR Rearrgd sarc not seen in PD Syn Sarc

(Overlap unduly emphasized in the literature)

SS18-SSX +ve only in PD SS

Syn Sarc, PD (RCs with atypia,large/staghorn BV, no myxoid stroma)



EEFT:Clues from ICC profile supporting Dx of Non-ES, BCOR Rearrngd sarcoma

.To conserve resources/reduce cost request 6/13 reported markers with working histologic Dx

Positive markers:CD99 can be +ve (rarely membranous)

.BCOR +ve CCNB3 +ve TLE1 +ve (-ve in CIC Rearg)

Negative markers: WT1 (N terminus Ab) Consistently -ve

SS18-SSX -ve(requested because of overlap with Syn Sarc)

(CCNB3 apparently -ve in BCOR ITD Sarcomas)

EEFT:Clues from ICC profile supporting Dx of Non-ES, BCOR Rearrngd sarcoma

With ICC profile supporting histologic Dx

Seek targeted genetic test(s) for BCOR Rearragd sarcoma

Details of genetic markers to be discussed

EEFTs:Two histologic subgroups of Non-ES

- A.
- BCOR-CCNB3 or BCOR-ITD sarcomas e.g. Primitive Myxoid Mesenchymal Tumor of Infancy (PMMTI) & 3 other subsets
- B.
- 1.FET-Non ETS (NFATC2/PATZ1) fusion sarcomas : two subsets,
- 2. D-SRCT (EWS-WT1)
- 3. CIC-DUX4 (or other gene) fusion sarcoma

EEFTs:Dx (Clues from routine Tumor Cell cytology/histology)

- 2nd subgroup of Non-ES:EWS-NFATC2

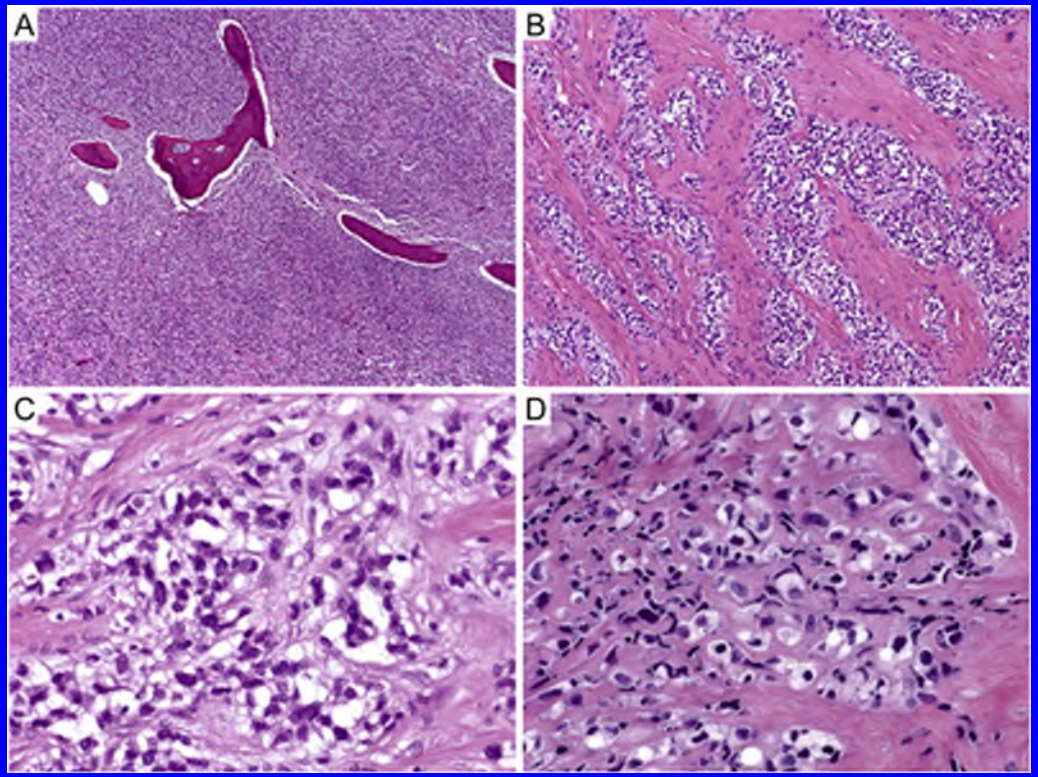
.Myoepithelioma-like pattern (stroma around cords/nests)

.cartilaginous foci (Perret R Mod Path 2020;33:1930)

.Focal stag-horn blood vessels

. (Diaz-Perez JA Hum Path 2019;90:45- Review of 49 cases)

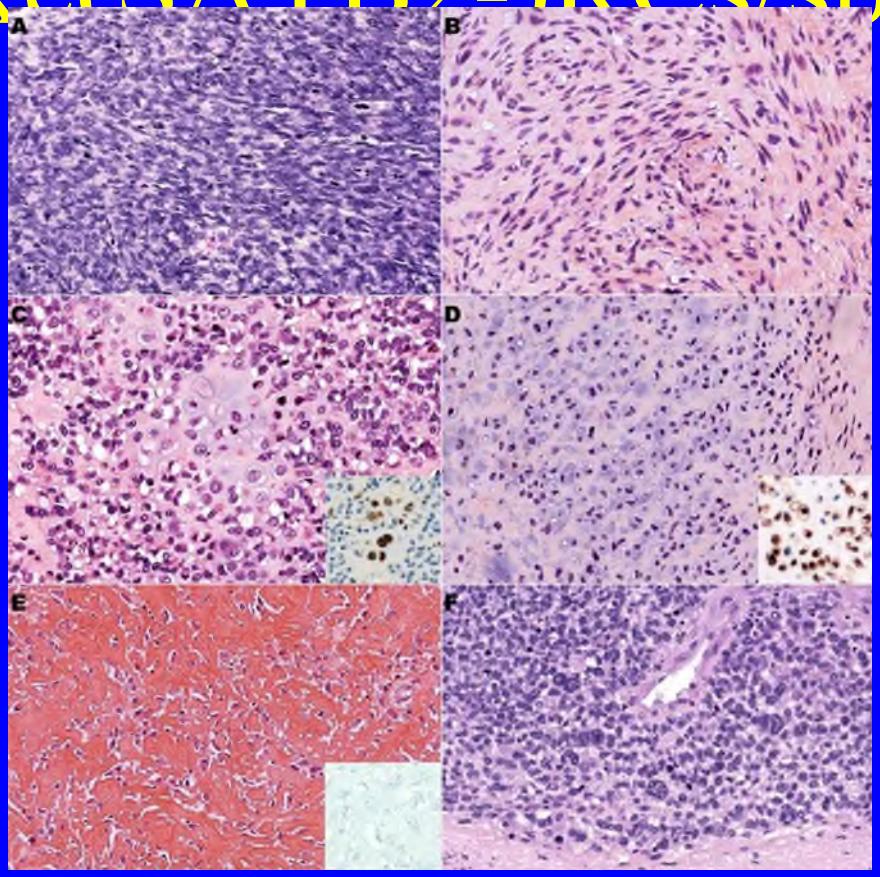
EWS/FUS-NFATc2 RCS :A-RC area,B-myoepith pattern,C,D nucl atypia :Diaz-Perez 2019



EEFTs

- Perret R EWS/FUS-NFATC2 Sarcomas (seven cases) Modern Pathol 2020;33:1930

EWS/FUS-NFATC2 sarc(PerretR 2020)c,d:cartilage(SP100+),e:ost-eoid-like(SATB2-)RCs/spindling



