Soft Tissue Tumors

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Frequently Asked Questions

1. **What is the role of molecular testing in undifferentiated round cell sarcomas?**
   - The differential for undifferentiated round cell sarcomas is broad. In fact, there are so many that the use of mnemonics is often employed just to remember the differential (Table 10.1). The role of molecular is to provide a definitive diagnosis on limited tissue samples to ensure appropriate treatment (e.g., neoadjuvant chemotherapy) and prognostic data when applicable.
   - Molecular testing is often considered an ancillary technique after an initial round of sorting with immunohistochemical stains (lymphoma/leukemia versus Ewing versus Rhabdomyosarcoma); however, many tumors can have overlapping histologic and immunohistochemical features, and in these cases the use of molecular testing is invaluable.
2. **How are undifferentiated round cell sarcomas classified? What are the most common fusions?**
   - Undifferentiated round cell sarcomas are classified as Ewing sarcoma, round cell sarcoma with *EWSR1*-

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non-ETS fusions, *CIC*-rearranged sarcoma, and sarcoma with *BCOR* genetic alterations [1] (Table 10.2).

- Ewing sarcoma (EWS) is the prototypical small round cell sarcoma that involves *EWSR1* on 22q12 with members of the ETS (E-26 transformation specific) transcription factors creating *EWSR1-ETS* fusions.
  - Most commonly involved fusion involves *FLI1* [2] on 11q24 in ~85% of cases followed by *ERG* on 21q22 in ~10% of cases [3].
  - Other less common fusions involved *ETV1* (ETS-variant gene 1) on 7p22 [4], *ETV4* (*ETS*-variant gene 4) on 17q12 [5], and *FEV* (fifth Ewing sarcoma variant) on 2q33 [6].
- Because *EWSR1* is a member of FET family, other members including *FUS* (Fused in Sarcoma) can rarely substitute for *EWSR1* creating FET/ETS fusions.
  - Known fusions include *FUS-ERG* and *FUS-FEV* [7, 8].
  - *TAF15* other member of FET family could, in theory, substitute for *FUS* or *EWSR1*.
- Round cell sarcoma with EWSR1-non-ETS fusions.
  - *EWSR1-NFATC2* and *EWSR1-PATZ1* tend to be not respond as well to neoadjuvant chemotherapy [9].
  - DNA methylations studies confirm distinct profiling from *EWSR1*-ETS and *CIC* or *BCOR* rearranged sarcomas [10, 11].
- *CIC*-rearranged sarcomas account for the vast majority of "Ewing-like" sarcomas.
  - Most commonly involve *CIC-DUX4* but other partners include *FOXO4* [12, 13], *NUTM1* [14], and *NUTM2A* [15].
  - Can also show trisomy 8 and *MYC* amplifications [16].
  - *CIC-LEUTX* can be seen as a subset of angiosarcomas [17].
  - Not chemosensitive like EWS and have a worse overall survival [18].

    Tend to affect the deep soft tissue of the trunk and lower extremities of young adults (third to fourth decade).

    Exception are the *CIC-NUTM1* fusion which involves the bones of young children [14].
  - Potential pitfall.

    A subset of angiosarcomas can show *CIC* gene abnormalities, most often mutations, but rearrangements have also been reported [17].

Table 10.1 Differential of small round cell tumors

Mnemonic	Tumor
M	Melanoma
R	Rhabdomyosarcoma (alveolar subtype)
S	Synovial sarcoma
L	Lymphoma/leukemia
E	Ewing sarcoma and other undifferentiated round cell tumors (BCOR, CIC, etc.)
M	Merkel cell carcinoma
O	Olfactory neuroblastoma
N	Neuroblastoma
S	Small cell carcinoma

Table 10.2 Undifferentiated round cell sarcomas

WHO terminology	Age	Sex	Location	Fusion(s)	IHC	Other
Ewing sarcoma	Peak incidence in second decade Older patients tend to be extraskeletal	M > F	Bones (long bones, pelvis, ribs) >> > soft tissue	*EWSR1-FLI* (~85%) *EWSR1-ERG* (~10%)	CD99 (strong, membranous), NKX2.2, FLI1 and ERG depending on fusion	Small % involves *FUS* from FET family) to other ETS genes (*ETV1*, *ETV4*, *FEV*)
Round cell sarcoma with *EWSR1*-non-ETS-fusions	Median age = fourth decade (*NFATC2*) Mean age = fifth decade (*PATZ1*)	M >> > F (*NFATC2*) M = F (*PATZ1*)	Long bones (*NFATC2*) Soft tissue (*PATZ1*)	*EWSR1-NFATC2* *FUS-NFATC2* *EWSR1-PATZ1*	CD99 (50%), NKX2.2 and focal AE1/AE3 (*NFATC2*) Co-expression of myogenic and neural markers (*PATZ1*)	*EWSR1-NFATC2* often shows concurrent amplification of 5′ probe by break-apart FISH
CIC-rearranged sarcoma	Median = third and fourth decades	M > F	Trunk and lower extremities deep soft tissue	CIC-DUX4 (95%)	CD99, ETV4, DUX4, WT1	Small % involves fusion to *FOXO4*, *LEUTX*, *NUTM1*, and *NUTM2* Trisomy 8 with *MYC* amplifications
Sarcoma with *BCOR* genetic alterations	Most common in first-second decade (>90%)	M >> > F	Bone > soft tissue	*BCOR-CCNB3* *BCOR*-ITD	CD99 (50%), BCOR, cyclin D1, SATB2, TLE1	Rare fusions include *BCOR-MAML3* and *BCOR-ZC3H7B*

IHC immunohistochemistry, *M* male, *F* female

- *BCOR*-rearranged sarcoma.
  - Most frequent is *BCOR-CCNB3*; but others include internal tandem duplication (*BCOR*-ITD), *KMT2D-BCOR*, *BCOR-ZC3H7B*, and *BCOR-MAML3* [19, 20].
  - Tend to affect the bones of child/adolescents (80% in first two decades) [21, 22].
  - More common in males and have a similar overall survival compared to EWS [19].

3. **Is FISH testing for Ewing sarcoma and other undifferentiated round cell sarcomas enough? What is the role of next-generation sequencing (NGS) in Ewing sarcoma and other undifferentiated small round cell sarcomas?**
   - FISH testing, primarily through break-apart assays, is no longer considered enough by most soft tissue pathologists. This is in part due to some overlapping histology and immunohistochemistry with non-ETS fusions and other round cell sarcomas with specific gene rearrangements that can have a different prognosis (see below). The role of NGS is to identify specific gene fusions that may dictate treatment and prognosis.
   - *EWSR1* break-apart FISH testing can sometimes create false positives in non-EWS tumors [23].
     - Often with tumors showing concurrent *SMARCB1* deletions since genes are located only 5.5 Mb from each other.
   - Around 50% of previously diagnosed undifferentiated round cell sarcomas, most commonly defined as negative for *EWSR1* rearrangement by FISH, have disease defining fusions [24–26], most commonly *CIC* and *BCOR*.
   - Because both *EWSR1* and *PATZ1* are located on the same chromosome and are ~2 mb away from each other, a FISH break-apart probe may result in a false negative due to the short distance between the inversion of the involved genes.
   - A subset of *CIC*-rearranged sarcomas may be missed using FISH as opposed to NGS testing [27–29].
     - Exact reason for false negatives is unknown, but it may be due to cryptic insertions beyond the resolution of the FISH assay.
4. **What are some of the limitations for using NGS on undifferentiated round cell sarcomas?**
   - Occasionally, *CIC*-rearranged sarcomas can be missed on RNA-based sequencing and this may be related to a failure of algorithmic analysis [29].
     - This may be in part due to repetitive sequences that can be seen with *DUX4* on chromosomes 4q35.2 and 10q26.3 and are filtered out by algorithms.
     - Use of "Grep" command may help detect fusion when missed by other programs such as FusionMap, FusionFinder, and ChimeraScan programs [30].
   - There is still a small subset of undifferentiated round cell sarcomas that lack an identifiable fusion. These cases may benefit from array-based DNA-methylation profiling to determine if they cluster with a known group (e.g., *CIC*, *BCOR*) or if it will change management (i.e., use of neoadjuvant chemotherapy).
5. **What is the role of molecular testing in adipocytic tumors?**
   - Many adipocytic neoplasms have specific molecular mutations or rearrangements (Table 10.3). The primary role for molecular testing is to confirm or establish the diagnosis in problematic situations (see below).
6. **What is the sensitivity of *MDM2* FISH for the diagnosis of ALT/WDL? What is the benefit of using FISH testing in problematic lipomatous tumors?**
   - Atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL) is defined by the amplification

Table 10.3 Alterations in lipomatous neoplasms

Neoplasm	Alteration	Molecular testing usually needed?	Role of testing if used
Lipoma	*HMGA2* rearrangements	No	Exclude WDL
Angiolipoma	*PRKD2* mutations	No	None
Hibernoma	Translocations and interstitial deletions of 11q that localize to *MEN1* and *AIP*	No	Exclude WDL
Lipoblastoma	*PLAG1* rearrangements	No	Exclude MLS and WDL
Spindle cell/ pleomorphic lipoma	*RB1* deletions	No	Exclude WDL
Chondroid lipoma	*MRTFB* rearrangement (previously called *MKL2*)	No	Exclude WDL and MLS
MLS	*DDIT3* rearrangements	Yes	For confirmation and exclude DDLS and other round cell sarcomas
WDL/DDLS	*MDM2* amplification	Yes	Exclude benign lipomatous neoplasms or confirm DDLS
Pleomorphic liposarcoma	Complex chromosomal aberrations	Yes	Exclude DDLS

WDL well-differentiated liposarcoma, *MLS*, myxoid liposarcoma, *DDLS* dedifferentiated liposarcoma

of *MDM2* originating from the region of 12q14-q15, and because of its relative increased sensitivity and specificity compared to other methods such as Q-PCR and immunohistochemistry, FISH is now considered the gold standard for diagnosis [31–33].
  - Sensitivity in literature is considered to be greater than 90% [31, 32]; however, many studies were done on non-problematic tumors.
  - Cytogenetic analysis often shows supernumerary ring and giant chromosomes that originate from the 12q14-q15 region [34].
- In problematic tumors, using the following criteria for *MDM2* FISH testing may identify up to 1/3 of cases that would otherwise be classified as lipoma [35, 36]:
  - Recurrent lipomas.
  - Tumors with equivocal cytologic atypia.
  - Retroperitoneal, intra-abdominal, and pelvic tumors.
  - Deep extremity tumors larger than 10 cm in patients over 50.

7. **When is it appropriate to use *MDM2* FISH testing in dedifferentiated liposarcoma (DDLPS)? Are there any potential pitfalls?**
   - Similar to ALT/WDLs, DDLPS are usually straightforward and do not need molecular testing if background WDL is present along with the high-grade component.
   - There are certain situations when testing is helpful, and these include:
     - When the differential includes primary retroperitoneal myxoid liposarcoma.
     - When then dedifferentiated component looks like another tumor such as myxoid liposarcoma or pleomorphic liposarcoma.
   - Potential pitfalls in dedifferentiated liposarcomas; the following situations might be misinterpreted as MDM2 amplification:
     - *STAT6*, which is associated with solitary fibrous tumors (*NAB2-STAT6* fusions) [37], is located in 12q13 and can show amplification by FISH [38] and can also show nuclear immunohistochemical staining for STAT6.
     - *DDIT3*, which is associated with myxoid liposarcoma (*FUS-DDIT3*) is located on 12q13.2, and DDLPS can show amplification of *DDIT3* in tumors that often have myxoid liposarcoma-like morphology [39].
   - Both ALTs and DDLPS can occasionally demonstrate multiple faint alphoid signals that represent satellite DNA of chromosome 12 [31].
     - This could be misinterpreted as gain of copy number.
8. **What molecular fusion is seen in myxoid liposarcoma (MLPS)? What other molecular alterations can be seen?**
   - Translocations of *FUS-DDIT3* [40] in >95% and *EWSR1-DDIT3* [41] in less than 5% are considered pathognomonic for MLPS.
     - Break-apart FISH for *DDIT3* is considered sensitive and specific [42].
   - Approximately 50% have *TERT* promoter mutations [43].
   - ~25% of PI3K/mTOR mutations, most often gain of function mutations [44].
   - Diagnosis of high-grade MLPS when >5% round cell change.
     - High-grade MLPS have higher rate of metastasis and death [45].
     - Presence of necrosis is also associated with adverse prognosis [45].
9. **What is the role of molecular testing in vascular tumors?**
   - Many vascular tumors have known mutations or rearrangements; however, molecular testing is really only performed in a handful of vascular tumors (Table 10.4),

Table 10.4 Commonly assessed molecular alterations in vascular lesions

WHO terminology	Common locations	Primary molecular alteration	Routine testing needed?	Role of testing if used
Epithelioid hemangioma	Head and neck, distal extremities, trunk	*FOS* or *FOSB* rearrangement	No but may be helpful in difficult cases	Exclude epithelioid angiosarcoma
Pseudomyogenic hemangioendothelioma	Lower and upper extremities, trunk	*SERPINE1-FOSB* and *ACTB-FOSB* rearrangements	No	Exclude epithelioid hemangioendothelioma and epithelioid sarcoma
Epithelioid hemangioendothelioma	Soft tissue, lung, liver	*WWTR1-CAMTA1* and *YAP-TFE3*	Yes and no	Exclude epithelioid angiosarcoma and also helpful if surrogate IHC for CAMTA1 not available
Secondary (radiation-induced) AS	Breast, other irradiated sites	*MYC* amplification	No	Equivocal cases where IHC may be considered false positive to exclude AVL

IHC immunohistochemistry, *AS* angiosarcoma, *AVL* atypical vascular lesion

and these are usually problematic or equivocal, borderline cases where the diagnosis is between benign and malignant and/or it will affect treatment/management.

