A PRACTICAL APPROACH TO ROUND CELL SARCOMAS

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Approach to round cell sarcomas

- Pay attention to age
- Pay attention to anatomic site
- Review radiology (bone tumors)
- Look for histologic clues
- Apply immunohistochemistry judiciously
- Apply molecular testing as needed

Typical age ranges for patients with round cell sarcomas

Age	Tumor types
Young children	Neuroblastoma, embryonal RMS
Adolescents	Alveolar RMS, Ewing sarcoma, *BCOR*-rearranged sarcoma, DSRCT
Young adults	Ewing sarcoma, *CIC*-rearranged sarcoma, poorly differentiated synovial sarcoma, DSRCT, mesenchymal chondrosarcoma, alveolar RMS, myxoid (round cell) liposarcoma

DSRCT, desmoplastic small round cell tumor; RMS, rhabdomyosarcoma

Distinctive anatomic sites for selected round cell sarcomas

Tumor type	Anatomic location
Embryonal RMS	Head and neck (orbit), genitourinary tract, retroperitoneum, biliary tree
Alveolar RMS	Head and neck (sinonasal)
DSRCT	Abdominal cavity
Neuroblastoma	Adrenal gland, retroperitoneum

DSRCT, desmoplastic small round cell tumor; RMS, rhabdomyosarcoma

Round cell sarcomas that often arise in bone

Ewing sarcoma

***BCOR*-rearranged sarcoma**

Mesenchymal chondrosarcoma

Histologic clues: distinctive blood vessels

Tumor type	Vascular pattern
Poorly differentiated synovial sarcoma	Staghorn (hemangiopericytoma-like)
Mesenchymal chondrosarcoma	Staghorn (hemangiopericytoma-like)
Myxoid (round cell) liposarcoma	Plexiform (crow’s feet)

Histologic clues: other distinctive features

Tumor type	Feature
CIC-rearranged sarcoma	Variable myxoid stroma
Myxoid (round cell) liposarcoma	Variable myxoid stroma
Alveolar rhabdomyosarcoma	Nested or alveolar architecture
Desmoplastic small round cell tumor	Nested, desmoplastic stroma

IHC in round cell sarcomas

Tumor type	KRT	DES	MYOG MYOD1	CD99	WT1	NKX2.2	ETV4	BCOR	SS18::SSX	DDIT3	PHOX2B
Ewing sarcoma	+	–	–	++	–	++	–	–	–	–	–
CIC-rearranged sarcoma	+	–	–	++	++	–	++	–	–	–	–
Sarcomas with *BCOR* alterations	–	–	–	+	–	–	–	++	–	–	–
PD synovial sarcoma	+	–	–	+	–	–	–	+	++	–	–
Alveolar RMS	+	++	++	+	+	–	–	–	–	–	–
High grade myxoid LPS	–	–	–	–	–	–	–	–	–	++	–
DSRCT	++	++	–	+	++	–	–	–	–	–	–
Neuroblastoma	–	–	–	–	–	–	–	–	–	–	++

Diagnostically useful gene fusions in round cell sarcomas

Tumor type	Gene fusions
Ewing sarcoma	*EWSR1::FLI1, EWSR1::ERG*
CIC-rearranged sarcoma	*CIC::DUX4*
BCOR-rearranged sarcoma	*BCOR::CCNB3*
Alveolar rhabdomyosarcoma	*PAX3::FOXO1, PAX7::FOXO1*
Desmoplastic small round cell tumor	*EWSR1::WT1*
Poorly differentiated synovial sarcoma	*SS18::SSX1, SS18::SSX2*
Myxoid (round cell) liposarcoma	*FUS::DDIT3, EWSR1::DDIT3*