EESFT: ICC profile supporting histologic working Dx of EWS-NFATC2 sarcoma (Diaz-Perez JA Hum Path 2019;90:45,Perret R Mod Path 2020;33:1930)

Positive results(49 cases including 4 of authors:Diaz-Perez)

CD99: +ve (~50%/42 cases), focal/diffuse membranous

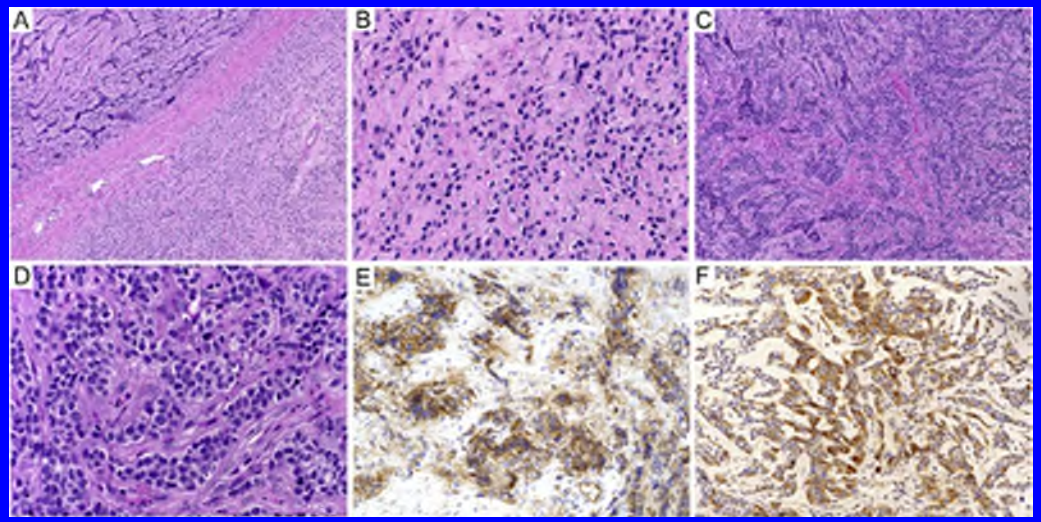
EMA, Keratin (dot-like, focal): +ve ~30% of 30 cases

NKX2.2, PAX7 (WHO 2020), SATB2: can be +ve

Notable recent addition to positive results(7 cases- Perret):

Aggrecan (proteoglycan of cartilage) -Diffuse cytoplasmic + 7/7 cases (Specificity 93%,Sensitivity 100%)

EWS-NFATC2 sarc-A,B: Hypocellular;C,D:myoepith-like foci ,E,F:CD99,membranous & EMA(Diaz-Perez 2019)



EWS/FUS-NFATC2 fusion:7 cases sarcoma (Perret R Mod Path 2020; 33:1930)

- a,b:Aggrecan strong, diffuse,cytoplasm in all cases;
- c,d:CD99 cytopl dot-like(c)/membranous (d)(all cases)
- e:Pan-keratin,cytopl, in 3 cases

EESFT: ICC profile supporting histologic working Dx of EWS-NFATC2 sarcoma

ICC profile supports working Dx of EWS-NFATC2 fusion sarcoma when

Epithelial markers (EMA, Keratin) are positive in a case with Myoepithelial tumor-like histology seen in a Bx (15/30cases with available data)

Aggrecan is diffusely positive with or without cartilaginous foci – Perret 2020 (Histology helpful when chondroid foci are seen)

Request test for genetic marker

EEFTs:Two histologic subgroups of Non-ES

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EESFT:ICC polyphenotypic profile:EWS-PATZ1 sarc(Chougule A AJSP 2019; 43:220-Review of 6 cases+2 of authors)

Positive results

.CD99:+ (50% cases) , patchy/diffuse membr/cytoplasmic.

.Myogenic markers: MyoD1,Myogenin,Desmin

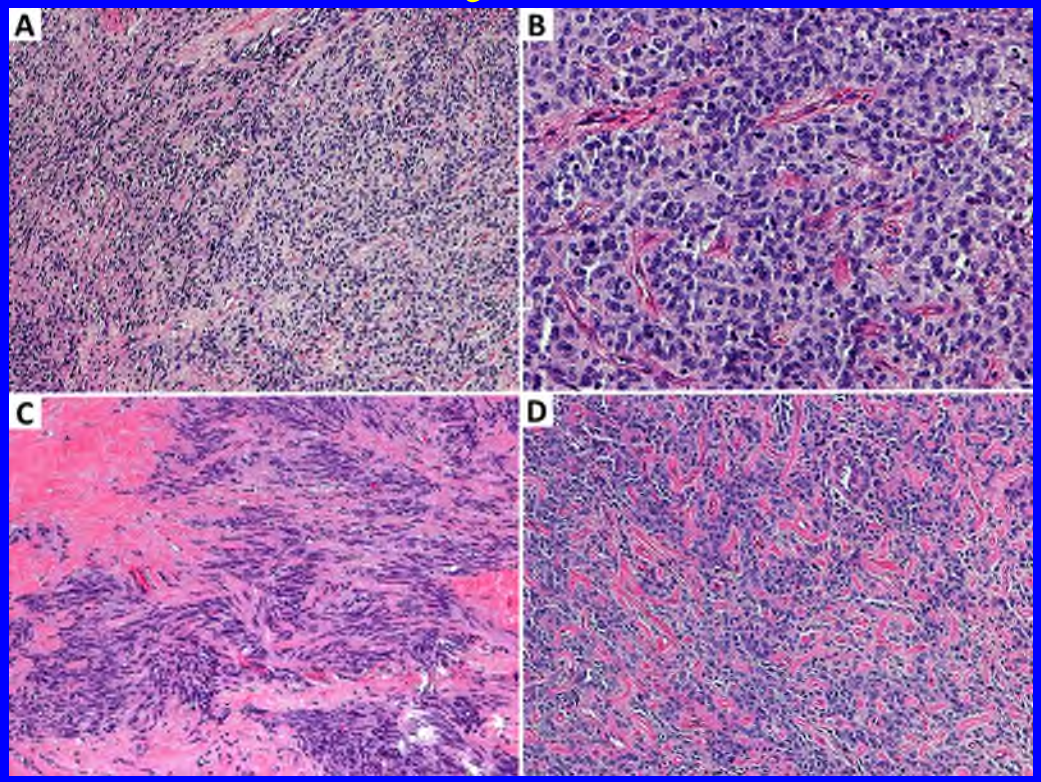
.Neural markers: S100,SOX10,Synapt,GFAP,NSE