10. **Are there any benign vascular tumors that may benefit from ancillary molecular testing?**
    - Epithelioid hemangioma is traditionally considered on spectrum and synonymous with the term angiolymphoid hyperplasia with eosinophilia (ALHE).
      - WHO no longer recommends the use of this terminology.
      - Interestingly, many cases of ALHE lack *FOS* or *FOSB* rearrangements [46].
    - Epithelioid hemangioma is characterized by recurrent fusion of *FOS* or *FOSB* genes in ~50% of cases [46].
      - FOS partners include *LMNA*, *MBNL1*, *VIM*, and l*incRNA* [47, 48].
      - FOSB partners include *ZFP36*, *WWTR1*, or *ACTB* [47, 49].
    - Molecular testing may be beneficial in cases that are referred to as atypical epithelioid hemangiomas [50], which can show some features such as solid growth and necrosis that would raise the differential of epithelioid angiosarcoma.
11. **What intermediate or low-grade vascular neoplasms are often confirmed with the use of molecular testing?**
    - Pseudomyogenic hemangioendothelioma (PMHE).
      - When originally described, it was called epithelioid sarcoma-like hemangioendothelioma [51].
      - Molecular testing can be helpful because the tumor is often confused for other entities such as carcinoma or an epithelioid sarcoma.
        Showing diffuse keratin (AE1/AE3) and vascular markers expression (ERG, FLI). About 50% express CD31 [52].
      - Characterized by *SERPINE1-FOSB* and *ACTB-FOSB* fusions [49, 53].
    - Epithelioid hemangioendothelioma (EHE).
      - Malignant vascular tumor that most commonly involves the somatic soft tissue but also visceral organs such as the lung and liver [54].
      - In classic cases, molecular testing is likely not needed; however, if surrogate IHC is not available or if there is a need to exclude epithelioid angiosarcoma, then molecular testing is helpful.
      - Characterized by *WWTR1-CAMTA1* fusion in >90% of cases [55] and *YAP1-TFE3* in approximately 10% of cases [56].
        CAMTA1 and TFE3 immunohistochemistry are often used as a surrogate for molecular testing [56, 57].
12. **What is the role of molecular testing in radiation-associated angiosarcoma?**
    - High-level *MYC* gene amplifications are characteristic of post-irradiation and chronic lymphedema-associated (Stewart-Treves) angiosarcoma [58].
    - Primary role of FISH testing is rule-in/-out angiosarcoma in difficult cases or when IHC is felt to represent a false positive [59].
      - ~25% of cases can show co-amplification of *FLT4* [58].
      - *FLT4* amplified lesion lack *KDR* and *PLCG1* mutations that can be seen in both secondary and primary angiosarcomas [17].
    - Potential pitfall is that a small subset of primary angiosarcomas can show both MYC overexpression by IHC and *MYC* amplification, so clinical context is needed [60].
13. **What is the role of molecular testing in skeletal muscle tumors?**
    - Molecular testing for sarcomas showing skeletal muscle differentiation (rhabdomyoblastic) is continuing to evolve. The main role of molecular testing is for confirmation of alveolar rhabdomyosarcoma and to exclude other sarcomas with specific rearrangements or in specific situations where there may be a clinical need for mutational status (e.g., *MYOD1* status in pediatric spindle cell rhabdomyosarcoma) or prognosis (e.g., congenital spindle cell rhabdomyosarcoma).
14. **What fusions are seen in alveolar rhabdomyosarcoma? What other alterations can be seen? How sensitive and specific is FISH testing and what are possible reasons for a negative result?**
    - Alveolar rhabdomyosarcoma (ARMS).
      - Second most common type of rhabdomyosarcoma [61].
      - Primitive round cell sarcoma characterized by *PAX3-FOXO1*, most commonly or *PAX7-FOXO1* fusions [62].
        Amplifications of *MYCN* and *CDK4* can often be seen in *PAX3-FOXO1* fusions [63, 64].
        Amplification of 1p36 which encompasses *PAX7* can be seen in *PAX7-FOXO1* fusions [65].
        Break-apart FISH for *FOXO1* is generally considered to be 100% specific [66] but still a subset (~15%) of ARMS that are negative.
        (a) Likely a combination of low-level fusions, cryptic fusions, and true fusion-negative cases [67].
      - *ALK* copy number gains can be seen but has not played a role in therapy [68, 69].
      - Prognosis worse than fusion-negative rhabdomyosarcoma and ERMS [70].

15. **What entities are encompassed under the umbrella term "sindle cell/sclerosing rhabdomyosarcoma"? What rearrangements and/or mutations are seen? How does this impact prognosis?**
    - Congenital spindle cell rhabdomyosarcoma.
      - Characterized by *VGLL2/NCOA2/CITED2* rearrangements.
        Fusions involve *VGLL2-CITED2, VGLL2-NCOA2, SRF-NCOA2, TEAD1-NCOA2* [71, 72].
      - Tend to present within first year and commonly involve the trunk and have a favorable prognosis.
        Exceptions are those with *MYOD1* mutations that tend to have a poor prognosis [72].
    - Spindle cell rhabdomyosarcoma with *FUS-TFCP2* and *EWSR1-TFCP2* fusions [11].
      - Can involve both bone and soft tissue, involve both children and adults, tend to be confused for an Ewing-like sarcoma, and behave aggressively.
    - Adult spindle cell/sclerosing rhabdomyosarcoma.
      - A subset shows *MYOD1* mutations [73, 74].
        Usually homozygous mutation in exon 1 (pL122R) but can also have heterozygous mutations.
        More commonly seen in sclerosing subtype and can also harbor coexistent *PIK3CA* mutations [74].
    - Histiocyte-rich rhabdomyoblastic tumor.
      - Provisional entity most often confused with spindle cell rhabdomyosarcoma [75].
      - Does not have *MYOD1* mutations, is usually encapsulated with surrounding lymphoid aggregates and a prominent histiocytic inflammatory infiltrate.
      - Good prognosis with no recurrences or metastasis [76].
      - Mentioned because angiomatoid fibrous histiocytoma would be on the histologic differential and molecular testing may be needed to exclude.
16. **What is the role of molecular testing in tumor of uncertain differentiation? What are the common fusions found in these tumors? What testing modalities are commonly employed and are there any limitations?**
    - Many soft tissue tumors of uncertain differentiation have unique molecular rearrangements that routinely primarily to confirm a diagnosis. See below for specifics on the individual tumors that are commonly tested.
    - Angiomatoid fibrous histiocytoma (AFH).
      - Primarily occurs in children and young adults (<40 years) primarily in the dermis/subcutis of the extremities, although can occur anywhere where normal lymph nodes are found [77].
      - Most frequent fusion is *EWSR1-CREB1* seen in >90% of cases [78].
        Less commonly *EWSR1-ATF1* and less commonly *FUS-ATF1*.
        FISH break-apart probes are generally sensitive for detecting either the *EWSR1* or the *FUS* rearrangement in AFH but up to 25% cases can be missed [79].
        (a) May possibly represent cryptic rearrangements not detectable through FISH probes or represent other fusion(s).
      - Immunohistochemistry.
        (a) Most show co-expression of EMA and desmin [77].
        (b) Recently, many have been shown to variably express ALK although this does not correlate with molecular alteration [80].
    - Synovial sarcoma (SS).
      - Primarily occurs in the deep soft tissue of the extremities of adolescents or young adults and vast majority occur before age 50 [81].
      - Monophasic subtype more common than biphasic.
      - Because synovial sarcomas express EMA and cyokeratins [82], molecular testing can help exclude sarcomatoid carcinoma, in cases with treatment effect where the original material is not available for review and in more poorly differentiated cases.
      - Most common fusion is *SS18-SSX1* between exon 10 of *SS18* and exon 6 of *SSX* and less commonly involves *SSX2*, *SSX4* or *SS18L-SSX1* [83, 84].
        Many centers employ the use of break-apart FISH which in some studies shows sensitivity of around 85% versus 95% when compared to PCR [83].
    - Alveolar soft part sarcoma.
      - Mainly affects young adults, most commonly involves the deep soft tissue of the extremities followed by the trunk [85].
        More commonly affects head and neck in children.
      - Characterized by *ASPSCR1-TFE3* fusion [86].
      - Molecular testing either by FISH or NGS only necessary in difficult or selected cases.
        Strong and diffuse TFE3 by IHC and classic morphology is considered sufficient for the diagnosis [87].
    - Clear cell sarcoma.
      - Mainly affects young adults, most commonly as deep-seated locations of the distal extremities, with the majority arising near the foot/ankle [88].
      - Because of the expression of melanocytic markers [89], immunohistochemical distinction from melanoma not possible and molecular has become the mainstay for definitive distinction.

  - Most common fusion involves *EWSR1-ATF1* most commonly between exon 8 of *EWSR1* and exon 4 of *ATF1* [90].
    Other variant translocations involve fusion of *EWSR1-CREB1* [89].
    Rare cases can also show concurrent BRAF mutations [91], further blurring the differential with melanoma.
- Extraskeletal myxoid chondrosarcoma.
  - Most commonly affects adults (median age 50 years) in the deep soft tissue of the proximal extremities and limb girdles [92].
  - Most commonly involves rearrangements of *NR4A3* with either *EWSR1* or *TAF15* [93].
    Rare fusions involving *TCF12-NR4A3* and *TFG-NR4A3* have also been identified [94, 95].
    More recently, a *HSPA8-NR4A3* fusion has been identified [96].
  - No other sarcoma has been found to have *NR4A3* fusions, so its detection is considered pathognomonic.
- Desmoplastic small round cell tumor (DSRCT).
  - Most commonly affects children and young adults in the abdomen/peritoneal cavity [97].
    Striking male predominance.
  - Because of its histologic overlap with other small round cell tumors (e.g., Ewing, alveolar rhabdomyosarcoma), the use of ancillary immunohistochemical stains, and molecular testing is often employed.
    Most cases show expression for keratins, desmin, and WT1 (C-terminus) [97].
    On molecular level, characterized by recurrent fusion most commonly involving first seven exons of *EWSR1* and exons 8–10 of *WT1* [98].
- Intimal sarcoma.
  - Malignant sarcoma involving the great vessels of the heart and is now considered the most common [99].
  - FISH for MD*M2* amplification is often necessary as these tumors can have non-distinctive histology.
  - The use of array-CGH can often also show amplification of *KIT* and *PDGFRA*, gain of *EGFR*, and loss of *CDKN2A* [99].

17. **What is the role of molecular testing in fibroblastic/myofibroblastic tumors? What are the common molecular alterations found in these tumors? What testing modalities are commonly employed and are there any limitations?**
    - Many fibroblastic/myofibroblastic tumors have distinct molecular mutations or fusions that are characteristics, but because ancillary testing methods such as immunohistochemistry are so cheap and have a faster turn-around-time, molecular testing is generally not needed. In addition, some of these tumors are benign and the cost of performing the molecular testing cannot be generally justified (Table 10.5). See below for specifics on the individual tumors for potential scenarios where ancillary molecular techniques may be employed.
    - Nodular fasciitis
      - Self-limited mesenchymal neoplasm that most commonly affects young adults of the subcutis of the upper extremities, trunk, head, and neck [100].
      - Most cases show classic histology and show myofibroblastic differentiation with actin positivity [101] in a "tram-track" pattern, so there is no need for molecular confirmation.
      - Difficult or unusual cases may benefit from molecular testing in order to exclude a more worrisome lesion.
        Most often characterized by rearrangements between *USP6* and *MYH9* [102].
        An exceptional case associated with multiple recurrences and metastatic disease has been associated with *PPP6R3-USP6* [103].
      - A subset of cellular fibromas of tendon sheath have been found to have *USP6* rearrangements [104].
    - *EWSR1-SMAD3*-positive fibroblastic tumor
      - Benign fibroblastic tumor that often involves the dermis/subcutis of acral sites [105].
      - Typically are positive for ERG but negative for SMA, CD34, CD31, and S100 [105].
        It is unclear if these tumors represent a spectrum of similar pediatric fibroblastic neoplasms, and indeed, some cases classified initially as one entity are sometimes re-classified as another based on molecular testing [106].
    - Soft tissue angiofibroma
      - Benign fibroblastic neoplasm affecting middle-aged adults in the extremities, most commonly the leg [107].
      - Characterized by *NCOA2* rearrangements most often to *AHRR* [108].
        Other fusion partners include *GTF2I-NCOA2* and *GAB1-ABL1* [109, 110].
      - In most cases, molecular testing is not needed to confirm the rearrangements.
        The main role of molecular testing is to exclude lesions that can have some histologic overlap and can behave more aggressively or have metastatic potential such as solitary

Table 10.5 Fibroblastic and myofibroblastic tumors with characteristic molecular findings that are not routinely tested

WHO classification	Age	Common location	Molecular	Routine molecular testing needed?
Nodular fasciitis	Young adults	Subcutis of upper extremities, trunk, head, and neck	*USP6* rearrangement	No
Fibrous hamartoma of infancy	Children	Axilla, trunk, extremities	*EGFR* mutations	No
Myofibroblastoma	Adults	Inguinal/groin area	Loss of *RB1*	No
Calcifying aponeurotic fibroma	Children, teenagers	Palmar surface of hands and fingers	*FN1-EGF* fusion	No
EWSR1-SMAD3-positive tumor	Wide age range	Hands and feet	*EWSR1-SMAD3*	Yes
Angiofibroma of soft tissue	Middle-aged adults	Extremities, most commonly the leg	*NCOA2* rearrangements	No
Cellular angiofibroma	Adults	Inguinal region	Loss of *RB1*	No
Acral fibromyxoma	Adults	Fingers and toes	Loss of *RB1*	No
Gardner fibroma	Children	Back, paraspinal, head, and neck	Germline *APC* mutation	No
Desmoid fibromatosis	Young adults	Extremities, abdominal wall, chest wall	*CTNNB1* mutations; germline *APC* in Gardner syndrome	No
Giant cell fibroblastoma/ dermatofibrosarcoma protuberans	Children/adults	Trunk, groin, extremities	*COL1A1-PDGFB*	No
Solitary fibrous tumor	Adults	Anywhere but more common in deep soft tissue	*NAB2-STAT6*	No
Inflammatory myofibroblastic tumor	Children, young adults	Abdominal viscera and soft tissue	*ALK*, *ROS*, *NTRK3* gene rearrangements	No
Infantile fibrosarcoma	Children <2 years	Extremities, trunk, head, and neck	*NTRK3* fusions	Yes
Low-grade fibromyxoid sarcoma	Young to middle aged adults	Extremities and trunk	*FUS-CREB3L2* or *FUS-CREB3L1*	No
Sclerosing epithelioid fibrosarcoma	Middle-aged to elderly adults	Extremities and trunk	*EWSR1-CREBL1*	No

fibrous tumor, low-grade fibromyxoid sarcoma, and a low-grade myxofibrosarcoma.

- Desmoid-type fibromatosis
  - Locally aggressive fibroblastic neoplasm with a propensity for local recurrence that primarily affects young adults and most commonly involves the extremities, trunk, and abdominal cavity [111].
  - Point mutations involved two codon of exon 3 of *CTNNB1* are found in the majority of sporadic tumors [112].

    Immunostain for beta-catenin often used as a surrogate marker.
  - Smaller percentage is associated with Gardner syndrome and show germline mutations in *APC* gene but can also show sporadic mutations [113].
- Giant cell fibroblastoma/dermatofibrosarcoma protuberans
  - Although listed separately in WHO, they are thought to be spectrums of the same neoplasm with the former arising primarily in children.
  - Lesions defined by *COL1A1-PDGFB* fusion.