WHO 2013

Tumors of bone

Ewing sarcoma

Tumors of soft tissue

Skeletal-muscle tumors

Embryonal rhabdomyosarcoma

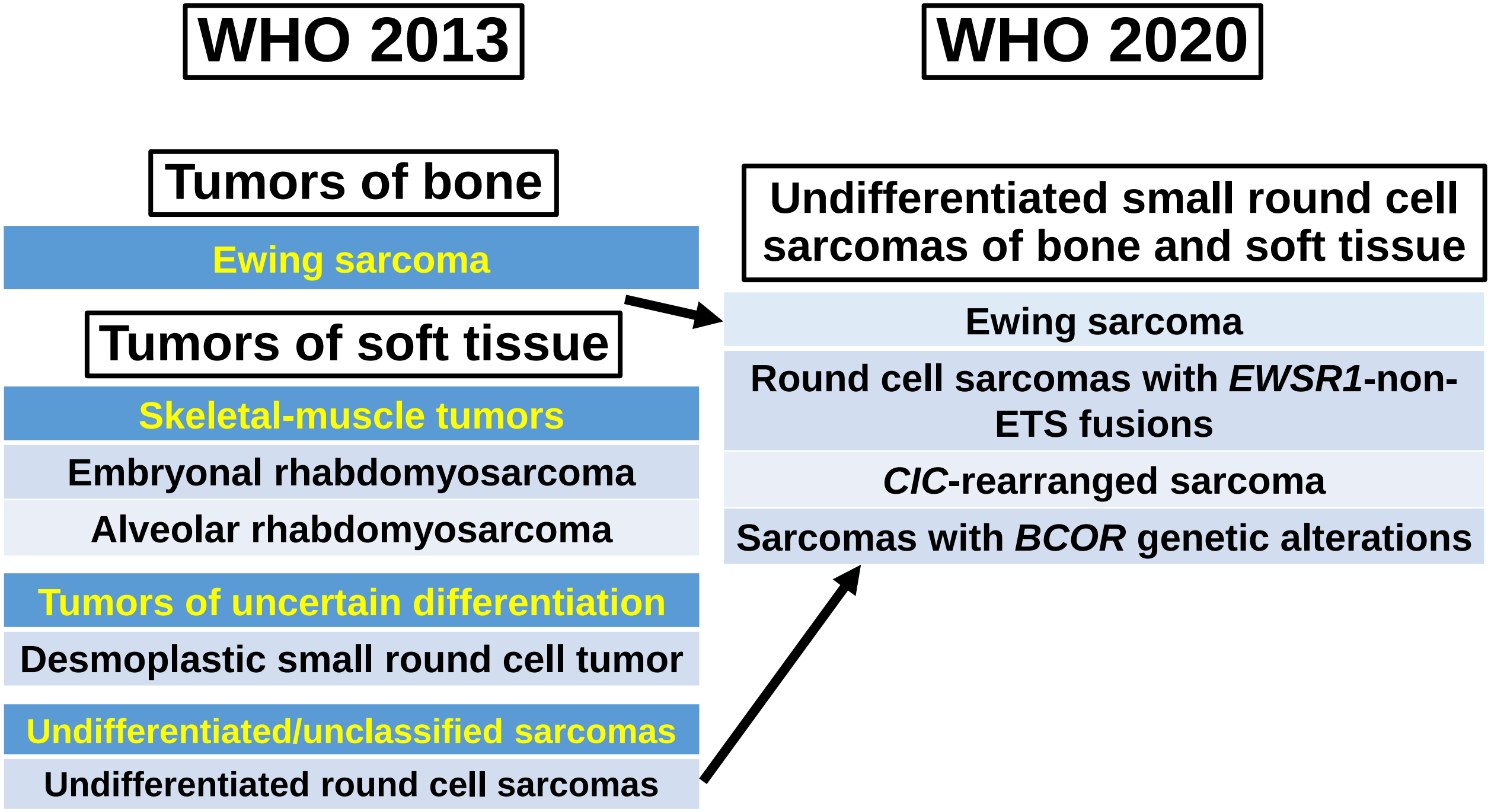
Alveolar rhabdomyosarcoma

Tumors of uncertain differentiation

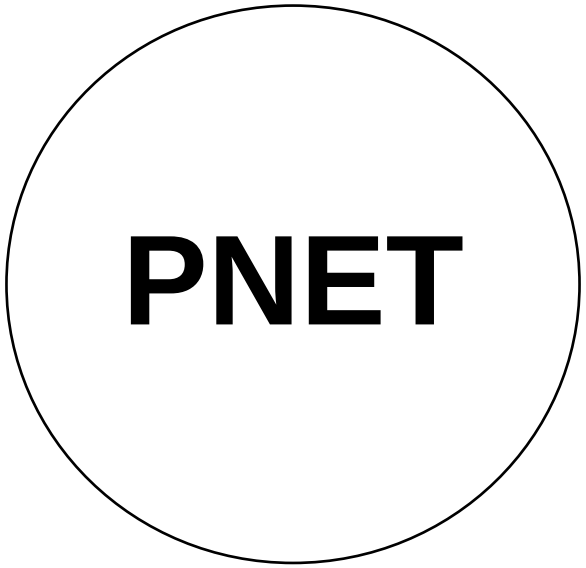
Desmoplastic small round cell tumor

Undifferentiated/unclassified sarcomas

Undifferentiated round cell sarcomas

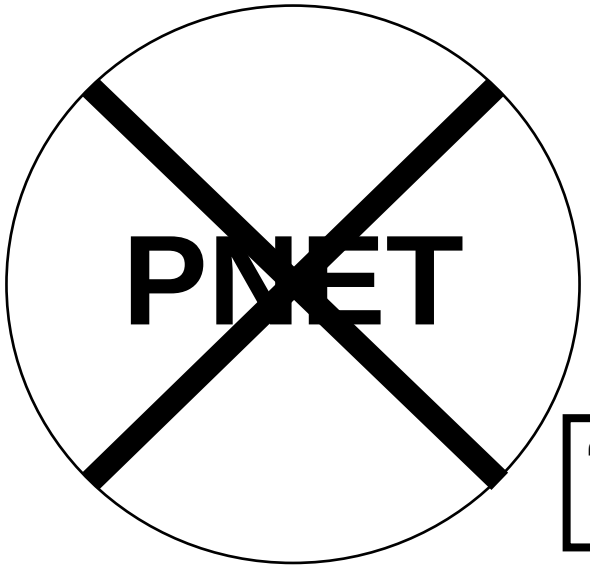

WHO 2013
WHO 2020
Tumors of bone
Ewing sarcoma
Tumors of soft tissue
Skeletal-muscle tumors
Embryonal rhabdomyosarcoma
Alveolar rhabdomyosarcoma
Tumors of uncertain differentiation
Desmoplastic small round cell tumor
Undifferentiated/unclassified sarcomas
Undifferentiated round cell sarcomas
Undifferentiated small round cell sarcomas of bone and soft tissue
Ewing sarcoma
Round cell sarcomas with EWSR1-non-ETS fusions
CIC-rearranged sarcoma
Sarcomas with BCOR genetic alterations

Peripheral PNET



Central PNET

Peripheral PNET → **Ewing sarcoma**



Even in GU and GYN pathology!?