.PAX7

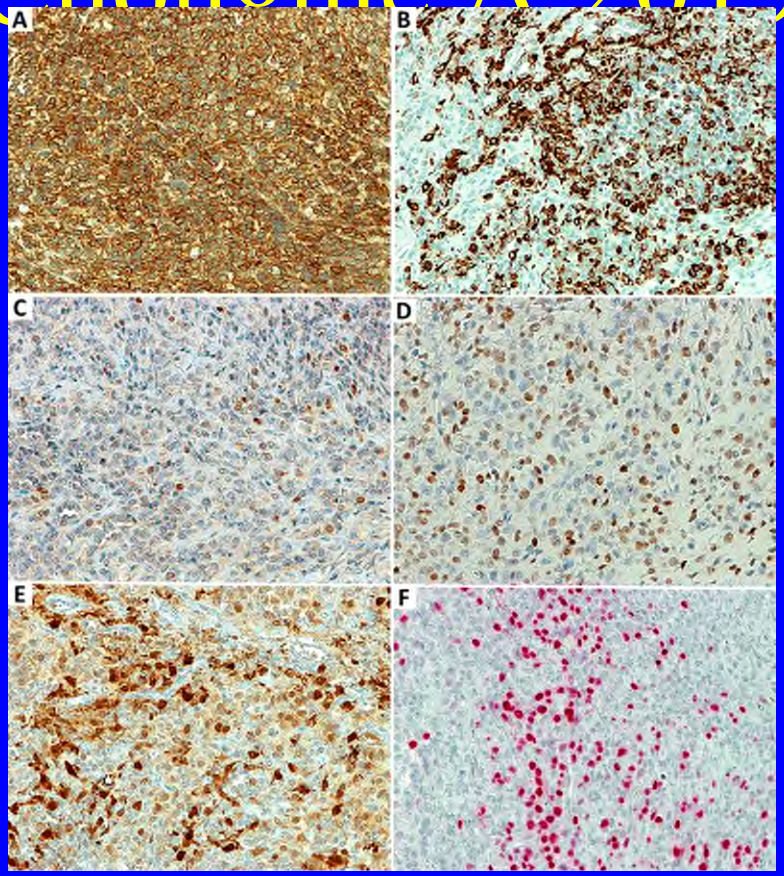
This polyphenotypic profile strengthens the working histologic Dx (Low MR- <1/10hpf, hyalinized capillaries and stroma) of EWS-PATZ1 sarcoma

Request test for genetic marker

EWS-PATZ1 Fusion Sarcoma:Chougule A 2019-RC, Spindle cells,Hyalinized str/capil



EWS-PATZ1 sarc: CD99, Des, Myog,MyoD1,S100,SOX10+ve Chougule A 2019



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EEFT:DX (Clues from NEXT polyphenotypic ICC profile- D-SRCT)

Lae ME AJSP 2002;26:823-Thirty-two cases of D-SRCT

.CD99 +ve (23% of 32 cases)

.WT1(C-terminus Ab)(N terminus of WT1 lost in EWS-WT1 fusion)* +ve (nucl) in 91%

.Desmin +ve (dot-like) in 81%

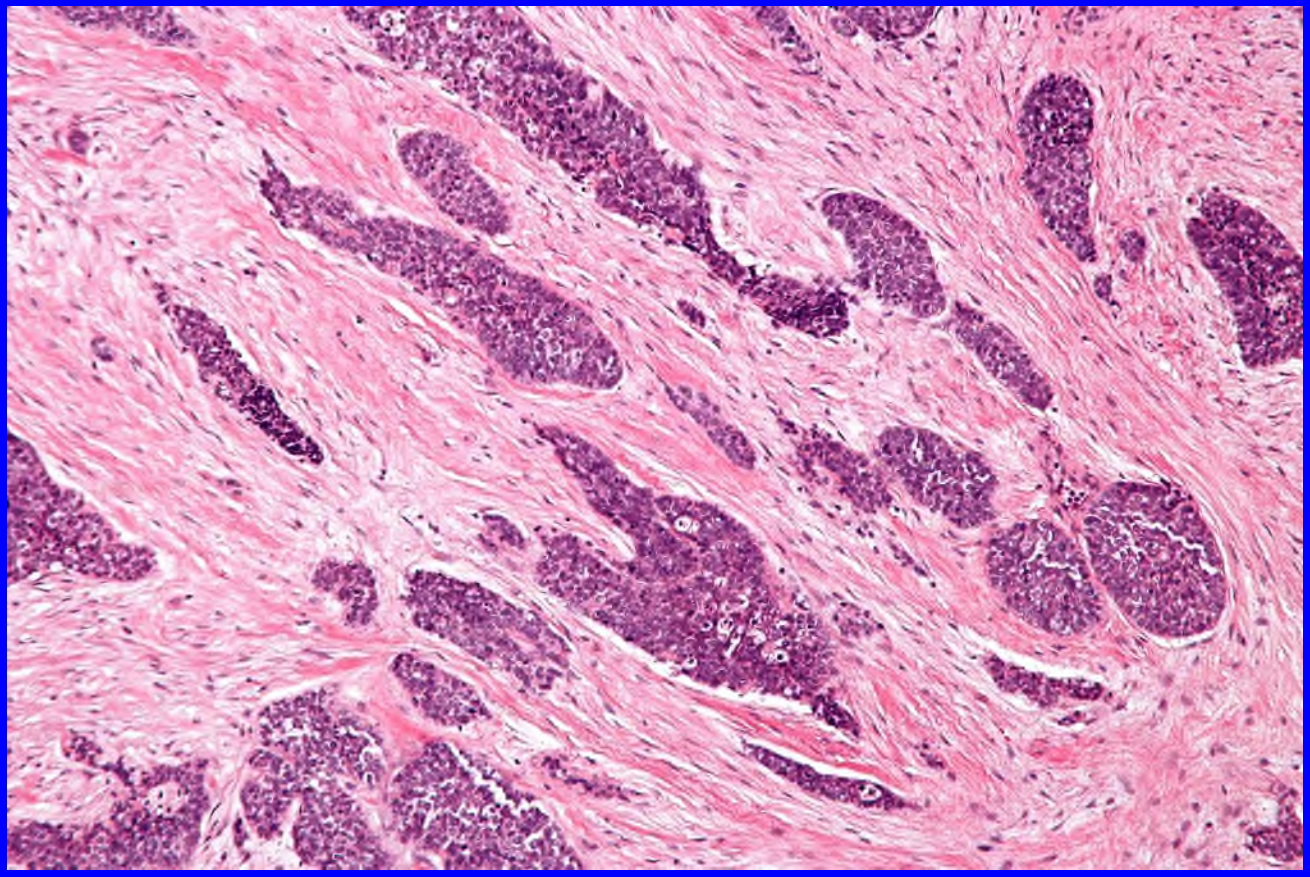
.NSE +ve in 84% Keratin +ve in 87%

.NKX2.2 +ve in 1/5 cases(Hung YP Mod Path 2016;29:370)

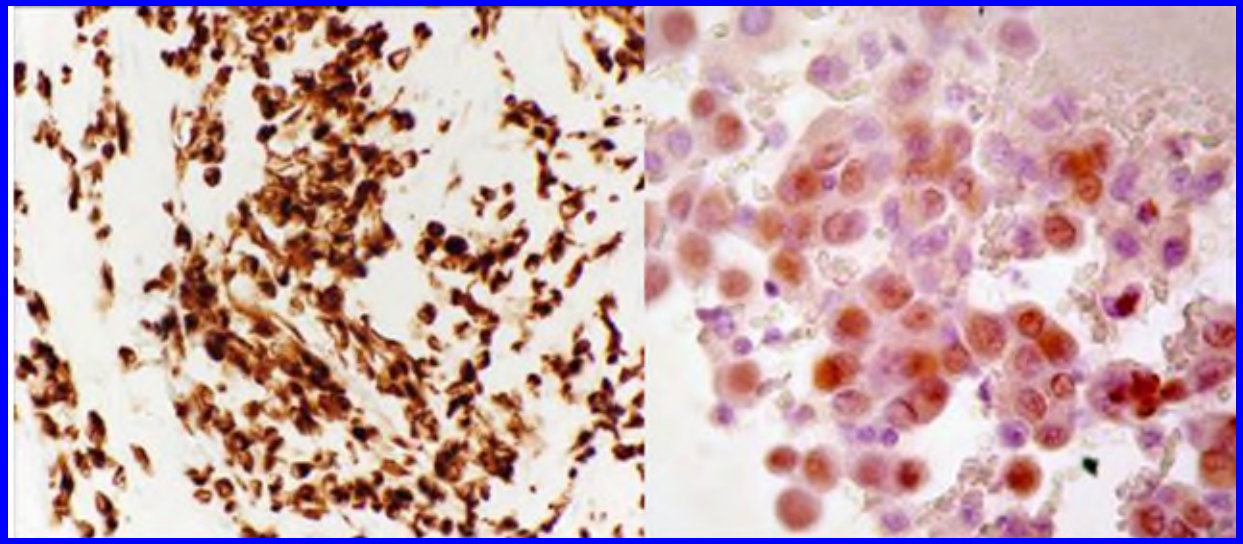
WT1+ only for C terminus Ab (Arnold MA AJSP 2014;38:220): not readily available (WT1 both types -ve in EWS-PATZ1 sarc)