    ~2% of cases may be cryptic, and another small percentage may show alternate fusions involving *PDGFB* including *COL6A3-PDGFD* and *EMILIN2-PDGFD* [114].
  - Reasons to perform break-apart FISH or NGS.

    Extensively myxoid lesion where classic architecture is not present.

    Metastatic/recurrent disease where original material is not available.

    Small samples where only "herringbone" pattern is present and the differential would include synovial sarcoma, MPNST, and fibrosarcomatous DFSP.
- Solitary fibrous tumor
  - Commonly affects adults and can occur at any site but more common in deep soft tissue and extrapleural locations [115].
  - NAB2-STAT6 fusion is pathognomonic [37].
  - Molecular testing not generally needed as STAT6 IHC is generally considered sensitive and specific [38].

    Dedifferentiated liposarcomas can occasionally show STAT6 expression, so this may be an instance where molecular testing is indicated.
- Infantile fibrosarcoma
  - Commonly affects children less than 2 years and involves the extremities, trunk, head and neck.

  - Most cases harbor an *ETV6-NTRK3* fusion [116]. Other cases have *NTRK1*, *NTRK2*, *BRAF*, and *MET* fusions [117–119].
  - Cases with *NTRK* rearrangements often show pan-TRK IHC expression [120].
- Low-grade fibromyxoid sarcoma (LGFMS)
  - Malignant fibroblastic neoplasm that most commonly affects young to middle-aged adults of the deep extremities and trunk [121].
  - Characterized by fusions most commonly involving *FUS-CREB3L2* or *FUS CREB3L1* [122].
    Less commonly can involve *EWSR1* [123].
  - MUC4 IHC is generally considered sensitive and specific for the diagnosis, so routine molecular testing is not needed [124].
    Selected cases tested when MUC4 IHC is negative or not available.
- Sclerosing epithelioid fibrosarcoma
  - Malignant fibroblastic neoplasm with subset that is related to LGFMS both histologically and molecularly and can have a similar age and site distribution [125].
  - Most common fusion is *EWSR1-CREB3L1* [126].
    Other fusions show *FUS* or *PAX5* with *CREB3L2*, *CREB3L3*, or *CREM* [127, 128].
  - Similarly to LGFMS, MUC4 IHC is considered sensitive and specific and is present in ~90% of cases [129], so routine molecular testing is not often needed.

Case 1

Case History

30-year-old previously healthy male presents with progressively worsening double vision for 1 month with associated vertigo and balance difficulties. This has been compounded by neuropathic back pain that radiates to the abdomen. An imaging CT shows a 9 cm chest wall mass that encases the eighth and ninth rib with a mild periosteal reaction without frank osseous invasion. The radiologic differential would be a malignant solitary fibrous tumor versus lymphoma versus, less likely metastatic disease.

Histologic Features

A CT-guided biopsy demonstrates a basaloid population of epithelioid cells with crush artifact and scattered admixed spindle cells (Fig. 10.1). Immunostains are positive for AE1/AE3 (focal), EMA (rare), and the spindled cells are positive for SOX10. The cells are negative for CD3, CD20, CK7, CK20, TTF1, desmin, chromogranin A, and SALL4.

Choice of Molecular Testing

Given the presence of admixed spindled cells that are SOX10 positive, the preliminary differential was a round cell sarcoma with an *EWSR1-PATZ1* fusion (*EWSR1*-non-ETS fusion). As this particular fusion is not responsive to traditional chemotherapy, a decision was made to perform RNA sequencing to confirm or rule out the fusion as opposed to simply using an *EWSR1* break-apart FISH probe which has a faster turn-around time.

Molecular Study

An Archer® NGS fusion study revealed an *ESWR1* (Exon 7)-*FLI1* (Exon 5) fusion. (The NGS fusion study was performed using 26 gene FusionPlex Sarcoma panel, ArcherDx, Boulder, CO; validated in the molecular diagnostic laboratory for clinical testing.)

Final Diagnosis

Ewing Sarcoma with *EWSR1-FLI1* fusion.

Case Discussion

This case demonstrates that although break-apart FISH would have detected the *EWSR1* rearrangement, RNA sequencing provided the correct information for the diagnosis and also provided the treating clinicians with the correct clinical information to begin EWS chemotherapy regimen that would not have been started if this case truly demonstrated a *PATZ1* fusion.

Case 2

Case History

A 13-year-old female noticed a lump in her groin for 2 weeks. She had a history of a neoplasm removed from her right knee 5 years ago. Ultrasound showed a 4 cm lymph node concerning for malignancy. Upon further investigation, the patient had been diagnosed with an angiomatoid fibrous histiocytoma. Given that lesion was removed years ago without recurrence, the current lesion is suspicious for metastasis.

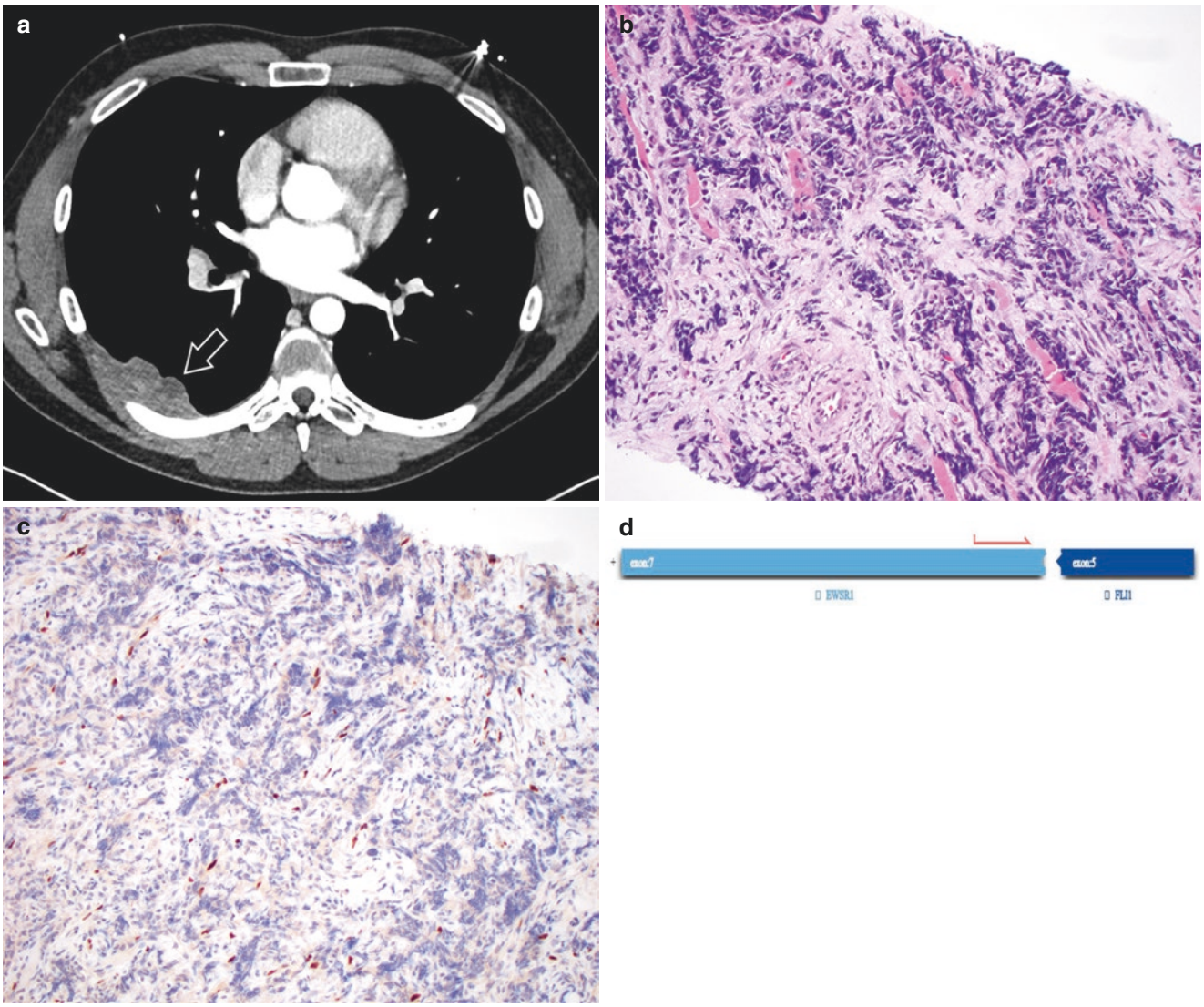

Fig. 10.1 Axial CT shows a posterior chest wall mass (**a**) that is composed of small round blue cells admixed with spindle cells (**b**) that are positive for SOX10 (**c**) that can of be seen in *EWSR1-PATZ1* fusion. Interestingly, however, NGS revealed an *EWSR1-FLI1* fusion (**d**)

Histologic Features

Sections show a tumor composed of spindled to epithelioid cells arranged in a syncytial pattern that is involving a lymph node (Fig. 10.2). Immunostains are positive for EMA and desmin.

Molecular Study

An Archer® NGS fusion study found an *EWSR1-ATF1* gene fusion.

Final Diagnosis

Metastatic angiomatoid fibrous histiocytoma.

Case Discussion

While angiomatoid fibrous histiocytomas can have surrounding lymphoid aggregates, the current case illustrates a rare metastasis to the locoregional lymph nodes that happens in <5% of cases. *EWSR1-CREB1* fusions are the most common fusions in angiomatoid fibrous histiocytoma, but *ATF1* fusions are more frequently associated with extrasomatic soft tissue cases. In the current case, FISH break-apart would have been an option. However, one potential limitation is that FISH testing cannot always discriminate between a simple terminal deletion of the 3' *EWSR1* and translocation involving the remaining 5′ portion of *EWSR1* with another gene. In addition, rare fusions involving *FUS* can happen and would be missed by that assay.

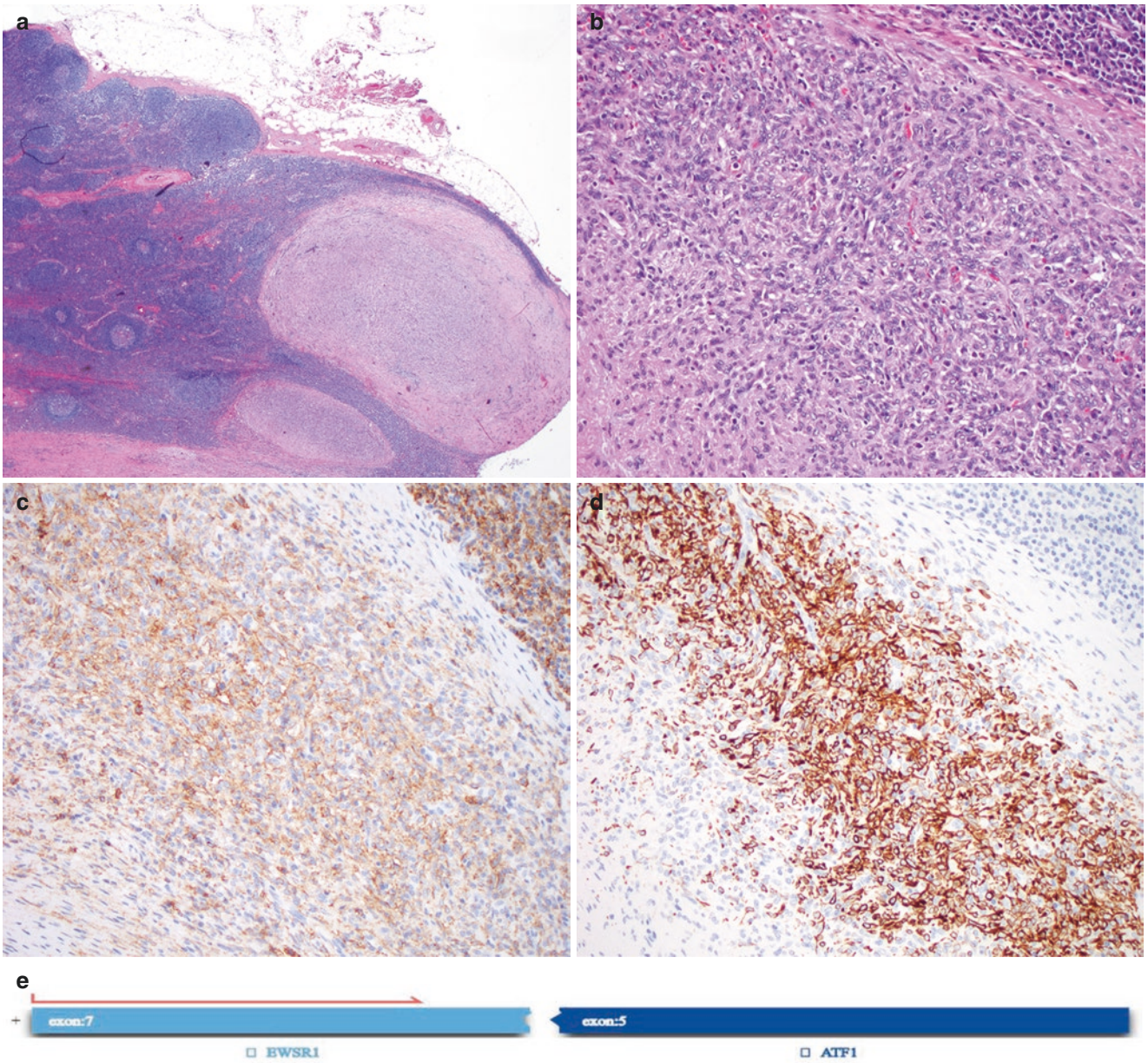

Fig. 10.2 Low power shows a metastatic deposit in a lymph node (**a**). Higher power shows uniform histiocytoid cells (**b**) that are positive for both EMA (**c**) and desmin (**d**). NGS reveal an *EWSR1-ATF1* fusion (**e**)

Case 3

Case History

A 25-year-old male presented with several week history of pleuritic chest pain. He was treated by his PCP with a trial of steroids and Z-Pak which failed to improve his symptoms. On initial examination, a right upper quadrant ultrasound demonstrated two hepatic masses. A follow-up MRI showed innumerable masses along the liver that appear external to liver parenchyma. There was also a large confluent mass anteriorly between the liver and the diaphragm that measured 10 cm.

Histologic Features

Core biopsy shows a malignant small round cell tumor arranged in nest and sheets with intervening fibrous stroma (Fig. 10.3). Immunostains are positive for AE1/AE3, Desmin, and CD99 (patchy) and are negative for WT1, CK5/6, MYOD1, CD45, Melan-A, and TTF1.