“Embryonic-type” neuroectodermal tumor
Flood et al. *Am J Surg Pathol* 2021

Central PNET →

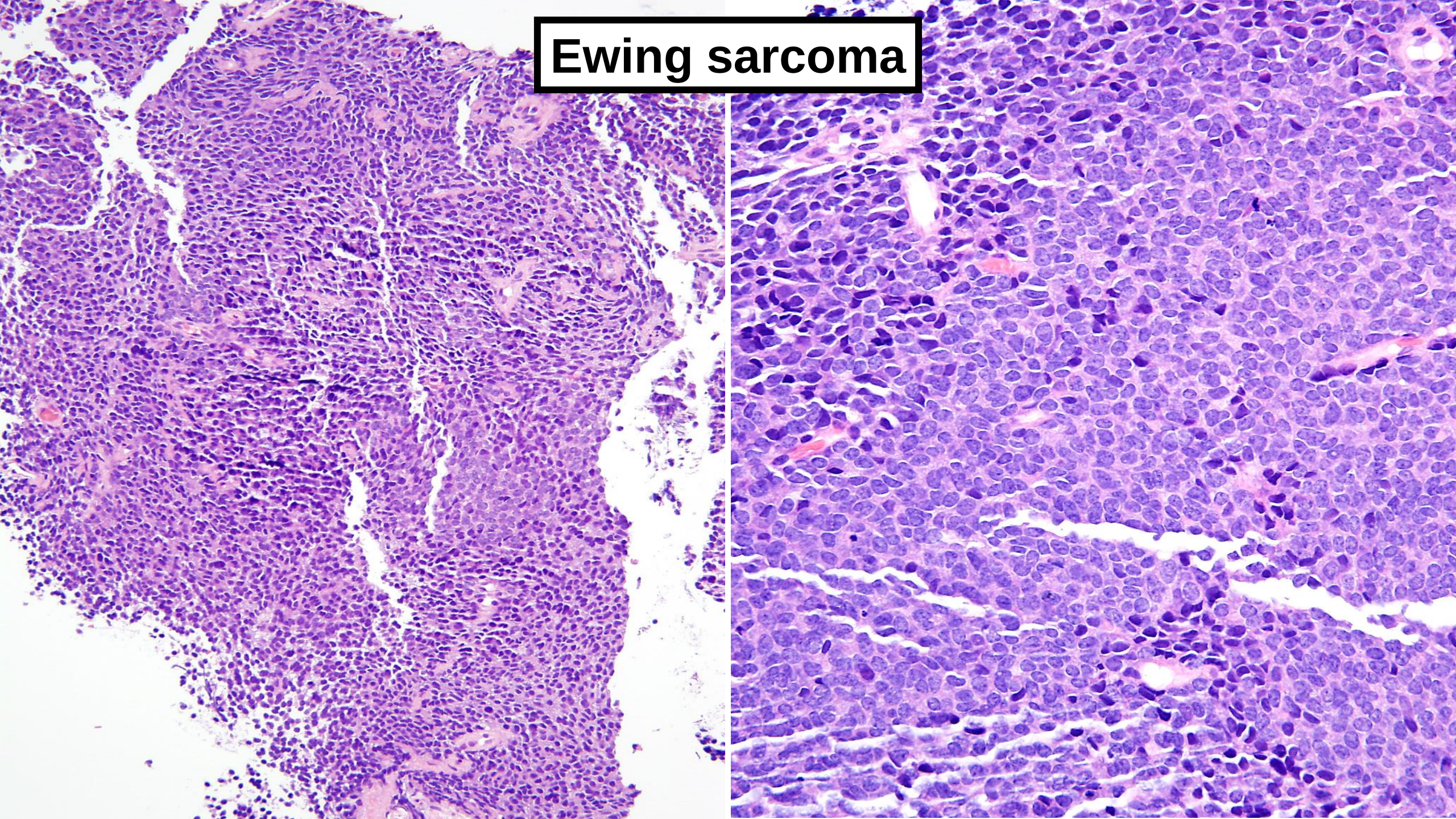
Embryonal tumor with multi-layered rosettes
High-grade gliomas and ependymomas
Atypical teratoid/rhabdoid tumor
CNS neuroblastoma with FOXR2 activation
High-grade neuroepithelial tumor with *MN1* alteration
High-grade neuroepithelial tumor with *BCOR* alteration