Polyphenotypic ICC profile helpful (correlation with site & desmoplasia: D-SRCT, No desmoplasia in Renal D-SRCT in children)

D-SRCT:islands of tumor cells and fibro-collagenous stroma



Wang LL 2021:C-terminus WT1: Nucl staining + (Histology & Cytology) in D-SRCT



EESFT:DX (Two EEFTs with overlap of polyphenotypic ICC profile)

.EWS-PATZ1 fusion sarcoma & D-SRCT

CD99 can be +ve in both, Desmin & NSE +ve in both

Keratin: +ve in D-SRCT,-ve in most EWS-PATZ1 cases

WT1:C-terminus nuclear +ve in D-SRCT(WT1 both types: –ve in EWS-PATZ1 fusion sarcoma

Site (abdominal in D-SRCT, ST in EWS-PATZ1) and histology (Fibrous septa around nests of primarily RCs helpful in D-SRCT-septa absent in renal D-SRCT & myxoid/fibrous stroma in EWS-PATZ1 can mimic that in Ds-SRCT- Davis 2020)

EESFT:DX (Two EEFTs with overlap of polyphenotypic ICC profile)

.EWS-PATZ1 fusion sarcoma & D-SRCT

Clear DD can be difficult in certain cases, but combination of primary site, histology, Keratin positivity in D-SRCT helpful in most cases.

Request test for genetic marker of more likely Dx

EESFT:DX (Positive results: ICC profile supporting Dx of CIC Rearrngd sarcoma)

Antonescu 2017 :115 cases

.CD99:Membranous/cytoplasmic +ve (84%)(diffuse in 23%, multi/focal in 61%)

.WT1 (N terminus):Diffuse nucl in 74% (48/65 cases tested)

Hung 2016 9:40 cases:

.ETV4:+ve (nucl in 90% cases & in 5% of 150 other RCSs) (Specificity/sensitivity 95/90%)-ETV of particular significance

.WT1 +ve (nucl in 95% & 19% of 189 other RCs) (Spec/sensity 95/81%) (Hung YP Mod Path 2016;29:1324)

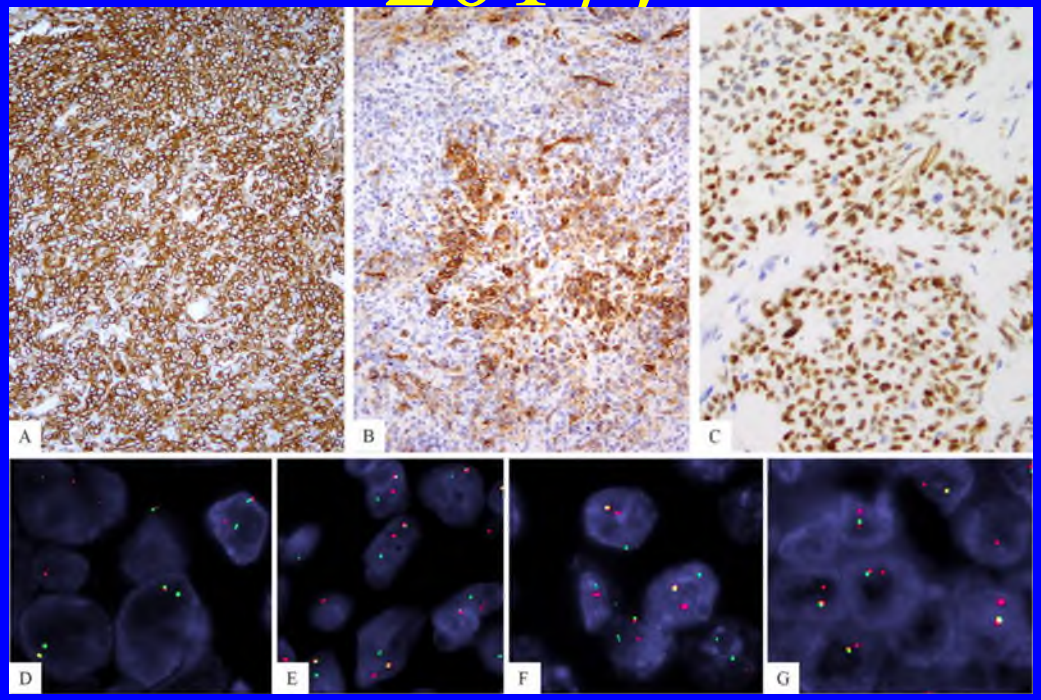
.AE1/AE3 & Desmin can be +ve (Antonescu 2017)

EESFT:DX (Positive results: ICC profile supporting CIC Rearrgd sarcoma)

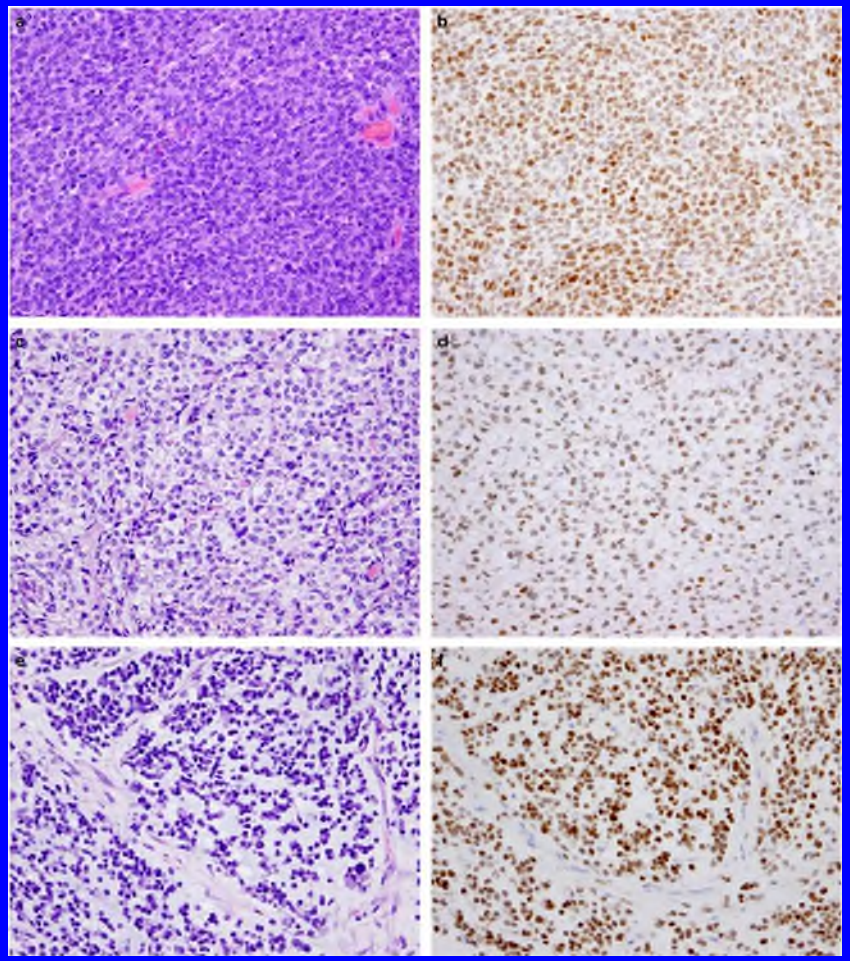
.ETV4 & WT1 both +ve (85% cases of CIC Rearrgd sarcomas) (Hung 2016)