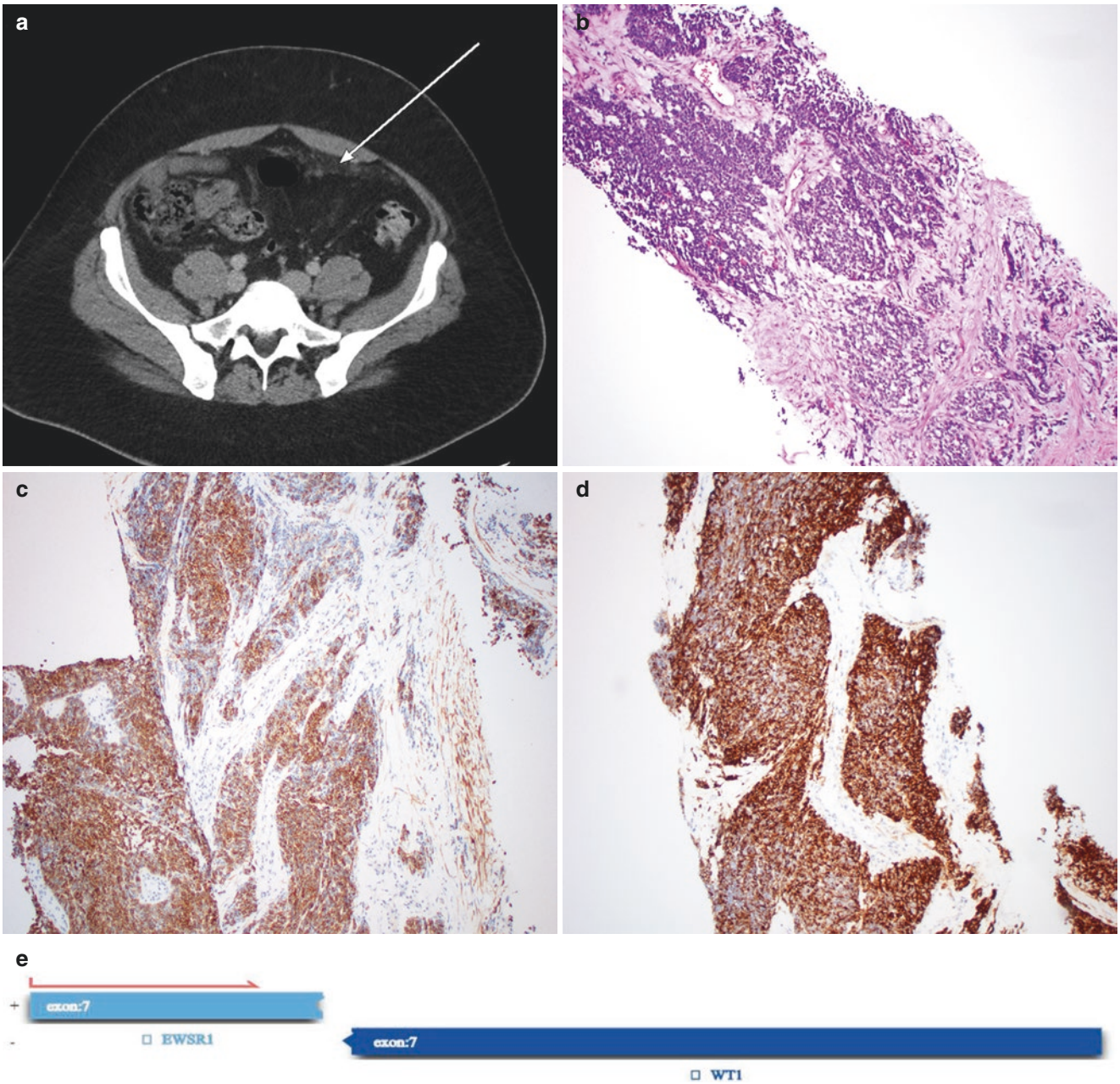

Fig. 10.3 Axial MRI shows multiple peritoneal surface masses throughout the abdomen (**a**). Biopsy shows a small round cell tumor growing in sheets with intervening fibrous stroma (**b**) that is strongly positive for AE1/AE3 (**c**) and desmin (**d**). NGS revealed an *EWSR1-WT1* fusion (**e**)

Molecular Study

An Archer® NGS fusion study revealed an *EWSR1-WT1* fusion.

Final Diagnosis

Desmoplastic small round cell tumor.

Case Discussion

The most common transcript for DSRCT involves the first 7 exons of *EWSR1* fusing to exons 8–10 of *WT1*; however variant translocations exist. This patient underwent 8 cycles of chemotherapy and repeat imaging revealed persistent abdominal lesions with persistent mediastinal lymphadenopathy. The patient is currently scheduled for resection of the mediastinal lesions with a plan for subsequent abdominal

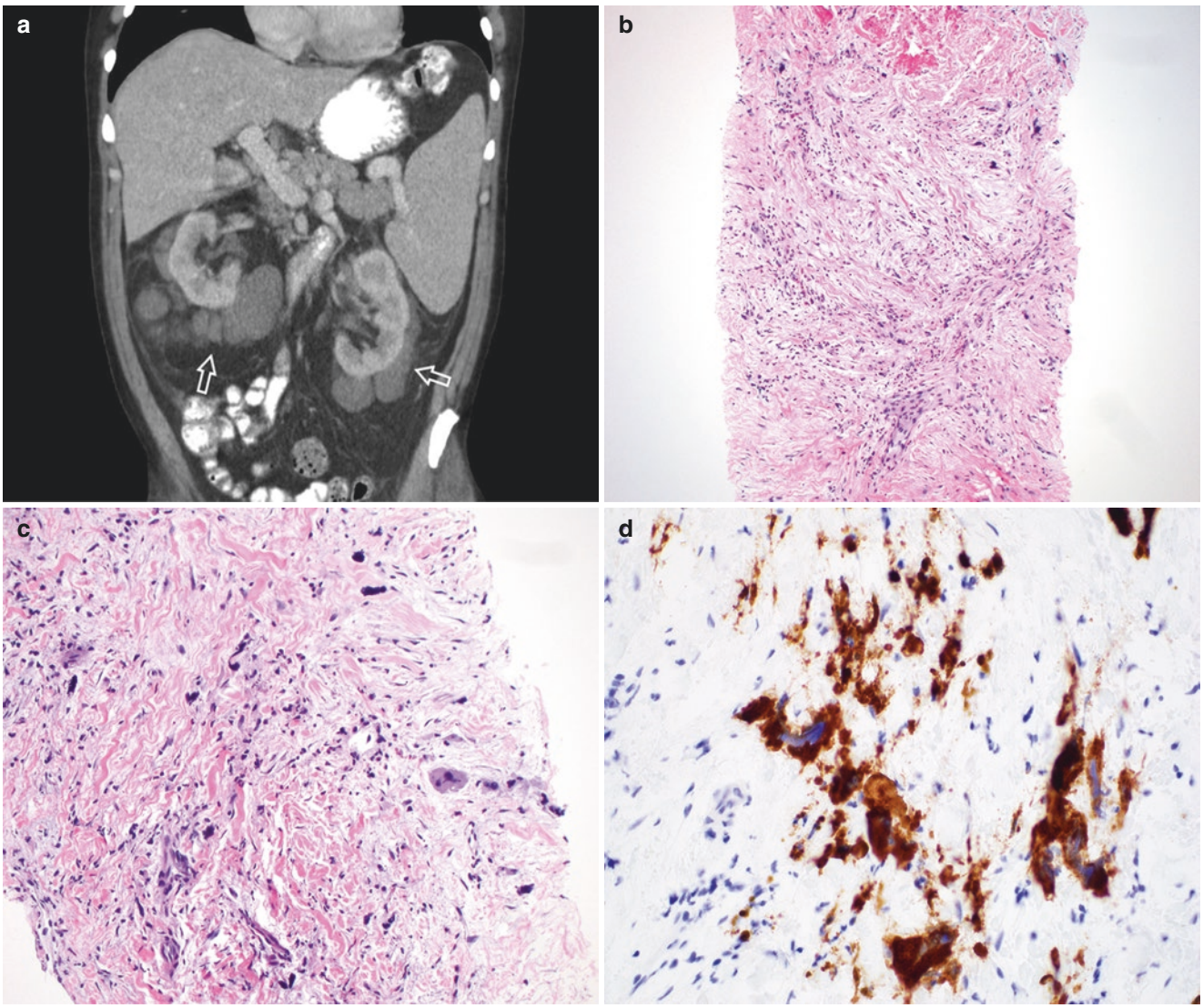

Fig. 10.4 Coronal CT shows multiple soft tissue masses bilaterally included around the kidneys (**a**). Biopsy of retroperitoneum at low power shows a fibrous spindle cell lesion with scattered enlarged and hyperchromatic cells (**b**). Higher power reveals the pleomorphic cells show smudgy chromatin and multinucleate forms (**c**) that are positive for CD61 (**d**)

exploration and cytoreductive surgery. Despite multimodality therapy, the 5-year overall survival rate is low.

Case 4

Case History

A 50-year-old male had a history of essential thrombocytosis diagnosed 20 years ago. His most recent blood cell counts revealed the following: WBC 3.3 K/μL, Hb 9.3 g/dL, Hct 38%, Plt 331 K/μL. He had had worsening anemia of the past year with increasing splenomegaly. A CT of the abdomen and pelvis revealed a 19.5 cm spleen as well as numerous soft tissue masses encompassing the right and left kidney but clinically the patient has been asymptomatic.

Histologic Features

Core biopsy shows a variably cellular lesion composed of small, bland spindled cells arranged in vague fascicles set in a myxo-collagenous stroma with scattered hyperchromatic pleomorphic cells with smudgy chromatin (Fig. 10.4). An immunostain is positive for CD61 and negative for MDM2.

FISH Results

Negative for *MDM2* amplification.

Final Diagnosis

Sclerosing extramedullary hematopoietic tumor.

Case Discussion

Sclerosing extramedullary hematopoietic tumor is an extramedullary complication associated with myeloproliferative neoplasms. The presence of atypical megakaryocytes in the retroperitoneum raises the differential of a well-differentiated liposarcoma; however, they stain appropriately with CD61 and FISH for *MDM2* is negative to help exclude a liposarcoma. In this case, the diagnosis would have been extremely difficult if the clinical history was not available.

Case 5

Case History

A 35-year-old female with no significant past medical history presented with a back mass that was clinically felt to be a lipoma or a cyst. Nothing was done at that time on initial presentation, and she returned to clinic a couple months later because the mass increased in size and it was excised by a surgeon.

Histologic Features

Sections show a cellular lesion composed of spindled and epithelioid cells arranged in sheets and nests with areas of peritheliomatous growth with associated necrosis, brisk mitotic activity and areas of clear cell and myxoid change. In addition, there are nodular areas of fascicular growth with intervening dense fibrous septa (Fig. 10.5). Immunostains are positive for CD99, FLI1 and WT1 and are negative for CD45, AE1/AE3, Cam5.2, PAX8, desmin, hmb-45, SOX10, and BCOR.

Molecular Study

An Archer® NGS fusion study revealed a fusion transcript of *CIC* (exon 20) and *FOXO4* (exon 2).

Final Diagnosis

CIC-rearranged sarcoma.

Case Discussion

CIC-rearranged sarcoma is an undifferentiated round cell sarcoma that most commonly involves a *CIC-DUX4* fusion. The nodular growth pattern in the current case along with the zone of necrosis and strong nuclear expression of WT1 is suggestive of a *CIC*-rearranged sarcoma. Interestingly the presence of clear cell change can sometimes be associated with *EWSR1-FEV*; however, because response to chemotherapy is dependent on the specific fusion transcript detected NGS testing is becoming the gold standard in the diagnosis of round cell sarcomas.

Case 6

Case History

An 80-year-old female with a left chest wall mass that was felt to be recurrent myxoid liposarcoma. She was originally diagnosed at an outside facility with a retroperitoneal primary myxoid liposarcoma 5 years ago and is status post two resections. The current lesion measures up to 16 cm. No history of molecular/FISH testing is found.

Histologic Features

Sections show a predominant low-grade myxoid adipocytic lesion with a plexiform vasculature and signet-type lipoblasts. A few sections demonstrate more increased cellularity and atypia with spindled cells arranged in fascicles with scattered pleomorphic forms and conspicuous mitotic activity. Within the cellular areas, focal osteoid formation is present (Fig. 10.6). An immunostain for MDM2 is focally positive.

FISH Results

Positive for *MDM2* amplification with a ratio of *MDM2* fluorescent signal to chromosome 12 centromere signal of 15.

Final Diagnosis

Dedifferentiated liposarcoma.

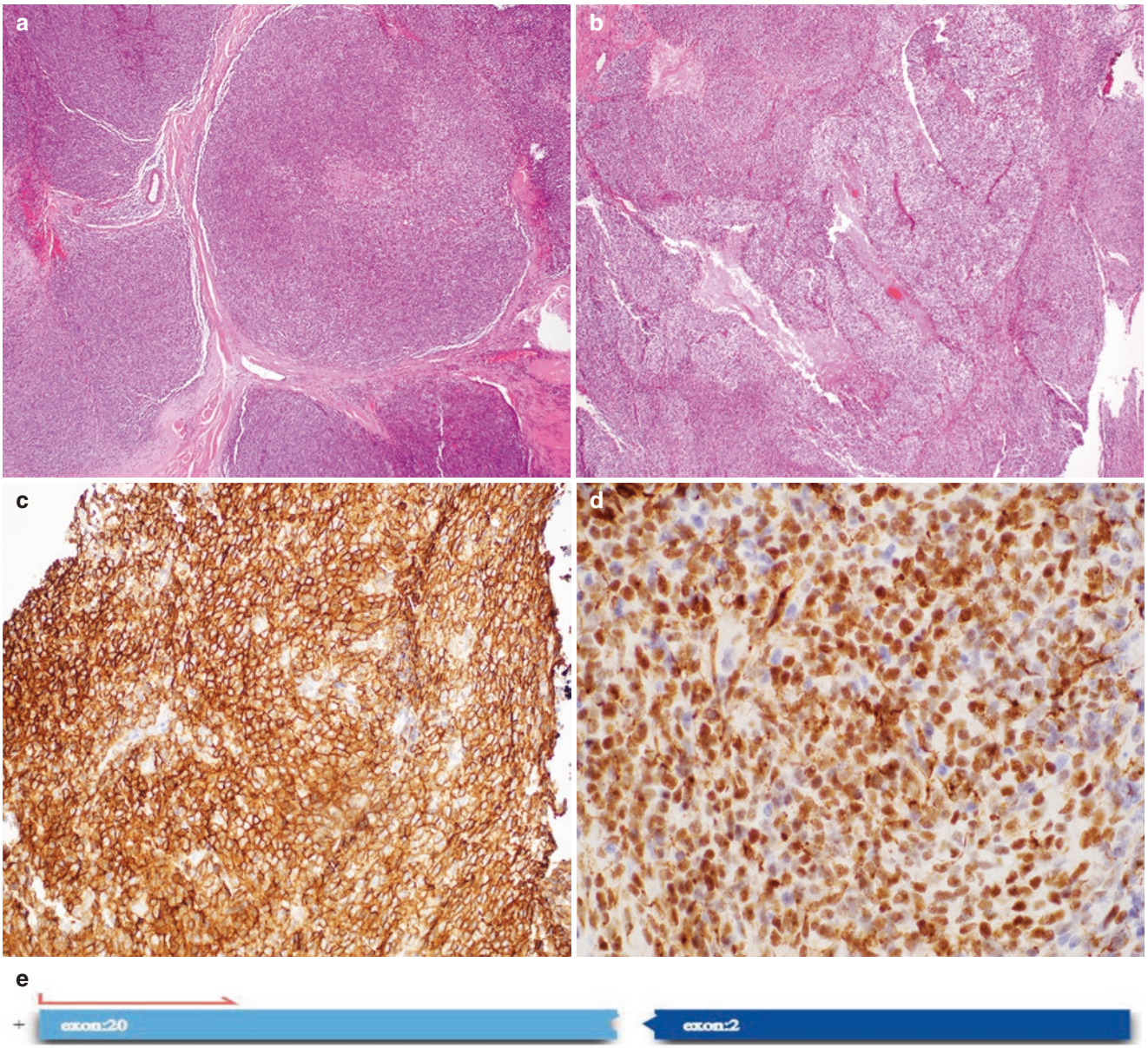

Fig. 10.5 Low power shows nodular growth pattern with intervening fibrous septa (**a**) and areas of clear cell change with zones of necrosis (**b**). Medium power shows strong, membranous CD99 (**c**) and nuclear WT1 (**d**). NGS revealed a fusion transcript of *CIC* (exon 20) and *FOXO4* (exon 2) (**e**)

Case Discussion

The patient's history of a retroperitoneal primary myxoid liposarcoma is noted. Although primary retroperitoneal myxoid liposarcomas do exist, they are rare and a retroperitoneal dedifferentiated liposarcoma with areas reminiscent of myxoid liposarcoma would be much more likely. As the current specimen showed a high-grade spindle cell component which would be unusual for a high-grade myxoid liposarcoma, FISH testing was employed and is amplified.