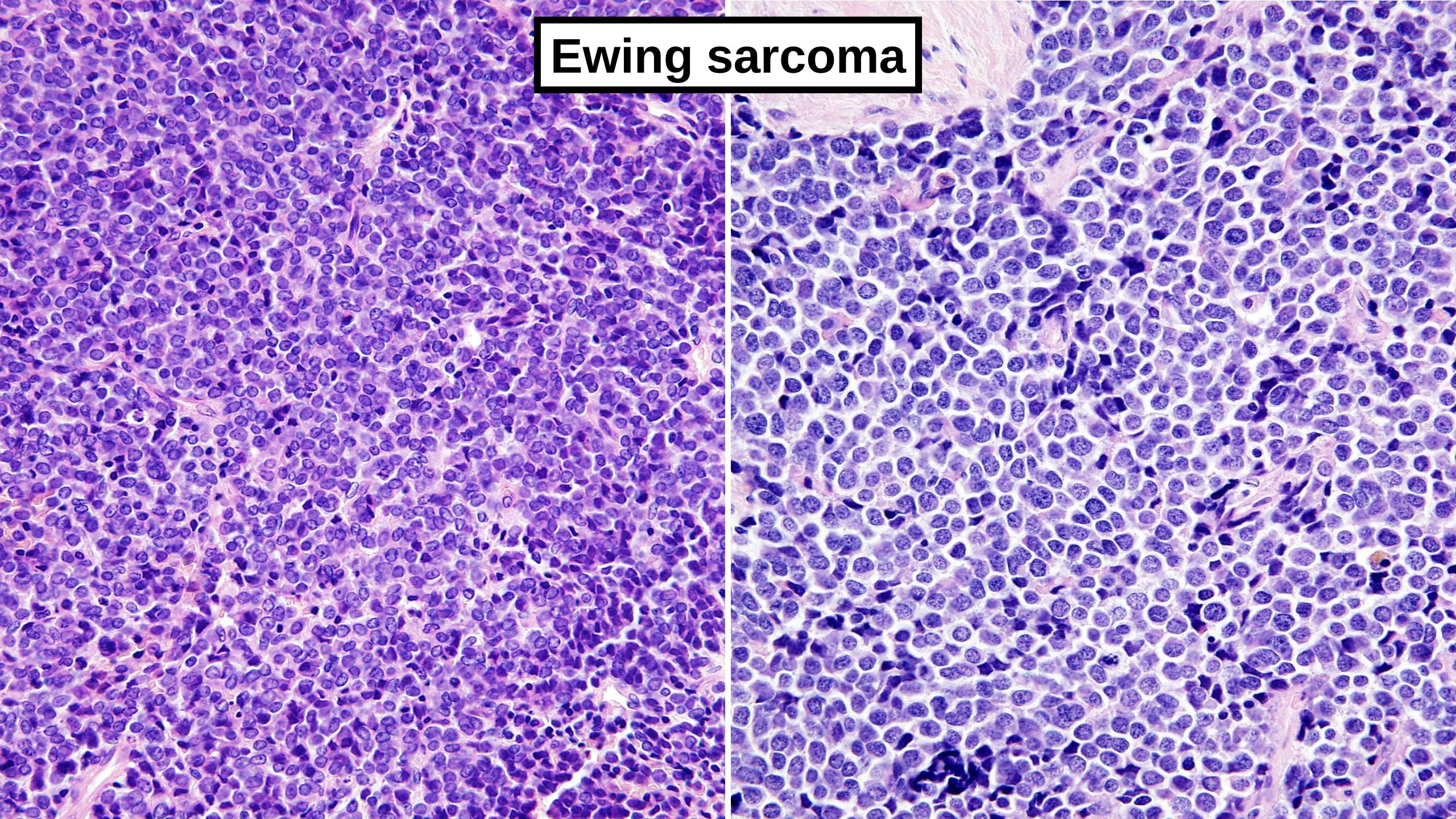
Sturm et al. *Cell* 2016

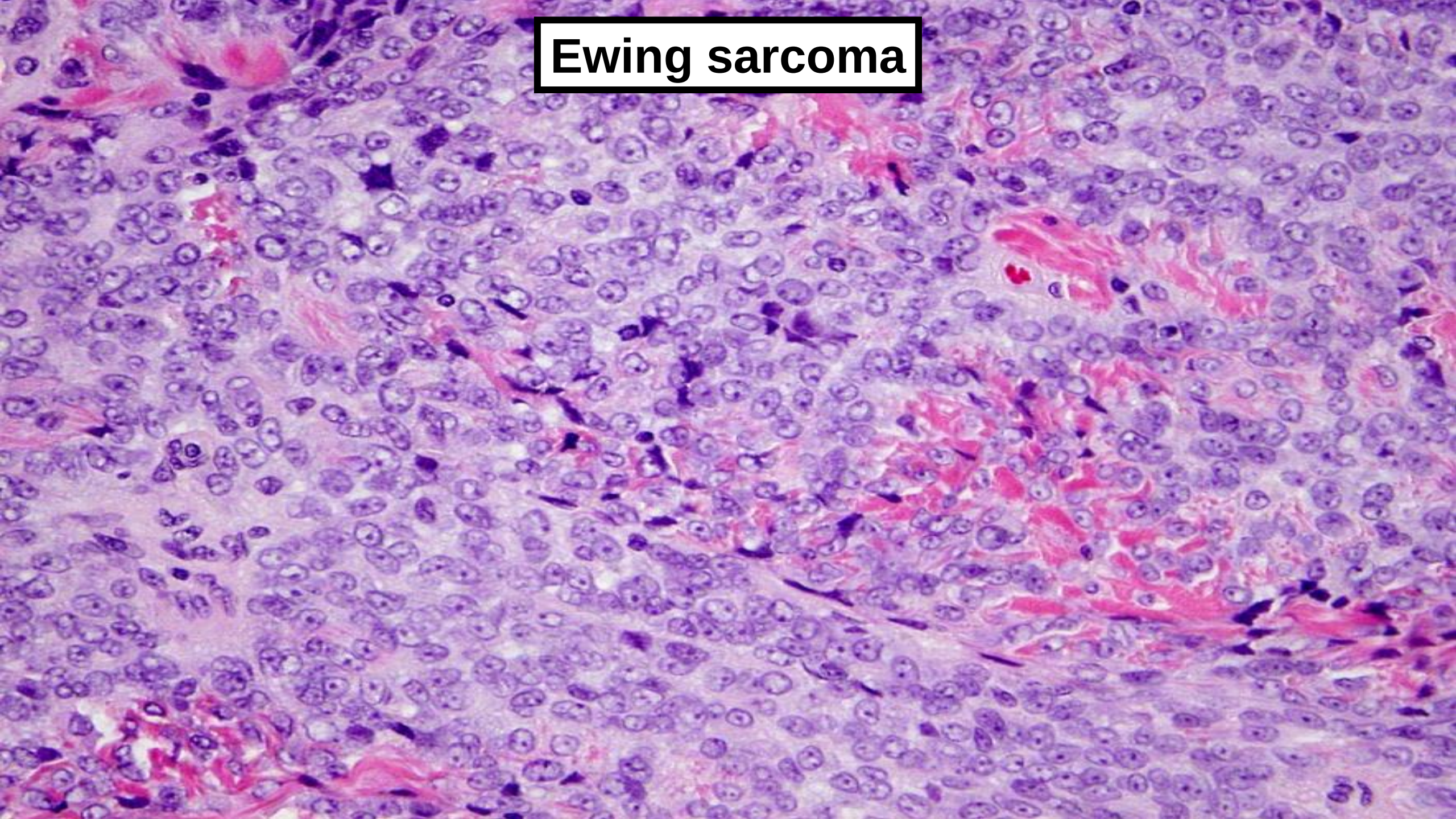
Ewing sarcoma

- Most common in children and young adults
- 2nd most common pediatric sarcoma
- Male predominance
- Presentation in bone more common in young patients
- Soft tissue presentation more common in older patients
- Most common soft tissue sites: lower extremities, paraspinal region, pelvis
- Treated aggressively with combination chemotherapy and surgery and/or radiation therapy