.DUX4:+ve (100% specific & sensitive in 5 cases tested,-ve all 76 cases of other RCSs - Siegele B AJSP 2017;41:423)

CIC rearrg:A,B-diff/focal CD99,membr;C-WT1 nucl,D,E-fused CIC-DUX4 (Antonescu 2017)

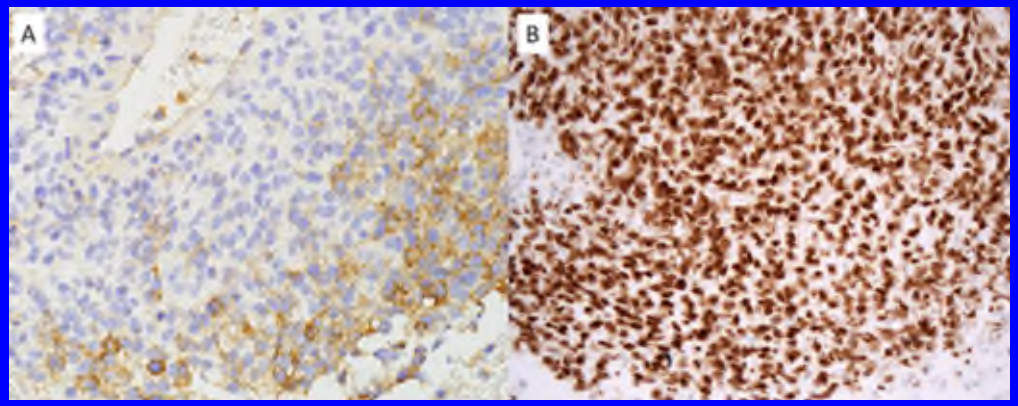


CIC Rearrgd sarc(Hung YP Mod Path 2016;29:1324)



- (a,c,e) CIC Rearrngd sarc; H&E (RCs + in c/e: myxoid stroma)
- (b,d,f) CIC Rearrgd sarc: ETV4-Diff nucl

Chebib I Cancer Cytopath 2016;124:350 CIC rearrng sarc:A-Focal cytoplasm CD99+,B-diffuse,nuclear WT1+



EESFT:DX (Clues from NEXT ICC profile supporting Dx of CIC Rearrngd sarcoma)

Negative result (Carter S Surg Path 2019;12:191):

TLE1

Myogenin

S100

BCOR

CCNB3

NKX2.2

PAX7

EESFT:DX (ICC profile supporting CIC Rearrgd sarcoma)

.WT1 & ETV4 both +ve (85% cases of CIC Rearrgd sarcomas) (Hung 2016)

.DUX4:+ve (100% specific & sensitive in 5 cases tested,-ve all 76 cases of other RCSs - Siegele B AJSP 2017;41:423)

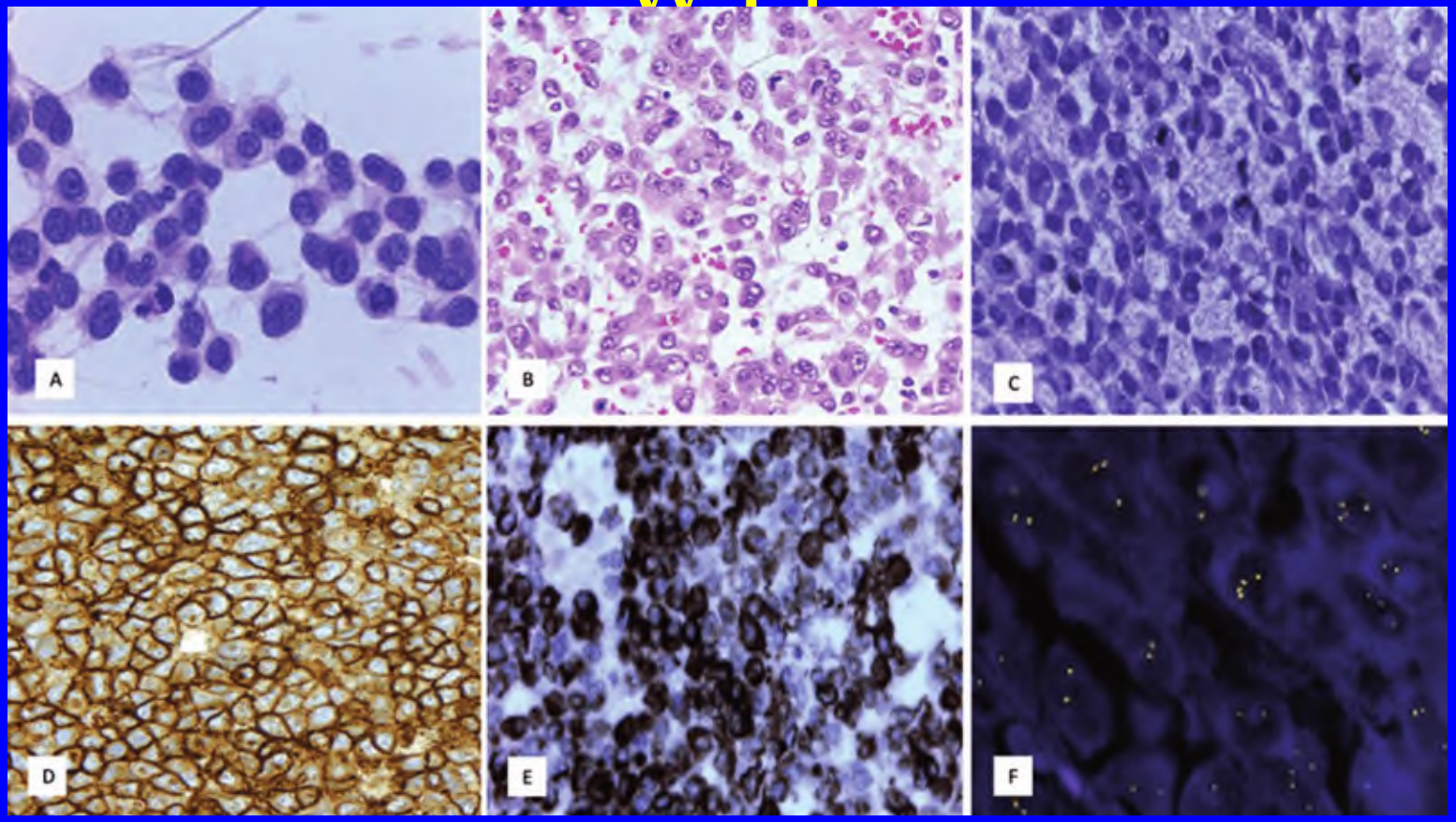
.CD99: membr/cytoplasm + ve in 84% cases (Antonescu 2017)

.TLE1,BCOR, CCNB3: -ve

Profile supportive of CIC Rearrgd sacrc (correlate with histology :atypia,high MR-mean 30/10 hpf,>10 in most,

Focal rhabdoid change).