Case 7

Case History

A 25-year-old female presented with a complaint of swelling and a slowly enlarging mass of the lower lip. Clinical exam demonstrated a mobile and well-circumscribed lesion suggestive of a benign process.

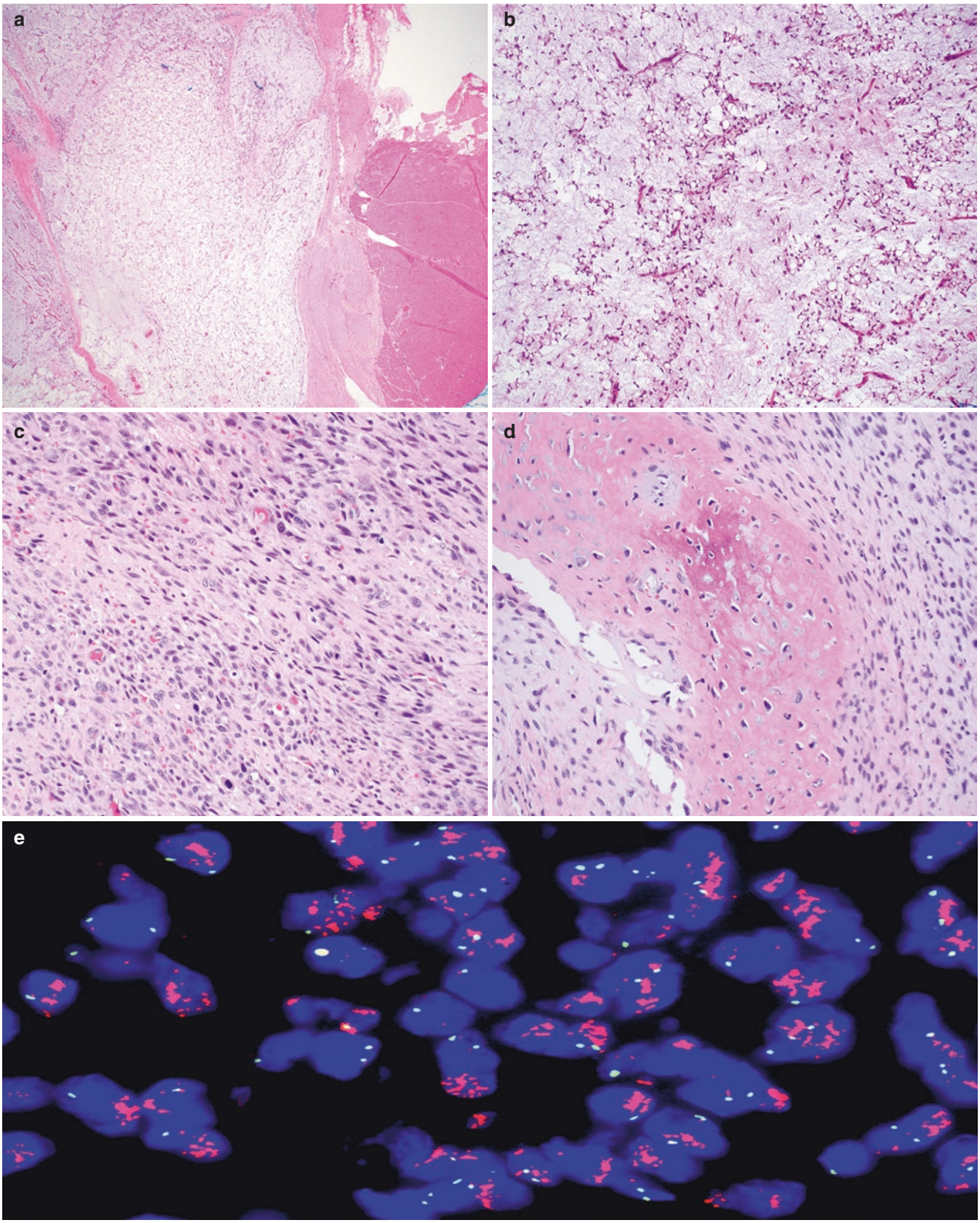

Fig. 10.6 Low power shows a nodular myxoid lesion (**a**) with plexiform vasculature and scattered signet-type lipoblasts (**b**). Higher power demonstrates spindled fascicular growth with conspicuous mitoses with pleomorphic forms (**c**) and osteoid formation (**d**). FISH testing demonstrates amplification of *MDM2* (**e**)

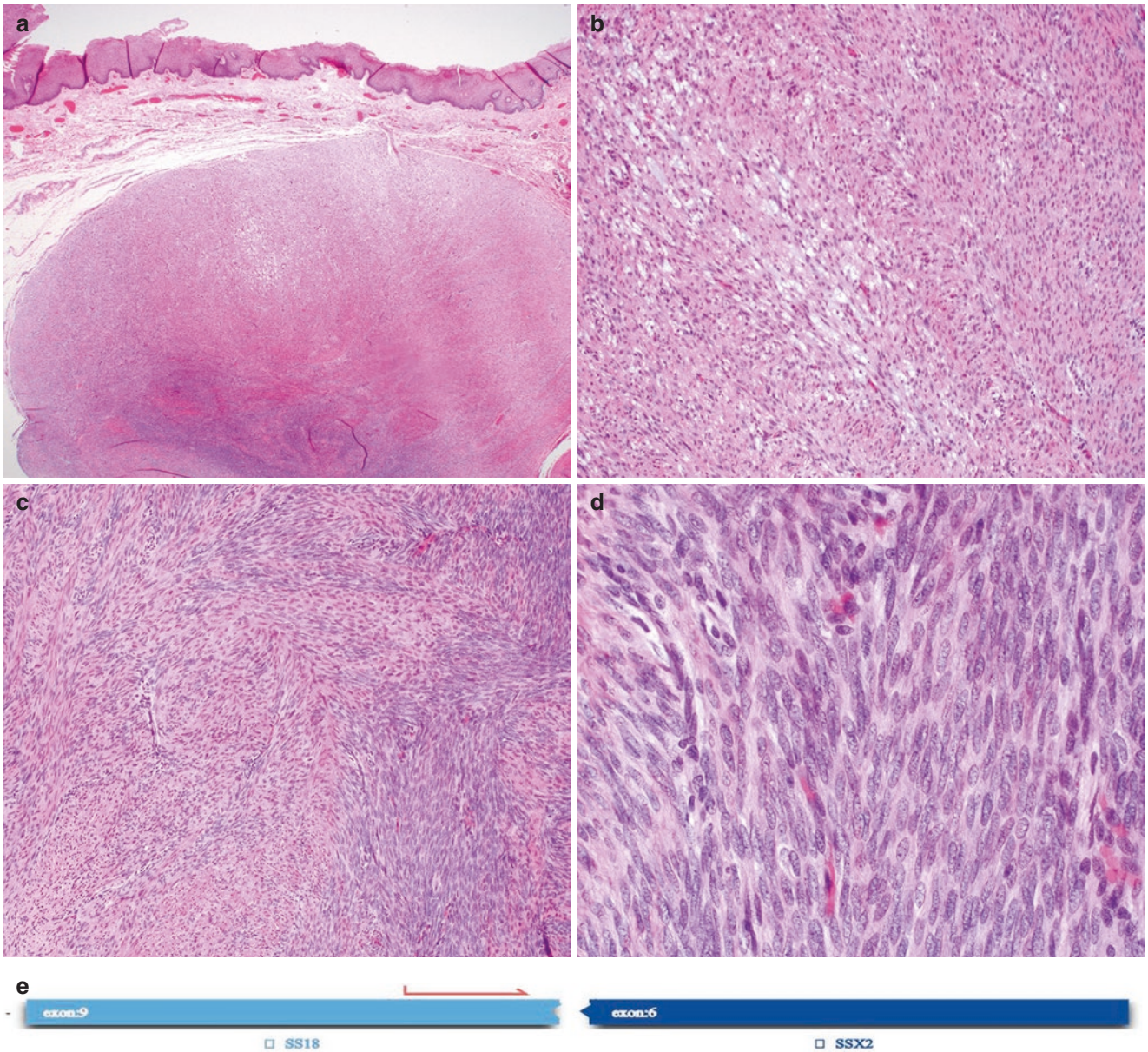

Fig. 10.7 Low power shows a well-circumscribed submucosal mass (**a**) that is composed of loose fibrous spindle cell areas (**b**) admixed with more cellular basophilic areas (**c**). Higher power shows the monotonous spindle cell population with overlapping nuclei (**d**). NGS revealed a *SS18-SSX2* fusion (**e**)

Histologic Features

Sections show a relatively well-circumscribed submucosal spindle cell lesion. The spindle cells show fascicular growth and are monomorphic with relatively bland elongated to wavy nuclei with finely granular chromatin, inconspicuous nucleoli, and minimal cytoplasm (Fig. 10.7). Immunostains are positive for EMA (patchy) and are negative for CD34, desmin, S100 protein, and STAT6.

Molecular Study

An Archer® NGS fusion study revealed a *SS18-SSX2* fusion transcript.

Final Diagnosis

Monophasic synovial sarcoma.

Case Discussion

The most common fusion in synovial sarcoma is *SS18-SSX1*, but a majority of *SS18-SSX2* are the monophasic subtype and they are more common in females. Less than 10% of synovial sarcomas involve the head and neck region and a well-circumscribed growth can give a false impression of a benign process. Although NGS was performed, break-apart FISH for *SS18* would have been a reasonable ancillary testing method choice.

Case 8

Case History

A 40-year-old female presented to her dermatologist with a left lower leg nodule. The clinical differential was broad and included lymphoma, a granulomatous process, a deep fungal infection versus a metastasis of unknown primary.

Histologic Features

A punch biopsy shows a cellular dermal-based mesenchymal neoplasm composed of basaloid cells growing in nests and sheets. The cells have scant cytoplasm and mitotic activity is conspicuous (Fig. 10.8). Immunostains are positive for desmin, myogenin, and myoD1 and are negative for AE1/AE3, S100, chromogranin, CK20, TTF1, TdT, and CD45.

Molecular Study

An Archer® NGS fusion study revealed a fusion transcript of *PAX3* (exon 7) and *FOXO4* (exon 2).

Final Diagnosis

Alveolar rhabdomyosarcoma.

Case Discussion

The patient underwent chemotherapy, and at the time of resection, the tumor was approximately 10% viable with negative margins and one lymph node positive for metastatic disease. Approximately 8 months later, she had a recurrence at the original site that was resection and most recent imaging studies have been negative.

Case 9

Case History

A 60-year-old female with a history of clear cell sarcoma that was diagnosed at an outside facility with break-apart FISH for *EWSR1*. She had lung metastases but had been on 4 cycles of pembrolizumab but developed a possible recurrence in the groin.

Histologic Features

Biopsy showed a subcutaneous clear cell neoplasm composed of relatively uniform cells arranged in pseudo-alveolar nests with surrounding fibrous septa (Fig. 10.9). Immunostains were positive for Melan-A and SOX10 and negative for AE1/AE3.

Molecular Study

An Archer® NGS fusion study revealed no database fusions.

Reflective FISH Results

Positive for *EWSR1* gene rearrangement in 33% of cells.

Final Diagnosis

Recurrent clear cell sarcoma.

Case Discussion

This is a challenging case that was discussed at length at the multidisciplinary tumor boards. It was suggested that perhaps there is potentially a DNA only fusion without an RNA transcript secondary to the immunotherapy that may account for the NGS testing being negative. Conversely, it is possible that the *EWSR1* FISH is a false positive. The FISH probe used in the most recent cytogenetic test is actually proximal to the 5′ portion of *EWSR1* and not a part of the gene itself. So, it is possible that the break is next to but not within the actual gene. That being said, the mutational burden was also low and other mutations commonly seen in melanoma (the main histologic differential) were not seen. Taken together, it was felt clinically that the lesion most likely represents clear cell sarcoma and the patient is currently continuing immunotherapy treatment.