Ewing sarcoma

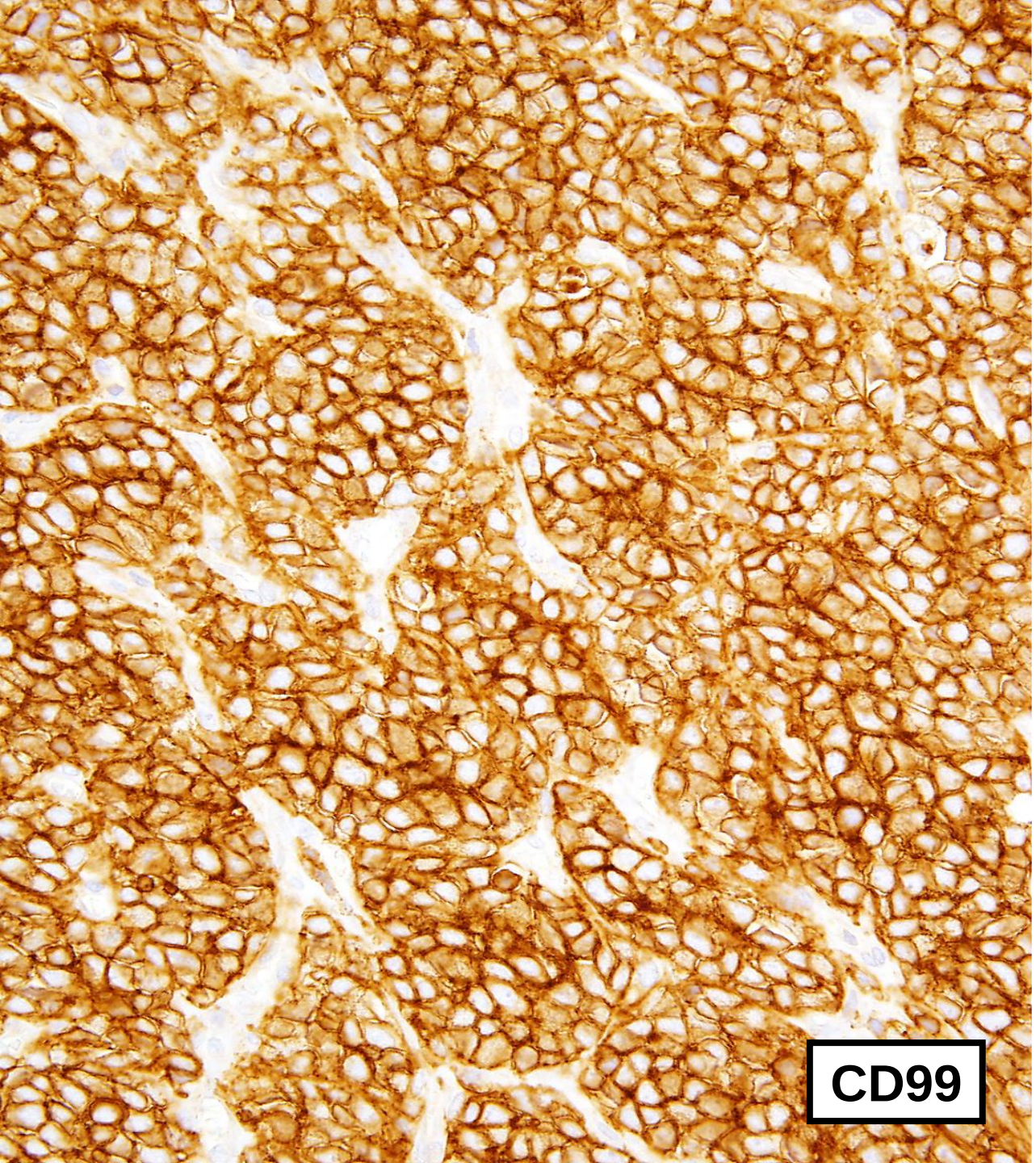


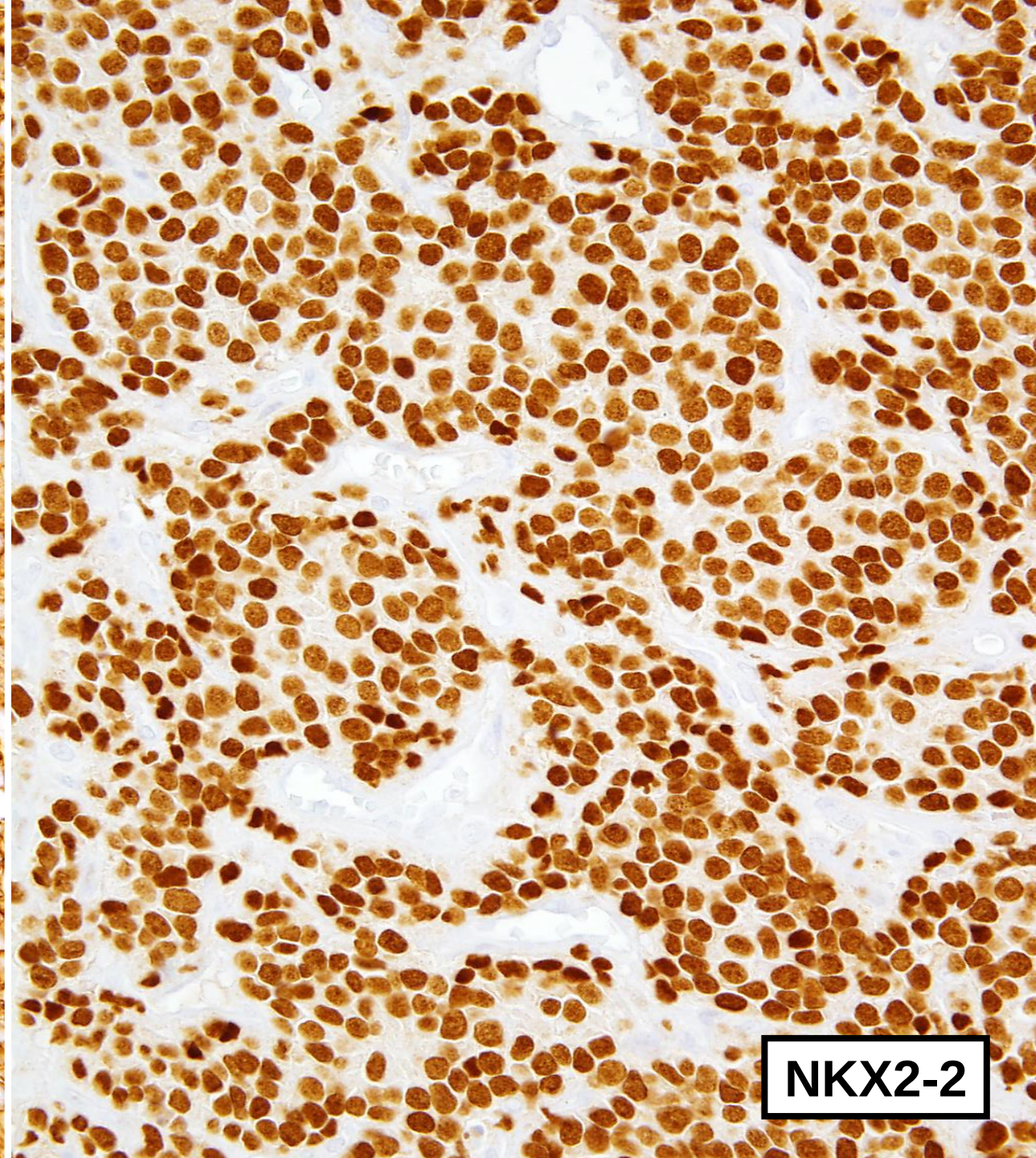

Ewing sarcoma


Ewing sarcoma

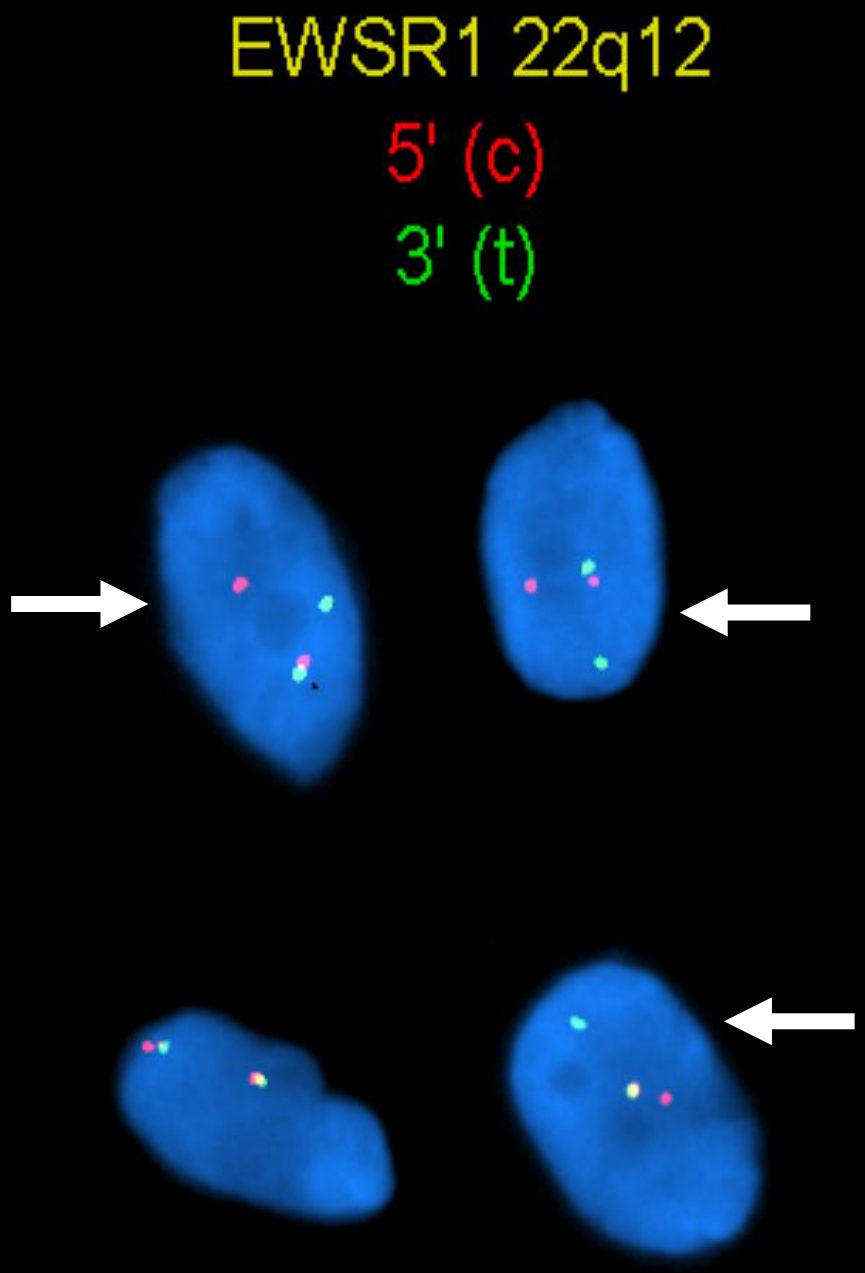
Marker	Positive	Comments
CD99	~100%	Strong, diffuse, membranous
NKX2-2	95%	Strong, diffuse, nuclear
FLI1	95%	Not specific
Keratins	30%	Focal or multifocal

Molecular genetics	
EWSR1::FLI1	90%
EWSR1::ERG	5-10%
Other *EWSR1::ETS* fusions	rare
FUS::ETS fusions	rare