CIC rearg sarc-Machado I:Ann Dx Path2016;22:25 Rhabd,CD99, WT1



CIC rearrg sarc (ICC marker of particular importance-ETV4)

.ETV4 nucl +ve irrespective of fusion partner,

.Underperformance (false -ve in 20-40% cases) of RT-PCR,FISH & sequencing for demonstration of CIC rearrangement (Davis 2020)

.ETV4 by ICC/ISH- surrogate marker for CIC rearg sarcoma cases with false -ve CIC rearrg

CIC rearrg sarc (ICC Marker of particular importance:ETV4)

.ETV >expression in 1 unclass RCS shown to have CIC rearrag on further investigation by break-apart FISH (Smith 2016)

.Shrinking subset of unclassified RCS due to demonstration of known markers by alternative method(s) and ?discovery of new fusions in future

EEFTs:Dx (Condensed panel for ICC profiles for EEFTs)

.Practical guideline for conserving resources: Panel (15-18 Abs from >25 reported in lit)

CD99 NKX2.2 BCOR CCNB3 WT1

ETV4 TLE1 AGGRECAN

NEURAL (NSE,SYNAPTPH), MUSCLE (MYOGEN,MYOD1)& EPITHELIAL (CK,HMWCK) MARKERS:2-3 OF EACH

SS18-SSX

(MORE WITH REPORTS OF NEW ONES)

Condensed ICC panels in EEFTs for supporting working histologic Dx

.CD99, NKX2.2, (ES);

.BCOR,CCNB3,TLE1, (BCOR Rearrgd);

.Aggrecan, EMA, Keratin (EWS-NFATC2);

.Myogenin, Desmin, NSE, Synaptophysin, (EWS-PATZ1);

.WT1(C terminus Ab) , Keratin (D-SRCT);

.ETV4,WT1(N term Ab): (CIC rearrgd)

(?Request stains useful for supporting working histologic Dx to conserve resources)

EEFTs:Dx (Clues from ICC profile)

Conserving resources with cautious optimism: choice related to available stains&type of case

?Also request stains with mostly -ve result
ES:WT1, BCOR

BCOR sarc:WT1,SS18-SSX

EWS-NFATC2:WT1 EWS-PATZ1:EMA

D-SRCT:WT1 (N terminus)

CIC sarc: TLE1,BCOR

EEFTs:Dx (Clues from ICC profile)

No working histologic Dx in a given case:

Request the most helpful stains for each entity as indicated previously.

(If ICC also is not helpful – general NGS +ve for known entity, new marker, no marker- Undifferentiated unclassified sarcoma)

Condensed ICC panels in EEFTs for supporting working histologic Dx

.CD99, NKX2.2, (ES);

.BCOR,CCNB3,TLE1, (BCOR Rearrgd);

.Aggrecan, EMA, Keratin (EWS-NFATC2);

.Myogenin, Desmin, NSE, Synaptophysin, (EWS-PATZ1);

.WT1(C terminus Ab) , Keratin (D-SRCT);

.ETV4,WT1(N term Ab): (CIC rearrgd)

(?Request stains useful for supporting working histologic Dx to conserve resources)

EEFTs:Dx (Clues from ICC profile)

Conserving resources with cautious optimism: choice related to available stains & type of case

Targeted ICC profiles can be supportive of histologic working Dx in most cases enabling targeted test for genetic marker

EEFTs:Dx

- MAKING A WORKING DX ON THE BASIS OF CLUES FROM REVIEW OF CLINICAL & HISTOLOGIC DATA & OF ICC PROFILE
- NEXT STEP IN APPROACH TO DX:

DEMONSTRATION OF MOLECULAR GENETIC MARKER FOR DEFINITIVE DX TO CONFIRM THE WORKING DX

EEFTs in children: bone, soft tissue (rarely viscera):Markers,

1.ES (FET-ETS fusion) : prototype, commonest
2.Round cell sarcomas (FET-Non ETS fusion)- two subsets: a. EWS/FUS-NFATC2 fusion
b.EWS-PATZ1 fusion

3. Desmoplastic SRCT:EWS-WT1 fusion (Desmoplasia absent in renal D-SRCT – Wang L AJSP 2007; 31:576)
4. BCOR rearranged sarcomas: 4 subsets

EEFTs

5. CIC rearranged sarcomas (CIC-DUX4 in 95%,DUX10/NUTM1/NUTM2A/FOXO4/LEUTX/DUX4L in rare cases) commonest non-ES RCS in adults.

EEFTs

4&5-BCOR & CIC rearrg sarc (Undiff RC features + Non FET-Non ETS fusion) hence distinct entities (cf-ES),

but included in Extended EFTs - RCs, undifferentiated by morphology

EEFTs (subsets of BCOR rearranged sarcomas)

- 4a. BCOR fusion sarc – partners-CCNB3 (90%),MAML3,ZC3H7B, KM2D
- 4b. ST Sarcomas with BCOR-ITD (internal tandem duplication of exon 16) referred to as Primitive Myxoid Mesenchymal Tumor of Infancy and/or Infantile Undiff RC sarc with BCOR-ITD
- 4c. CCSK (BCOR-ITD ~90%) (BCOR-CCNB3 or YWHAE-NUTZ2B remaining cases
- 4d. Endom stromal sarcomas, CNS neuroepithelial tumor (beyond scope of this presentation)

EEFTs

6.Undifferentiated (unclassified) sarcomas with no distinctive genetic markers or ICC profiles: mostly soft tissues, occur at all ages(WHO 2020):

Histologic subsets: Round, Spindle, Pleomorphic & Epithelioid cell.

Loss of SMARCA4 , SMARCB1 in isolated cases of undifferentiated (unclassified) sarcomas

EEFTs: Details of gene fusions in ES

- Fusion btw gene of FET/TET family (EWSR1, FUS/TLS, TAF15) and a gene from ETS family (FLI1, ERG, E1AF, ETV1, FEV): EWS-FLI1 (85%), ERG (10%), ETV1, E1AF, FEV (each <1%), FUS/TLS-ERG (<1%) (ETS family has 27 members- Riggi 2021)
- Gold standard: molecular genetic marker (EWS-FLI1 fusion or other fusions above)
- Gene fusion type not associated with histologic subtype or behavior

EEFTs:Dx:ES-Chromosomal Translocations/Fusion transcripts

- ES: t(11;22)(q24;q12):EWS-FLI1 (85%)
- t(21;22)(q12;q12):EWS-ERG (10%)
- t(2;22)(q33;q12):EWS-FEV (<1%)
- t(7;22)(q22;q12):EWS-ETV1 (<1%)
- t(17;22)(q12;q12):EWS-E1AF (<1%)
- t(16;21)(p11;q22):FUS-ERG (<1%)
- t(2;16)(q35;p11):FUS-FEV (<1%)

EEFTs:Dx: Chromosomal abnormalities/fusion transcripts

.RCS: t(20;22)(q13;q12):EWS-NFATC2,

t(16;20)(p11;q13):FUS-NFATC2

*Inversion(22q12):EWS-PATZ1(very close proximity of genes on chromosome 22 :<sensitivity of FISH test)

.D-SRCT:t(11;22)(q13;q12):EWS/N terminus - WT1/C terminus) fusion* (WT1 ICC +ve only for C terminus Ab)

EEFTs:Dx: Chromosomal abnormalities/fusion transcripts

.CIC rearrg:t(4;19)(q35;q13)-CIC-DUX4

.BCOR-CCNB3:(Inversion of short arm X resulting in fusion of nearby genes)

a)BCOR-ITD (Exon16):No transl/inversion

EEFTs:Molecular genetic marker-GOLD STANDARD

.Methods: FISH,RT-PCR,DNA-RNA NGS

.Quantity & quality: Material to be submitted

.Fresh snap frozen: best choice

.Sections from FFPE tissue (Neutral, buffered, nonrecycled formalin avoiding long fixation): acceptable (retrospective studies in EEFTs)