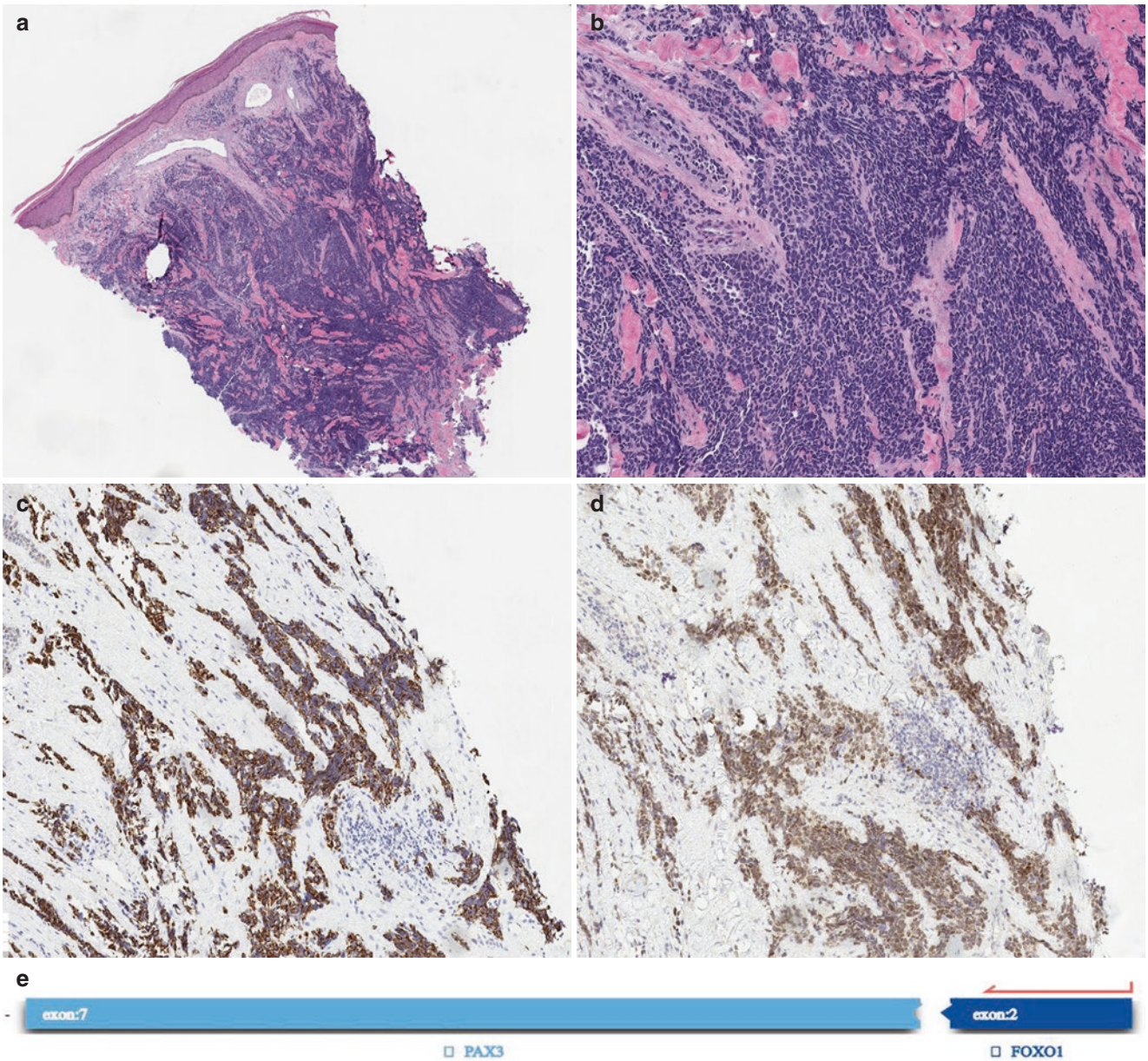

Fig. 10.8 Low power shows a cellular dermal basaloid neoplasm (**a**). Higher power shows a small round cell tumor growing in sheets and nests (**b**). The tumor cells are positive for desmin (**c**) and myo-D1 (**d**). NGS revealed a *PAX3-FOXO1* fusion (**e**)

Case 10

Case History

A 75-year-old male presented with a right posterior thigh mass. A CT exam showed a solid enhancing intramuscular mass that measured 5.2 cm. A CT biopsy was performed for diagnosis given the concern for a soft tissue sarcoma.

Histologic Features

A biopsy shows a myxoid mesenchymal neoplasm composed of monotonous spindled cells with admixed more epithelioid cells growing in reticular cords (Fig. 10.10). Immunostains show patchy EMA and are negative for AE1/AE3, S100 and synaptophysin.

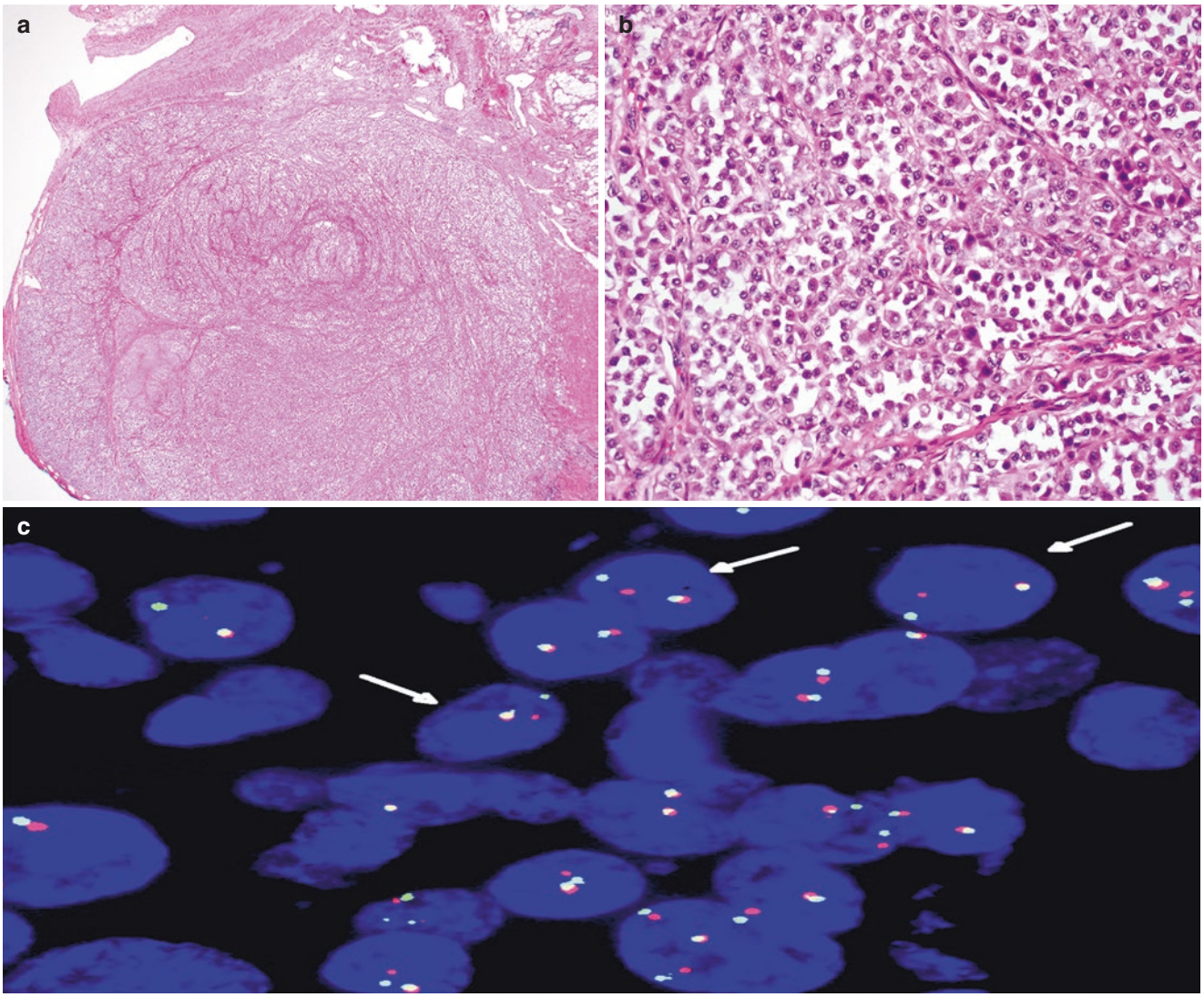

Fig. 10.9 Low power shows a subcutaneous clear cell neoplasm with intervening fibrous septa (**a**). Higher power shows a relatively monotonous population of epithelioid cells arranged in pseudo-alveolar nests (**b**). FISH testing demonstrates an *EWSR1* rearrangement (**c**)

Molecular Study

An Archer® NGS fusion study revealed an *EWSR1-NR4A3* fusion.

Final Diagnosis

Extraskeletal myxoid chondrosarcoma.

Case Discussion

The growth pattern of the cells is highly suggestive of an extraskeletal myxoid chondrosarcoma and the absence keratin and myoepithelial markers would make myoepithelioma less likely. However, because the immunophenotype of EMC is often non-distinct, routine molecular testing is performed when this diagnosis is entertained and the finding of a *NR4A3* rearrangement is pathognomonic.

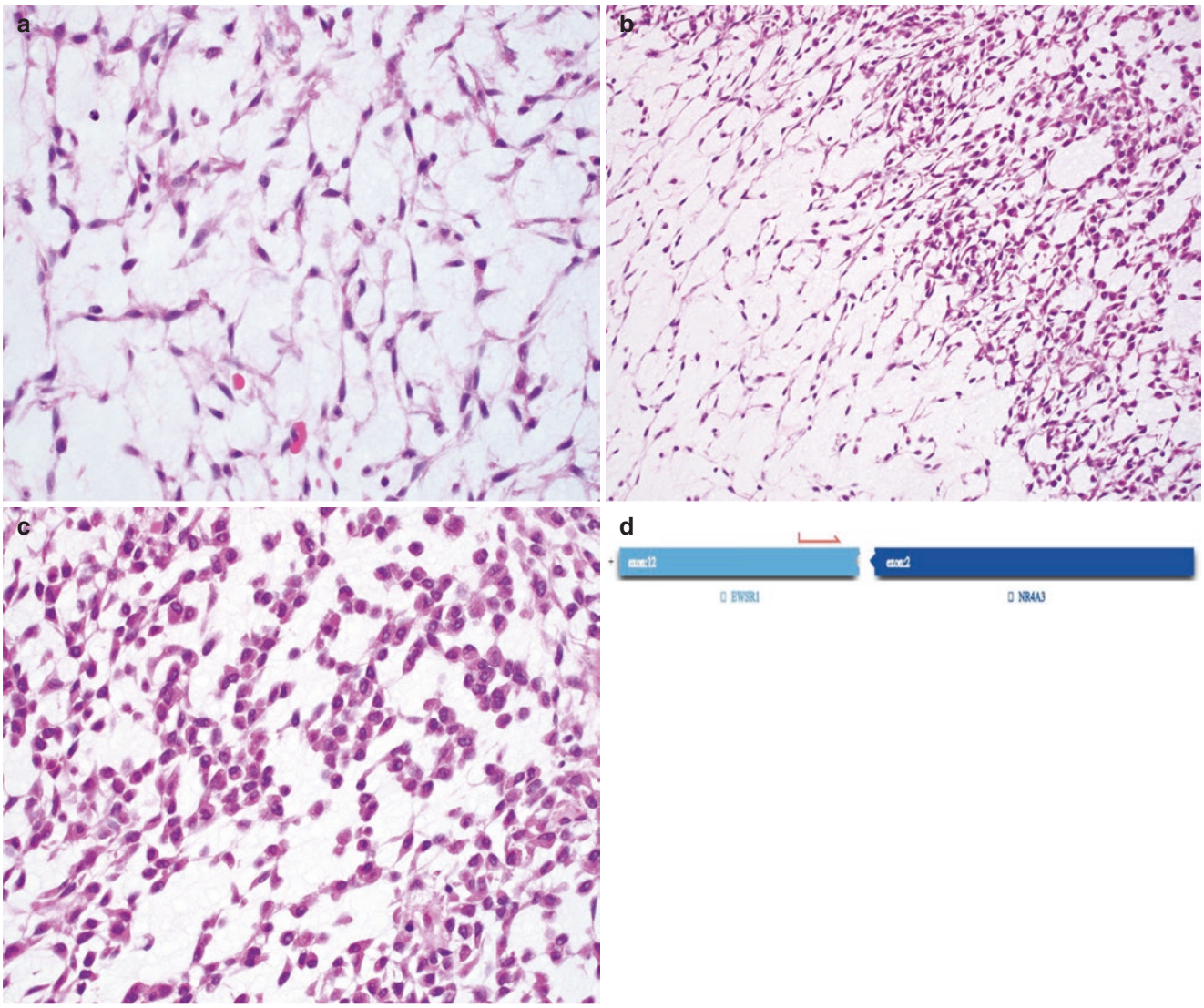

Fig. 10.10 Sections show a myxoid spindle lesion with eosinophilic cytoplasm growing in cords (**a**) with a transition to more cellular areas (**b**) with the cells becoming more epithelioid (**c**). NGS revealed an *EWSR1-NR4A3* fusion (**d**)

References

1. Board WCoTE. Soft tissue and bone tumours: international agency for research on cancer; 2020.
2. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature. 1992;359(6391):162–5.
3. Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, et al. The Ewing family of tumors – a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med. 1994;331(5):294–9.
4. Jeon IS, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, et al. A variant Ewing's sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. Oncogene. 1995;10(6):1229–34.
5. Kaneko Y, Yoshida K, Handa M, Toyoda Y, Nishihira H, Tanaka Y, et al. Fusion of an ETS-family gene, EIAF, to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy. Genes Chromosomes Cancer. 1996;15(2):115–21.
6. Peter M, Couturier J, Pacquement H, Michon J, Thomas G, Magdelenat H, et al. A new member of the ETS family fused to EWS in Ewing tumors. Oncogene. 1997;14(10):1159–64.
7. Shing DC, McMullan DJ, Roberts P, Smith K, Chin SF, Nicholson J, et al. FUS/ERG gene fusions in Ewing's tumors. Cancer Res. 2003;63(15):4568–76.
8. Ng TL, O'Sullivan MJ, Pallen CJ, Hayes M, Clarkson PW, Winstanley M, et al. Ewing sarcoma with novel translocation t(2;16) producing an in-frame fusion of FUS and FEV. J Mol Diagn. 2007;9(4):459–63.
9. Bridge JA, Sumegi J, Druta M, Bui MM, Henderson-Jackson E, Linos K, et al. Clinical, pathological, and genomic features of EWSR1-PATZ1 fusion sarcoma. Mod Pathol. 2019;32(11):1593–604.
10. Koelsche C, Kriegsmann M, Kommoss FKF, Stichel D, Kriegsmann K, Vokuhl C, et al. DNA methylation profiling distinguishes Ewing-like sarcoma with EWSR1-NFATc2 fusion from Ewing sarcoma. J Cancer Res Clin Oncol. 2019;145(5):1273–81.