CD99

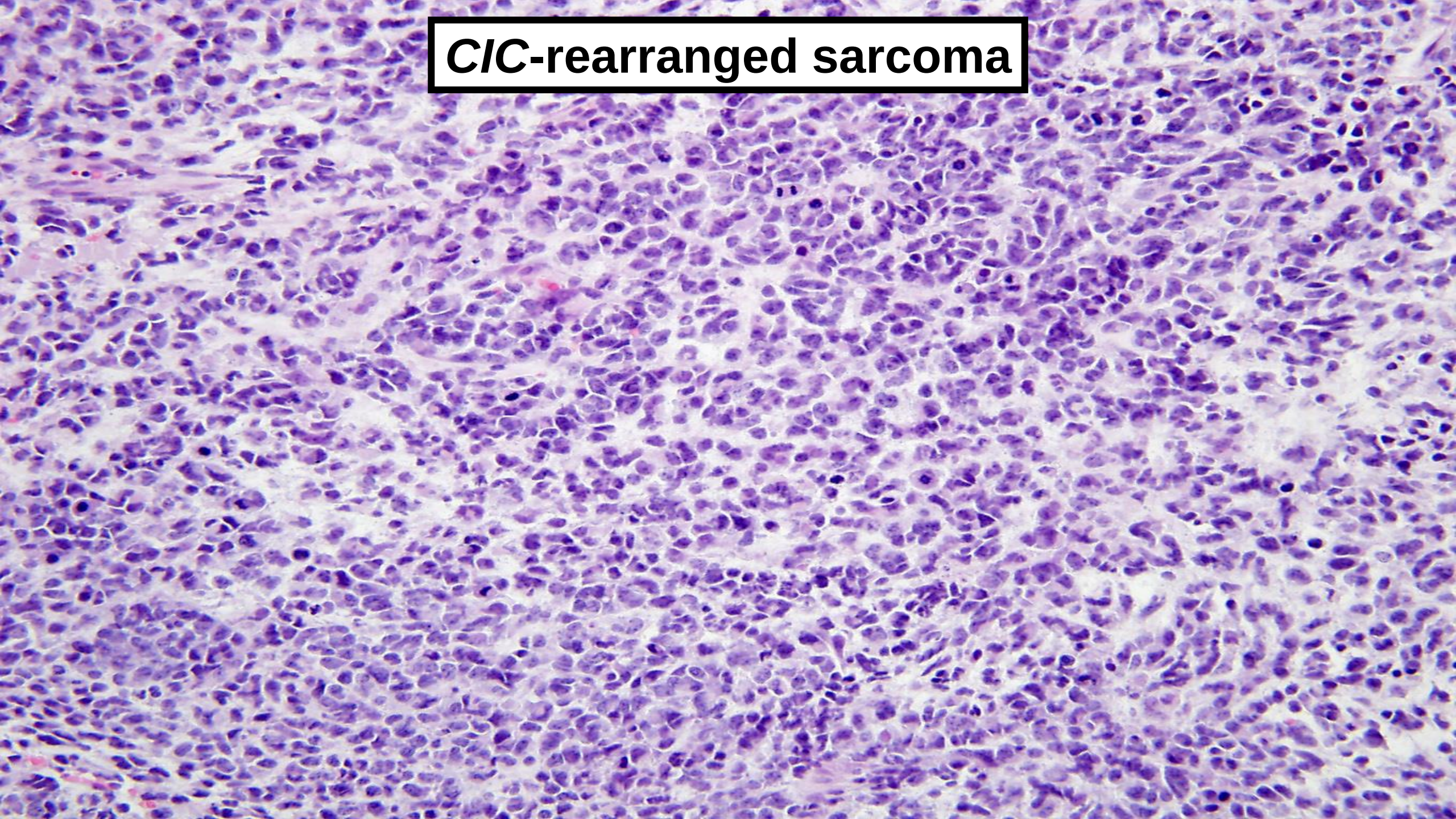

NKX2-2

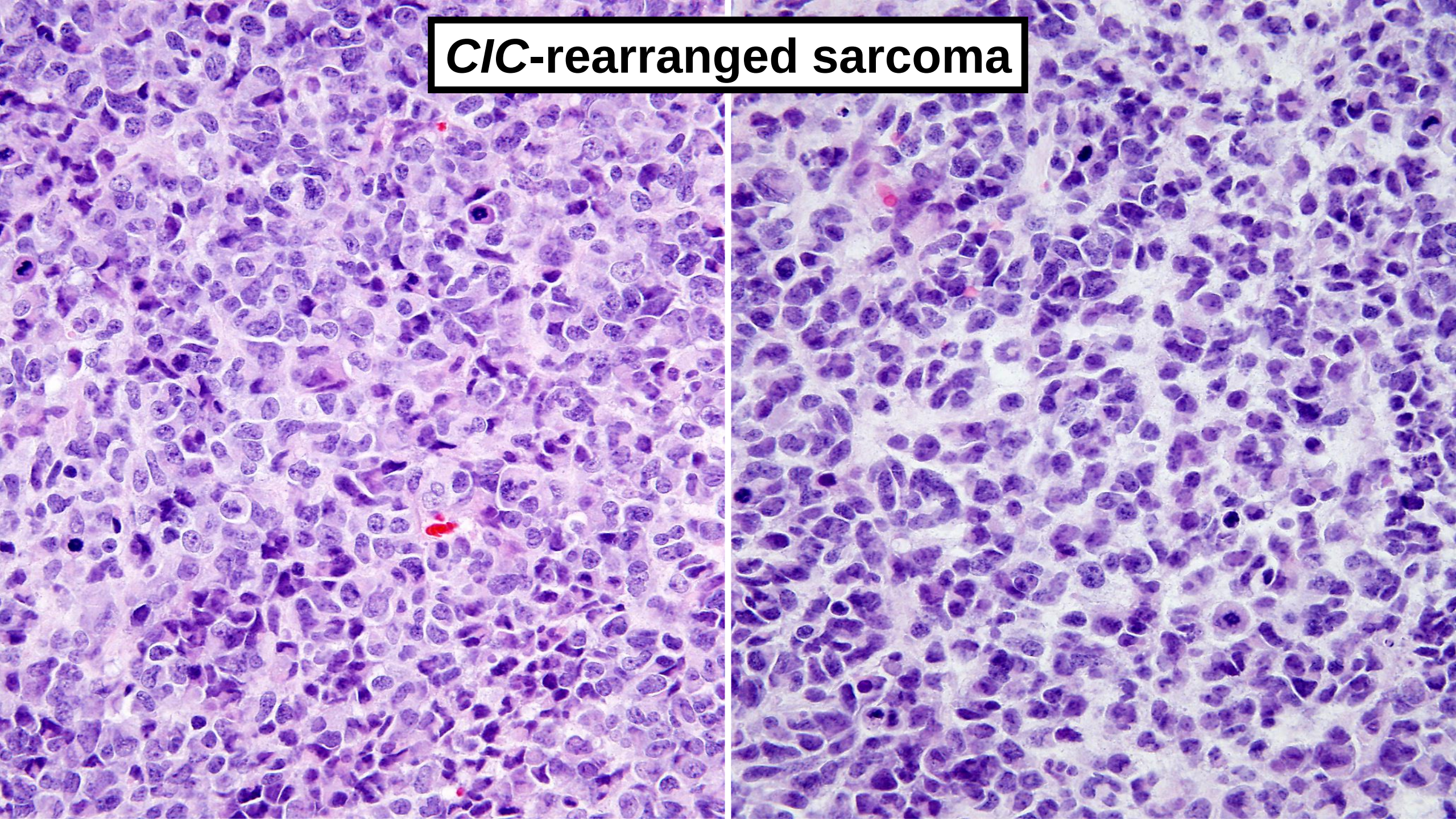
FISH

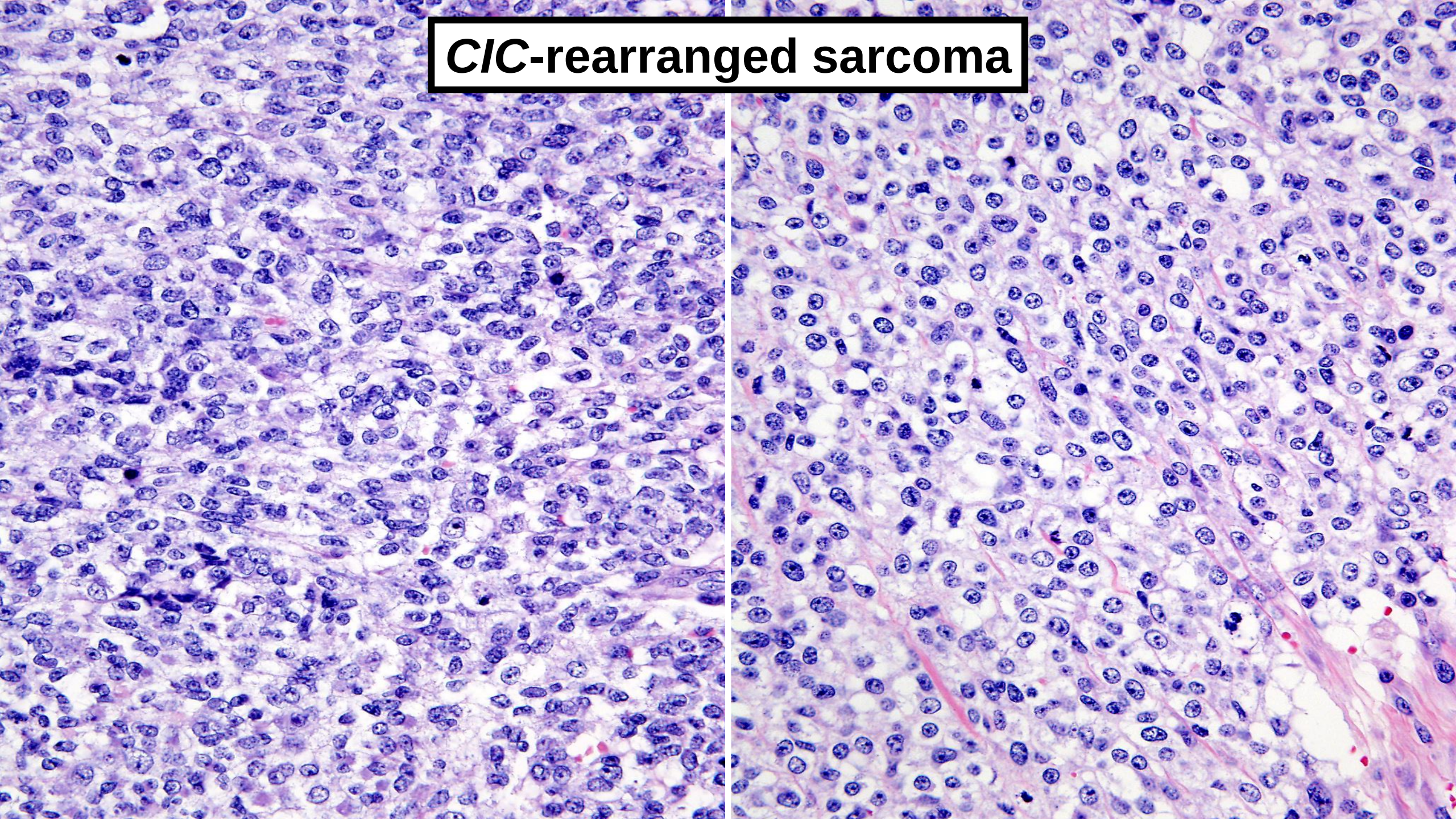


CIC-rearranged sarcoma

- **Most common “Ewing-like” (“undifferentiated”) round cell sarcoma that lacks *EWSR1* gene fusions (at least 70%)**
- **Majority with *CIC::DUX4* fusions**
- **Wide age range; peak in young adults**