EEFTs:Approach to Dx-NGS

- Type of sample for whole genome vs targeted NGS

- High mol weight DNA from fresh tissue required for whole genome sequencing
- Low mol weight DNA from FFPE tissue (unrecycled buffered neutral formalin) acceptable for targeted sequencing

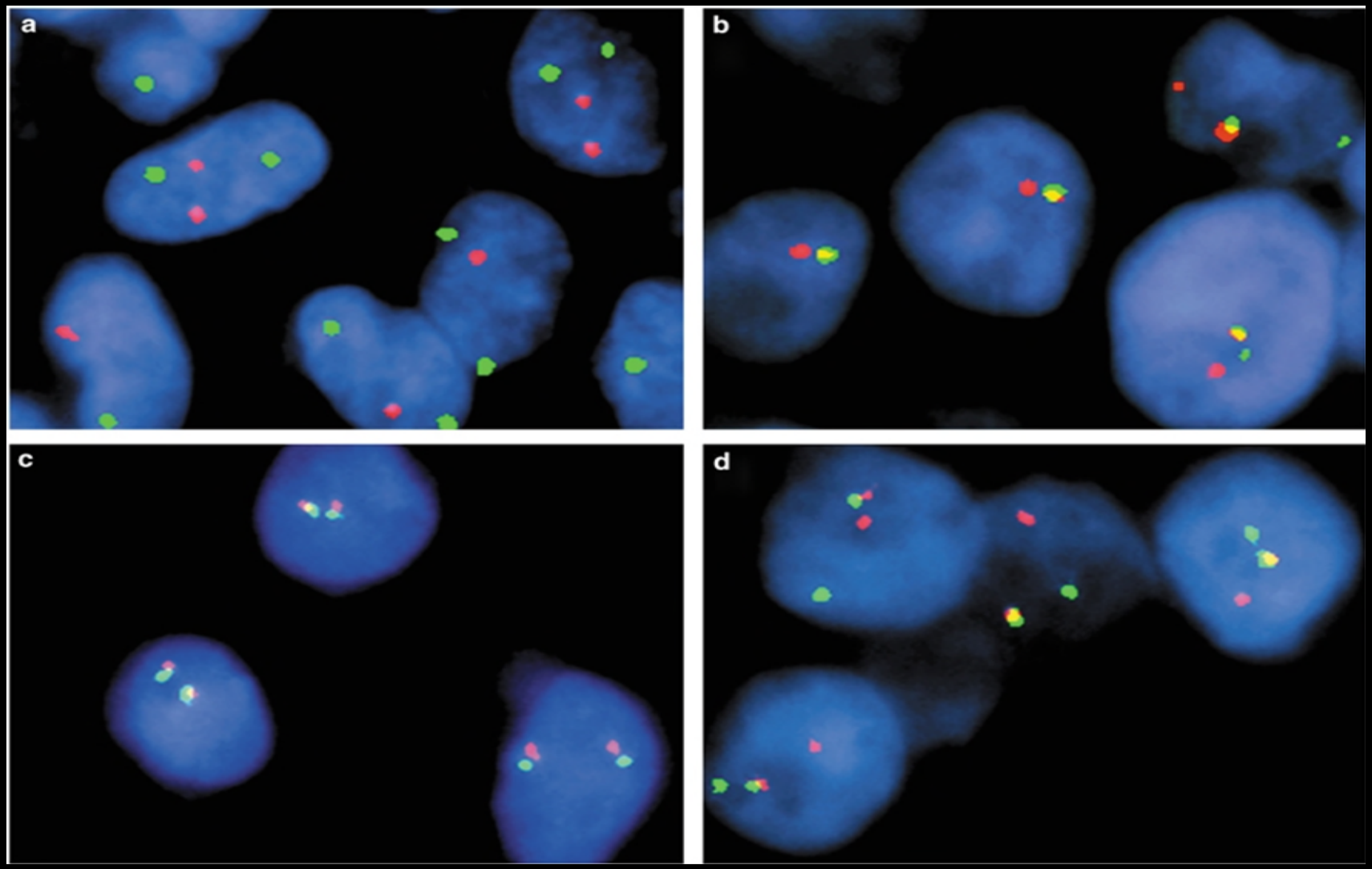
ADVANTAGES OF FISH TECHNIQUE

- FISH: Small sample: imprint/FNA smear or sections from BUFFERED-formalin fixed tissue.
- Sensitive and specific
- Short turnaround time (1-3 days)
- Moleculo-morphologic test:visualization of marker
- Suitable for resource-deficient labs

(Limitation:Targeted approach with limited no. of commercially available probes)

NEXT FIGURE

Figure 1 : Bridge RS Molecular diagnosis of ES/PNET in routinely processed tissue: Modern Pathology 2006;19:1



- Representative FISH results. (**a**) FISH-F-negative case of URCS with split EWS (red) and FLI1 (green) signals. (**b**) FISH-F-positive EWS/PNET case with fused signals. (**c**) FISH-BA-negative case of small cell carcinoma with normal fused EWS (centromeric red; telomeric green) signals. (**d**) FISH-BA-positive EWS/PNET case with split signals in most nuclei.

GENETIC MARKERS IN EEFTs

- FALSE NEGATIVE RESULTS
- Reduced sensitivity of FISH method in EWS-PATZ1 RCS due to proximity of gene partners on chromosome 22 .

Underperformance of FISH/RT-PCR/NGS in 20-40% cases of CIC Rearrgd Sarc

ES with uncommon/rare fusion partners

EEFTs: Tests for genetic marker

.Unavailability of FISH

.RT-PCR , the next choice

EFFECT OF FIXATIVE ON RT-PCR PRODCUTS (GUILLOU L: HUM PATHOL 2001; 32:105)

- RNA extraction from paraffin blocks in 64 cases of synovial sarcoma for SYT – SSX gene fusion transcripts.
- RT-PCR products in 91.5% tumors fixed in buffered formalin.
- Products in 0% tumors fixed in Bouin's fixative.

EEFTs: Tests for genetic marker for each entity-Davis JL Curr Treat Options in Oncolgy 2020;21:90

.ES: FISH or RT-PCR (If -ve due to uncommon fusion partners: RNA based NGS)

.BCOR Rearrgd sarc: a)FISH or RT-PCR, RNA based NGS for uncommon fusion partners b) DNA NGS for BCOR-ITD

.EWS-NFATC2: RNA based NGS reqd

.EWS-PATZ1: Reduced sensitivity of FISH due to close proximity of fusion partners –

Negative result for common genetic marker for ES in RC sarc

- Rare ES genetic marker (e.g.EWS-ETV1)
- Alternative Dx on histologic reassessment to be confirmed by genetic marker for non-ES entity in EEFTs or other SRCT
- Need for alternative method (nested RT-PCR , NGS)
- Needle bx: Inadequacy (necrosis, sclerosis, inappropriate fixation, delayed delivery)
- Inappropriate tissue handling: mol path lab