11. Watson S, Perrin V, Guillemot D, Reynaud S, Coindre JM, Karanian M, et al. Transcriptomic definition of molecular subgroups of small round cell sarcomas. J Pathol. 2018;245(1):29–40.
12. Solomon DA, Brohl AS, Khan J, Miettinen M. Clinicopathologic features of a second patient with Ewing-like sarcoma harboring CIC-FOXO4 gene fusion. Am J Surg Pathol. 2014;38(12):1724–5.
13. Sugita S, Arai Y, Tonooka A, Hama N, Totoki Y, Fujii T, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol. 2014;38(11):1571–6.
14. Le Loarer F, Pissaloux D, Watson S, Godfraind C, Galmiche-Rolland L, Silva K, et al. Clinicopathologic features of CIC-NUTM1 sarcomas, a new molecular variant of the family of CIC-fused sarcomas. Am J Surg Pathol. 2019;43(2):268–76.
15. Sugita S, Arai Y, Aoyama T, Asanuma H, Mukai W, Hama N, et al. NUTM2A-CIC fusion small round cell sarcoma: a genetically distinct variant of CIC-rearranged sarcoma. Hum Pathol. 2017;65:225–30.
16. Smith SC, Buehler D, Choi EY, McHugh JB, Rubin BP, Billings SD, et al. CIC-DUX sarcomas demonstrate frequent MYC amplification and ETS-family transcription factor expression. Mod Pathol. 2015;28(1):57–68.
17. Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Recurrent CIC gene abnormalities in Angiosarcomas: a molecular study of 120 cases with concurrent investigation of PLCG1, KDR, MYC, and FLT4 gene alterations. Am J Surg Pathol. 2016;40(5):645–55.
18. Antonescu CR, Owosho AA, Zhang L, Chen S, Deniz K, Huryn JM, et al. Sarcomas with CIC-rearrangements are a distinct pathologic entity with aggressive outcome: a clinicopathologic and molecular study of 115 cases. Am J Surg Pathol. 2017;41(7):941–9.
19. Kao YC, Owosho AA, Sung YS, Zhang L, Fujisawa Y, Lee JC, et al. 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. Am J Surg Pathol. 2018;42(5):604–15.
20. Specht K, Zhang L, Sung YS, Nucci M, Dry S, Vaiyapuri S, et al. Novel BCOR-MAML3 and ZC3H7B-BCOR gene fusions in undifferentiated small blue round cell sarcomas. Am J Surg Pathol. 2016;40(4):433–42.
21. Krskova L, Kabickova E, Drahokoupilova E, Kopeckova K, Plank L, Vitkova P, et al. An undifferentiated sarcoma with BCOR-CCNB3 fusion transcript – pathological and clinical retrospective study. Neoplasma. 2018;65(4):630–6.
22. Yamada Y, Kuda M, Kohashi K, Yamamoto H, Takemoto J, Ishii T, et al. Histological and immunohistochemical characteristics of undifferentiated small round cell sarcomas associated with CIC-DUX4 and BCOR-CCNB3 fusion genes. Virchows Arch. 2017;470(4):373–80.
23. Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Secondary EWSR1 gene abnormalities in SMARCB1-deficient tumors with 22q11-12 regional deletions: potential pitfalls in interpreting EWSR1 FISH results. Genes Chromosomes Cancer. 2016;55(10):767–76.
24. Mantilla JG, Ricciotti RW, Chen E, Hoch BL, Liu YJ. Detecting disease-defining gene fusions in unclassified round cell sarcomas using anchored multiplex PCR/targeted RNA next-generation sequencing-molecular and clinicopathological characterization of 16 cases. Genes Chromosomes Cancer. 2019;58(10):713–22.
25. Rekhi B, Kembhavi P, Mishra SN, Shetty O, Bajpai J, Puri A. Clinicopathologic features of undifferentiated round cell sarcomas of bone & soft tissues: an attempt to unravel the BCOR-CCNB3- & CIC-DUX4-positive sarcomas. Indian J Med Res. 2019;150(6):557–74.
26. Italiano A, Chen CL, Thomas R, Breen M, Bonnet F, Sevenet N, et al. Alterations of the p53 and PIK3CA/AKT/mTOR pathways in angiosarcomas: a pattern distinct from other sarcomas with complex genomics. Cancer. 2012;118(23):5878–87.
27. Yoshida A, Arai Y, Kobayashi E, Yonemori K, Ogura K, Hama N, et al. CIC break-apart fluorescence in-situ hybridization misses a subset of CIC-DUX4 sarcomas: a clinicopathological and molecular study. Histopathology. 2017;71(3):461–9.
28. Hung YP, Fletcher CD, Hornick JL. Evaluation of ETV4 and WT1 expression in CIC-rearranged sarcomas and histologic mimics. Mod Pathol. 2016;29(11):1324–34.
29. Kao YC, Sung YS, Chen CL, Zhang L, Dickson BC, Swanson D, et al. ETV transcriptional upregulation is more reliable than RNA sequencing algorithms and FISH in diagnosing round cell sarcomas with CIC gene rearrangements. Genes Chromosomes Cancer. 2017;56(6):501–10.
30. Panagopoulos I, Gorunova L, Bjerkehagen B, Heim S. The "grep" command but not FusionMap, FusionFinder or ChimeraScan captures the CIC-DUX4 fusion gene from whole transcriptome sequencing data on a small round cell tumor with t(4;19)(q35;q13). PLoS One. 2014;9(6):e99439.
31. Kashima T, Halai D, Ye H, Hing SN, Delaney D, Pollock R, et al. Sensitivity of MDM2 amplification and unexpected multiple faint alphoid 12 (alpha 12 satellite sequences) signals in atypical lipomatous tumor. Mod Pathol. 2012;25(10):1384–96.
32. Sirvent N, Coindre JM, Maire G, Hostein I, Keslair F, Guillou L, et al. Detection of MDM2-CDK4 amplification by fluorescence in situ hybridization in 200 paraffin-embedded tumor samples: utility in diagnosing adipocytic lesions and comparison with immunohistochemistry and real-time PCR. Am J Surg Pathol. 2007;31(10):1476–89.
33. Clay MR, Martinez AP, Weiss SW, Edgar MA. MDM2 and CDK4 immunohistochemistry: should it be used in problematic differentiated lipomatous tumors?: a new perspective. Am J Surg Pathol. 2016;40(12):1647–52.
34. Dal Cin P, Kools P, Sciot R, De Wever I, Van Damme B, Van de Ven W, et al. Cytogenetic and fluorescence in situ hybridization investigation of ring chromosomes characterizing a specific pathologic subgroup of adipose tissue tumors. Cancer Genet Cytogenet. 1993;68(2):85–90.
35. Clay MR, Martinez AP, Weiss SW, Edgar MA. MDM2 amplification in problematic lipomatous tumors: analysis of FISH testing criteria. Am J Surg Pathol. 2015;39(10):1433–9.
36. Zhang H, Erickson-Johnson M, Wang X, Oliveira JL, Nascimento AG, Sim FH, et al. Molecular testing for lipomatous tumors: critical analysis and test recommendations based on the analysis of 405 extremity-based tumors. Am J Surg Pathol. 2010;34(9):1304–11.
37. Robinson DR, Wu YM, Kalyana-Sundaram S, Cao X, Lonigro RJ, Sung YS, et al. Identification of recurrent NAB2-STAT6 gene fusions in solitary fibrous tumor by integrative sequencing. Nat Genet. 2013;45(2):180–5.
38. Doyle LA, Tao D, Marino-Enriquez A. STAT6 is amplified in a subset of dedifferentiated liposarcoma. Mod Pathol. 2014;27(9):1231–7.
39. Mantilla JG, Ricciotti RW, Chen EY, Liu YJ, Hoch BL. Amplification of DNA damage-inducible transcript 3 (DDIT3) is associated with myxoid liposarcoma-like morphology and homologous lipoblastic differentiation in dedifferentiated liposarcoma. Mod Pathol. 2019;32(4):585–92.
40. Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature. 1993;363(6430):640–4.
41. Panagopoulos I, Lassen C, Isaksson M, Mitelman F, Mandahl N, Aman P. Characteristic sequence motifs at the breakpoints of the hybrid genes FUS/CHOP, EWS/CHOP and FUS/ERG in myxoid liposarcoma and acute myeloid leukemia. Oncogene. 1997;15(11):1357–62.

42. Narendra S, Valente A, Tull J, Zhang S. DDIT3 gene break-apart as a molecular marker for diagnosis of myxoid liposarcoma – assay validation and clinical experience. Diagn Mol Pathol. 2011;20(4):218–24.
43. Koelsche C, Renner M, Hartmann W, Brandt R, Lehner B, Waldburger N, et al. TERT promoter hotspot mutations are recurrent in myxoid liposarcomas but rare in other soft tissue sarcoma entities. J Exp Clin Cancer Res. 2014;33:33.
44. Trautmann M, Cyra M, Isfort I, Jeiler B, Kruger A, Grunewald I, et al. Phosphatidylinositol-3-kinase (PI3K)/Akt signaling is functionally essential in myxoid liposarcoma. Mol Cancer Ther. 2019;18(4):834–44.
45. Antonescu CR, Tschernyavsky SJ, Decuseara R, Leung DH, Woodruff JM, Brennan MF, et al. Prognostic impact of P53 status, TLS-CHOP fusion transcript structure, and histological grade in myxoid liposarcoma: a molecular and clinicopathologic study of 82 cases. Clin Cancer Res. 2001;7(12):3977–87.
46. Huang SC, Zhang L, Sung YS, Chen CL, Krausz T, Dickson BC, et al. Frequent FOS gene rearrangements in epithelioid hemangioma: a molecular study of 58 cases with morphologic reappraisal. Am J Surg Pathol. 2015;39(10):1313–21.
47. Adam J, Adamova D, Aggarwal MM, Rinella GA, Agnello M, Agrawal N, et al. Measurement of pion, kaon and proton production in proton-proton collisions at [formula: see text] TeV. Eur Phys J C Part Fields. 2015;75(5):226.
48. van IDG, de Jong D, Romagosa C, Picci P, Benassi MS, Gambarotti M, et al. Fusion events lead to truncation of FOS in epithelioid hemangioma of bone. Genes Chromosomes Cancer. 2015;54(9):565–74.
49. Agaram NP, Zhang L, Cotzia P, Antonescu CR. Expanding the Spectrum of genetic alterations in pseudomyogenic hemangioendothelioma with recurrent novel ACTB-FOSB gene fusions. Am J Surg Pathol. 2018;42(12):1653–61.
50. Antonescu CR, Chen HW, Zhang L, Sung YS, Panicek D, Agaram NP, et al. ZFP36-FOSB fusion defines a subset of epithelioid hemangioma with atypical features. Genes Chromosomes Cancer. 2014;53(11):951–9.
51. Billings SD, Folpe AL, Weiss SW. Epithelioid sarcoma-like hemangioendothelioma. Am J Surg Pathol. 2003;27(1):48–57.
52. Hornick JL, Fletcher CD. Pseudomyogenic hemangioendothelioma: a distinctive, often multicentric tumor with indolent behavior. Am J Surg Pathol. 2011;35(2):190–201.
53. Walther C, Tayebwa J, Lilljebjorn H, Magnusson L, Nilsson J, von Steyern FV, et al. A novel SERPINE1-FOSB fusion gene results in transcriptional up-regulation of FOSB in pseudomyogenic haemangioendothelioma. J Pathol. 2014;232(5):534–40.
54. Weiss SW, Enzinger FM. Epithelioid hemangioendothelioma: a vascular tumor often mistaken for a carcinoma. Cancer. 1982;50(5):970–81.
55. Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, et al. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. Genes Chromosomes Cancer. 2011;50(8):644–53.
56. Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Genes Chromosomes Cancer. 2013;52(8):775–84.
57. Doyle LA, Fletcher CD, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. Am J Surg Pathol. 2016;40(1):94–102.
58. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. Genes Chromosomes Cancer. 2011;50(1):25–33.
59. Ginter PS, Mosquera JM, MacDonald TY, D'Alfonso TM, Rubin MA, Shin SJ. Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. Hum Pathol. 2014;45(4):709–16.
60. Shon W, Sukov WR, Jenkins SM, Folpe AL. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. Mod Pathol. 2014;27(4):509–15.
61. Newton WA Jr, Gehan EA, Webber BL, Marsden HB, van Unnik AJ, Hamoudi AB, et al. Classification of rhabdomyosarcomas and related sarcomas. Pathologic aspects and proposal for a new classification – an intergroup rhabdomyosarcoma study. Cancer. 1995;76(6):1073–85.
62. Rudzinski ER, Anderson JR, Chi YY, et al. Histology, fusion status, and outcome in metastatic rhabdomyosarcoma: A report from the Children's Oncology Group. Pediatr Blood Cancer. 2017;64(12):10.1002/pbc.26645. https://doi.org/10.1002/pbc.26645.
63. Hachitanda Y, Toyoshima S, Akazawa K, Tsuneyoshi M. N-myc gene amplification in rhabdomyosarcoma detected by fluorescence in situ hybridization: its correlation with histologic features. Mod Pathol. 1998;11(12):1222–7.
64. Ragazzini P, Gamberi G, Pazzaglia L, Serra M, Magagnoli G, Ponticelli F, et al. Amplification of CDK4, MDM2, SAS and GLI genes in leiomyosarcoma, alveolar and embryonal rhabdomyosarcoma. Histol Histopathol. 2004;19(2):401–11.
65. Duan F, Smith LM, Gustafson DM, Zhang C, Dunlevy MJ, Gastier-Foster JM, et al. Genomic and clinical analysis of fusion gene amplification in rhabdomyosarcoma: a report from the children's oncology group. Genes Chromosomes Cancer. 2012;51(7):662–74.
66. Downs-Kelly E, Shehata BM, Lopez-Terrada D, Weaver J, Patel RM, Hartke M, et al. The utility of FOXO1 fluorescence in situ hybridization (FISH) in formalin-fixed paraffin-embedded specimens in the diagnosis of alveolar rhabdomyosarcoma. Diagn Mol Pathol. 2009;18(3):138–43.
67. Barr FG, Qualman SJ, Macris MH, Melnyk N, Lawlor ER, Strzelecki DM, et al. Genetic heterogeneity in the alveolar rhabdomyosarcoma subset without typical gene fusions. Cancer Res. 2002;62(16):4704–10.
68. van Gaal JC, Flucke UE, Roeffen MH, de Bont ES, Sleijfer S, Mavinkurve-Groothuis AM, et al. Anaplastic lymphoma kinase aberrations in rhabdomyosarcoma: clinical and prognostic implications. J Clin Oncol. 2012;30(3):308–15.
69. Wierdl M, Tsurkan L, Chi L, Hatfield MJ, Tollemar V, Bradley C, et al. Targeting ALK in pediatric RMS does not induce antitumor activity in vivo. Cancer Chemother Pharmacol. 2018;82(2):251–63.
70. Rudzinski ER. Histology and fusion status in rhabdomyosarcoma. Am Soc Clin Oncol Educ Book. 2013:425–8.
71. Mosquera JM, Sboner A, Zhang L, Kitabayashi N, Chen CL, Sung YS, et al. Recurrent NCOA2 gene rearrangements in congenital/infantile spindle cell rhabdomyosarcoma. Genes Chromosomes Cancer. 2013;52(6):538–50.
72. Alaggio R, Zhang L, Sung YS, Huang SC, Chen CL, Bisogno G, et al. A molecular study of pediatric spindle and sclerosing rhabdomyosarcoma: identification of novel and recurrent VGLL2-related fusions in infantile cases. Am J Surg Pathol. 2016;40(2):224–35.
73. Szuhai K, de Jong D, Leung WY, Fletcher CD, Hogendoorn PC. Transactivating mutation of the MYOD1 gene is a frequent event in adult spindle cell rhabdomyosarcoma. J Pathol. 2014;232(3):300–7.
74. Agaram NP, Chen CL, Zhang L, LaQuaglia MP, Wexler L, Antonescu CR. Recurrent MYOD1 mutations in pediatric and adult sclerosing and spindle cell rhabdomyosarcomas: evidence for a common pathogenesis. Genes Chromosomes Cancer. 2014;53(9):779–87.

75. Martinez AP, Fritchie KJ, Weiss SW, Agaimy A, Haller F, Huang HY, et al. Histiocyte-rich rhabdomyoblastic tumor: rhabdomyosarcoma, rhabdomyoma, or rhabdomyoblastic tumor of uncertain malignant potential? A histologically distinctive rhabdomyoblastic tumor in search of a place in the classification of skeletal muscle neoplasms. Mod Pathol. 2019;32(3):446–57.
76. Bourgeau M, Martinez AP. Histiocyte-rich rhabdomyoblastic tumor: a report of two cases and a review of the differential diagnoses. Virchows Arch. 2021;478(2):367–73.
77. Fanburg-Smith JC, Miettinen M. Angiomatoid "malignant" fibrous histiocytoma: a clinicopathologic study of 158 cases and further exploration of the myoid phenotype. Hum Pathol. 1999;30(11):1336–43.
78. Antonescu CR, Dal Cin P, Nafa K, Teot LA, Surti U, Fletcher CD, et al. EWSR1-CREB1 is the predominant gene fusion in angiomatoid fibrous histiocytoma. Genes Chromosomes Cancer. 2007;46(12):1051–60.
79. Tanas MR, Rubin BP, Montgomery EA, Turner SL, Cook JR, Tubbs RR, et al. Utility of FISH in the diagnosis of angiomatoid fibrous histiocytoma: a series of 18 cases. Mod Pathol. 2010;23(1):93–7.
80. Cheah AL, Zou Y, Lanigan C, Billings SD, Rubin BP, Hornick JL, et al. ALK expression in angiomatoid fibrous histiocytoma: a potential diagnostic pitfall. Am J Surg Pathol. 2019;43(1):93–101.
81. Ladanyi M, Antonescu CR, Leung DH, Woodruff JM, Kawai A, Healey JH, et al. Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. Cancer Res. 2002;62(1):135–40.
82. Pelmus M, Guillou L, Hostein I, Sierankowski G, Lussan C, Coindre JM. Monophasic fibrous and poorly differentiated synovial sarcoma: immunohistochemical reassessment of 60 t(X;18)(SYT-SSX)-positive cases. Am J Surg Pathol. 2002;26(11):1434–40.
83. Amary MF, Berisha F, Bernardi Fdel C, Herbert A, James M, Reis-Filho JS, et al. Detection of SS18-SSX fusion transcripts in formalin-fixed paraffin-embedded neoplasms: analysis of conventional RT-PCR, qRT-PCR and dual color FISH as diagnostic tools for synovial sarcoma. Mod Pathol. 2007;20(4):482–96.
84. Storlazzi CT, Mertens F, Mandahl N, Gisselsson D, Isaksson M, Gustafson P, et al. A novel fusion gene, SS18L1/SSX1, in synovial sarcoma. Genes Chromosomes Cancer. 2003;37(2):195–200.
85. Wang H, Jacobson A, Harmon DC, Choy E, Hornicek FJ, Raskin KA, et al. Prognostic factors in alveolar soft part sarcoma: a SEER analysis. J Surg Oncol. 2016;113(5):581–6.
86. Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, et al. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene. 2001;20(1):48–57.
87. Argani P, Lal P, Hutchinson B, Lui MY, Reuter VE, Ladanyi M. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. Am J Surg Pathol. 2003;27(6):750–61.
88. Enzinger FM. Clear-cell sarcoma of tendons and aponeuroses. An analysis of 21 cases. Cancer. 1965;18:1163–74.
89. Hisaoka M, Ishida T, Kuo TT, Matsuyama A, Imamura T, Nishida K, et al. Clear cell sarcoma of soft tissue: a clinicopathologic, immunohistochemical, and molecular analysis of 33 cases. Am J Surg Pathol. 2008;32(3):452–60.
90. Panagopoulos I, Mertens F, Debiec-Rychter M, Isaksson M, Limon J, Kardas I, et al. Molecular genetic characterization of the EWS/ATF1 fusion gene in clear cell sarcoma of tendons and aponeuroses. Int J Cancer. 2002;99(4):560–7.
91. Park BM, Jin SA, Choi YD, Shin SH, Jung ST, Lee JB, et al. Two cases of clear cell sarcoma with different clinical and genetic features: cutaneous type with BRAF mutation and subcutaneous type with KIT mutation. Br J Dermatol. 2013;169(6):1346–52.
92. Enzinger FM, Shiraki M. Extraskeletal myxoid chondrosarcoma. An analysis of 34 cases. Hum Pathol. 1972;3(3):421–35.
93. Panagopoulos I, Mertens F, Isaksson M, Domanski HA, Brosjo O, Heim S, et al. Molecular genetic characterization of the EWS/CHN and RBP56/CHN fusion genes in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer. 2002;35(4):340–52.
94. Agaram NP, Zhang L, Sung YS, Singer S, Antonescu CR. Extraskeletal myxoid chondrosarcoma with non-EWSR1-NR4A3 variant fusions correlate with rhabdoid phenotype and high-grade morphology. Hum Pathol. 2014;45(5):1084–91.
95. Hisaoka M, Ishida T, Imamura T, Hashimoto H. TFG is a novel fusion partner of NOR1 in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer. 2004;40(4):325–8.
96. Urbini M, Astolfi A, Pantaleo MA, Serravalle S, Dei Tos AP, Picci P, et al. HSPA8 as a novel fusion partner of NR4A3 in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer. 2017;56(7):582–6.
97. Lae ME, Roche PC, Jin L, Lloyd RV, Nascimento AG. Desmoplastic small round cell tumor: a clinicopathologic, immunohistochemical, and molecular study of 32 tumors. Am J Surg Pathol. 2002;26(7):823–35.
98. Ladanyi M, Gerald W. Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. Cancer Res. 1994;54(11):2837–40.
99. Neuville A, Collin F, Bruneval P, Parrens M, Thivolet F, Gomez-Brouchet A, et al. Intimal sarcoma is the most frequent primary cardiac sarcoma: clinicopathologic and molecular retrospective analysis of 100 primary cardiac sarcomas. Am J Surg Pathol. 2014;38(4):461–9.
100. Meister P, Buckmann FW, Konrad E. Extent and level of fascial involvement in 100 cases with nodular fasciitis. Virchows Arch A Pathol Anat Histol. 1978;380(2):177–85.
101. Montgomery EA, Meis JM. Nodular fasciitis. Its morphologic spectrum and immunohistochemical profile. Am J Surg Pathol. 1991;15(10):942–8.
102. Erickson-Johnson MR, Chou MM, Evers BR, Roth CW, Seys AR, Jin L, et al. Nodular fasciitis: a novel model of transient neoplasia induced by MYH9-USP6 gene fusion. Lab Investig. 2011;91(10):1427–33.
103. Guo R, Wang X, Chou MM, Asmann Y, Wenger DE, Al-Ibraheemi A, et al. PPP6R3-USP6 amplification: novel oncogenic mechanism in malignant nodular fasciitis. Genes Chromosomes Cancer. 2016;55(8):640–9.
104. Carter JM, Wang X, Dong J, Westendorf J, Chou MM, Oliveira AM. USP6 genetic rearrangements in cellular fibroma of tendon sheath. Mod Pathol. 2016;29(8):865–9.
105. Kao YC, Flucke U, Eijkelenboom A, Zhang L, Sung YS, Suurmeijer AJH, et al. Novel EWSR1-SMAD3 gene fusions in a group of acral fibroblastic spindle cell neoplasms. Am J Surg Pathol. 2018;42(4):522–8.
106. Al-Ibraheemi A, Folpe AL, Perez-Atayde AR, Perry K, Hofvander J, Arbajian E, et al. Aberrant receptor tyrosine kinase signaling in lipofibromatosis: a clinicopathological and molecular genetic study of 20 cases. Mod Pathol. 2019;32(3):423–34.
107. Marino-Enriquez A, Fletcher CD. Angiofibroma of soft tissue: clinicopathologic characterization of a distinctive benign fibrovascular neoplasm in a series of 37 cases. Am J Surg Pathol. 2012;36(4):500–8.
108. Jin Y, Moller E, Nord KH, Mandahl N, Von Steyern FV, Domanski HA, et al. Fusion of the AHRR and NCOA2 genes through a recurrent translocation t(5;8)(p15;q13) in soft tissue angiofibroma results in upregulation of aryl hydrocarbon receptor target genes. Genes Chromosomes Cancer. 2012;51(5):510–20.
109. Arbajian E, Magnusson L, Mertens F, Domanski HA, Vult von Steyern F, Nord KH. A novel GTF2I/NCOA2 fusion gene empha-

sizes the role of NCOA2 in soft tissue angiofibroma development. Genes Chromosomes Cancer. 2013;52(3):330–1.
110. Bekers EM, Groenen P, Verdijk MAJ, Raaijmakers-van Geloof WL, Roepman P, Vink R, et al. Soft tissue angiofibroma: Clinicopathologic, immunohistochemical and molecular analysis of 14 cases. Genes Chromosomes Cancer. 2017;56(10):750–7.
111. Crago AM, Denton B, Salas S, Dufresne A, Mezhir JJ, Hameed M, et al. A prognostic nomogram for prediction of recurrence in desmoid fibromatosis. Ann Surg. 2013;258(2):347–53.
112. Lazar AJ, Tuvin D, Hajibashi S, Habeeb S, Bolshakov S, Mayordomo-Aranda E, et al. Specific mutations in the beta-catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol. 2008;173(5):1518–27.
113. Miyaki M, Konishi M, Kikuchi-Yanoshita R, Enomoto M, Tanaka K, Takahashi H, et al. Coexistence of somatic and germ-line mutations of APC gene in desmoid tumors from patients with familial adenomatous polyposis. Cancer Res. 1993;53(21):5079–82.
114. Dadone-Montaudie B, Alberti L, Duc A, Delespaul L, Lesluyes T, Perot G, et al. Alternative PDGFD rearrangements in dermatofibrosarcomas protuberans without PDGFB fusions. Mod Pathol. 2018;31(11):1683–93.
115. Enzinger FM, Smith BH. Hemangiopericytoma. An analysis of 106 cases. Hum Pathol. 1976;7(1):61–82.
116. Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PH. A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet. 1998;18(2):184–7.
117. Flucke U, van Noesel MM, Wijnen M, Zhang L, Chen CL, Sung YS, et al. TFG-MET fusion in an infantile spindle cell sarcoma with neural features. Genes Chromosomes Cancer. 2017;56(9):663–7.
118. Kao YC, Fletcher CDM, Alaggio R, Wexler L, Zhang L, Sung YS, et al. Recurrent BRAF gene fusions in a subset of pediatric spindle cell sarcomas: expanding the genetic spectrum of tumors with overlapping features with infantile fibrosarcoma. Am J Surg Pathol. 2018;42(1):28–38.
119. Suurmeijer AJH, Dickson BC, Swanson D, Zhang L, Sung YS, Cotzia P, et al. A novel group of spindle cell tumors defined by S100 and CD34 co-expression shows recurrent fusions involving RAF1, BRAF, and NTRK1/2 genes. Genes Chromosomes Cancer. 2018;57(12):611–21.
120. Rudzinski ER, Lockwood CM, Stohr BA, Vargas SO, Sheridan R, Black JO, et al. Pan-Trk immunohistochemistry identifies NTRK rearrangements in pediatric mesenchymal tumors. Am J Surg Pathol. 2018;42(7):927–35.
121. Folpe AL, Lane KL, Paull G, Weiss SW. Low-grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes: a clinicopathologic study of 73 cases supporting their identity and assessing the impact of high-grade areas. Am J Surg Pathol. 2000;24(10):1353–60.
122. Mertens F, Fletcher CD, Antonescu CR, Coindre JM, Colecchia M, Domanski HA, et al. Clinicopathologic and molecular genetic characterization of low-grade fibromyxoid sarcoma, and cloning of a novel FUS/CREB3L1 fusion gene. Lab Investig. 2005;85(3):408–15.
123. Lau PP, Lui PC, Lau GT, Yau DT, Cheung ET, Chan JK. EWSR1-CREB3L1 gene fusion: a novel alternative molecular aberration of low-grade fibromyxoid sarcoma. Am J Surg Pathol. 2013;37(5):734–8.
124. Doyle LA, Moller E, Dal Cin P, Fletcher CD, Mertens F, Hornick JL. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. Am J Surg Pathol. 2011;35(5):733–41.
125. Meis-Kindblom JM, Kindblom LG, Enzinger FM. Sclerosing epithelioid fibrosarcoma. A variant of fibrosarcoma simulating carcinoma. Am J Surg Pathol. 1995;19(9):979–93.
126. Arbajian E, Puls F, Magnusson L, Thway K, Fisher C, Sumathi VP, et al. Recurrent EWSR1-CREB3L1 gene fusions in sclerosing epithelioid fibrosarcoma. Am J Surg Pathol. 2014;38(6):801–8.
127. Dewaele B, Libbrecht L, Levy G, Brichard B, Vanspauwen V, Sciot R, et al. A novel EWS-CREB3L3 gene fusion in a mesenteric sclerosing epithelioid fibrosarcoma. Genes Chromosomes Cancer. 2017;56(9):695–9.
128. Arbajian E, Puls F, Antonescu CR, Amary F, Sciot R, Debiec-Rychter M, et al. In-depth genetic analysis of sclerosing epithelioid fibrosarcoma reveals recurrent genomic alterations and potential treatment targets. Clin Cancer Res. 2017;23(23):7426–34.
129. Doyle LA, Wang WL, Dal Cin P, Lopez-Terrada D, Mertens F, Lazar AJ, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. Am J Surg Pathol. 2012;36(10):1444–51.