EEFTs:Dx:ES-Chromosomal Translocations/Fusion transcripts

- ES:1. t(11;22)(q24;q12):EWS-FLI1 (85%)
- 2. t(21;22)(q12;q12):EWS-ERG (10%)
- 3. t(2;22)(q33;q12):EWS-FEV (<1%)
- 4. t(7;22)(q22;q12):EWS-ETV1 (<1%)
- 5. t(17;22)(q12;q12):EWS-E1AF (<1%)
- 6. t(16;21)(p11;q22):FUS-ERG (<1%)
- 7. t(2;16)(q35;p11):FUS-FEV (<1%)
- Seek transcripts 3 to 7 in a case -ve for 1&2

EEFTs: Tests for genetic marker for each entity (Davis JL Curr Treat Options in Oncolgy 2020;21:90

.D-SRCT: FISH, RT-PCR

.CIC Rearrgd sarc: Positive result by FISH,RT-PCR or DNA/RNA based NGS diagnostic , but false -ve results by FISH,RT-PCR & DNA/RNA based NGS in 20-40% cases :ETV4 & WT1 ICC particularly helpful in such cases (ETV4 surrogate ICC marker)

Next Generation Sequencing

.Advantages:

Only small amount of material reqd

Most definitive test with availability of appropriate material

.Disadvantages:

High cost ($ 2-3 K)

Long turnaround time (In house 10 days, reference lab 2-3 weeks)

EEFTs: Biologic relevance of genes involved in fusions & ICC profiles

. EWS;ETS family-FLI1,ERG,FEV;NonETS Family(NFATC2,PATZ1,SP3),FUS,CIC, DUX4,CCNB3,BCOR etc.

.ICC:gene overexpression(e.g.FLI1,BCOR)

- All genes Involved in one or more of the following: embryonic development, cell proliferation, differentiation, transcriptional repression

EEFTs:ES-Pathobiologic &Clinical relevance of fusion transcript-Riggi N 2021

- Molecular genetic markers: Not just Gold Standard for Dx of EEFTs, but also of pathobiologic & clinical relevance

- Genetic marker of ES being explored/studied for its possible significance in
- a)Tumorigenesis of ES and
- b)Designing targeted Rx

EEFTs:ES-Pathobiologic &Clinical relevance of fusion transcript-Riggi N 2021

- EWS-FLI1 is an aberrant transcription factor (transcription of genetic info from DNA to mRNA) for inducing expression of genes with oncogenic properties
- EWS & ETS factors are implicated in DNA repair, cell differentiation & cell cycle control
- Role in tumorigenesis: Formation of ES-like tumors after injecting EWS-FLI1 expressing NIH3T3 cells into SCID mice (Thompson AD Oncogene 1999;18:5506)

EEFTs:Pathobiologic &Clinical relevance of fusion transcripts-Riggi N 2021;Antonescu 2017;WHO 2020

- Oncogenic action of ES fusion transcript also related to epigenetic mechanisms such as modification of chromatin architecture to allow access of genomic DNA to regulatory transcription proteins (chromatin packages long DNA into compact structure)

- EWS-FLI1 fusion transcript is considered to play a primary role in ES tumorigenesis.

- Role in tumorigenesis of DUX4 dysregulation in the setting of its fusion with CIC or upregulation of BCOR in BCOR-ITD sarcomas is poorly defined.

EEFTs:ES-Pathobiologic &Clinical relevance of fusion transcript-Riggi N 2021

- Clinical relevance of ES fusion transcript
- Disordered structure & lack of enzymatic activity of fusion transcript do not lend themselves to targeted Rx
- Targeted Rx to be directed to epigenetic mechanisms of action of FET-ETS fusion transcripts
- Novel approach to Rx being explored

EEFTs:ES-Histogenesis (Riggi 2021)

- ES is a paradigm for solid tumor development after a single genetic arrangement.
- Mesenchymal stem cell: possible cell of origin which provides favorable ‘soil’ required for inducing transformation (Riggi N Cancer Res 2005;65:11459)
- ES may also arise from neural crest cells (von Lovetzow C PLoS One 2011;6:e19305) or other pluripotent cells

EEFTs:Dx of Established SRCTs

CRUCIAL IMPORTANCE OF ACCURATE SPECIFIC Dx:

SPECIFIC Rx CAN BE GIVEN

THERAPY OF SRCTs

- NB: CISPLATIN (C), VP-16, CYTOXAN (CX), ADRIAMYCIN (A)
- PNE: VINCRISTINE (V), ACTINOMYCIN (AC), IFOSFAMIDE (I), VP-16, CX, PLATINUM
- ES: SAME AS PNE EXCEPT PLATINUM
- RMS: V, DACTINOMYCIN, CX, VP-16, C
- NHL: CX, A, V, PREDNISONE, METHOTREXATE

EEFTs:ES-Pathobiologic &Clinical relevance of fusion transcript-Riggi N 2021

- ES being treated currently by variable combinations of ChemoRx (doxorubicin,etoposide,vincristine etc),surgery & radiation (Davis 2020).
- Non-ES tumors of family being given same Rx as for ES - variable survival rates: ES 76%,D-SRCT 15-30%,BCOR fusion & BCOR-ITD 72-90%,CIC rearrng 44%,EWS-NFATC2 RCS 90%,EWS-PATZ1-No data
- Specific Dx helpful in prediction of prognosis in the case being signed out.

Summary:SRCTS & EEFTs in children:Steps in Dx

.Assumption for this presentation: Non-EEFTs in the broad group of established SRCTs are ruled out

.Proper triage of needle core bx

.Collect data on the case for clues: clinical, histologic (& EM) features: Working Dx (ES or type of Non-ES)

.Request ICC markers to strengthen the working histologic Dx

Summary:SRCTS & EEFTs in children:Steps in Dx

.Correlate the features from each source with those of other sources (Clinical data Histology, ICC profile) for firm working Dx

.Request targeted tests for genetic marker to confirm the firm working Dx

.Pre-established access to reference lab reqd

Basic articles on ES & Approach to Dx of SRCTs in Children

1. Kissane JM, Askin FB, Foulkes M et al Ewing's Sarcoma of Bone-Clinicopathologic study of 303 cases from Intergroup Ewing's Sarcoma Study Hum Path 1983;14:773

2.Shimada H, Newton WA, Soule EH et al Pathologic features of Extraosseous Ewing's Sarcoma- A report from Intergroup Rhabdomyosarcoma study Hum Path 1988;19:442

3.Dickman P Ewing's Sarcoma/Primitive Neuroectodermal Tumor Path Case Rev 2000;5:60

4.Joshi VV, Balarezo F, Hicks MJ Approach to SRCTs of Childhood Path Case Reviews 2000;5:26

Recent & Key Publications on EEFT (only first author given)

1. Antonescu C. Round cells sarcomas Histopathology 2014;64:26
2. Carter CS Non-Ewing Small Round Cell Tumors Surgical Pathol 2019;12:191
3. WHO Editorial Board WHO Classification of Soft Tissue and Bone 5th Edition, 2020
4. Davis JL Small Round Blue Cell Sarcoma other than Ewing Sarcoma Current Treat Options in Oncol 2020;21:90
5. Riggi N. Ewing's Sarcoma NEJM 2021;384:154
6. Miettinen M New fusion sarcomas Hum Path 2019;86:57

Recent & Key Publications on EEFT (Only first authors given)

7. Parham D Fibroblastic and myofibroblastic tumors in children F1000Res 2018;7:F1000 Rev-1963

8. Parham D Modern Dx of small cell malignancies in children Surg Path 2010;3:515

9. Rajwanshi A Malignant small round cell tumors J Cytol 2009;26:1

10. Kim NR Utility of EM in small round cell tumors

11. Folpe A Diversity in Ewing Family Tumors AJSP 2005;29:1025

12. Schmidt D PNET and its distinction from Ewing's sarcoma Cancer 1991;68:2251.