- **Deep soft tissue of extremities and trunk most frequent**
- **Bone and visceral sites rare**
- **Much more aggressive clinical course and worse survival than Ewing sarcoma; currently treated similarly**


CIC-rearranged sarcoma


CIC-rearranged sarcoma


CIC-rearranged sarcoma

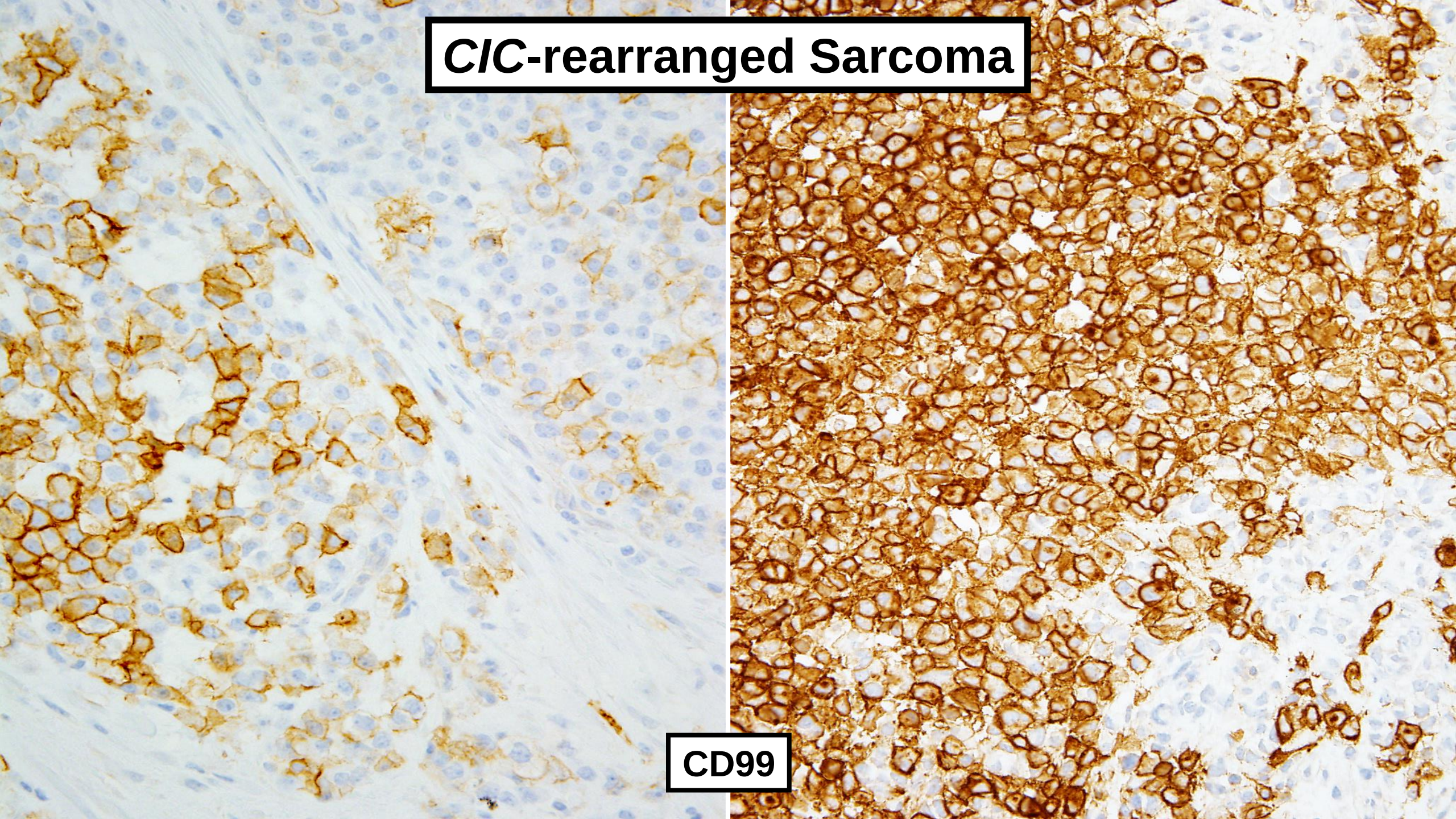
CIC-rearranged sarcoma: IHC

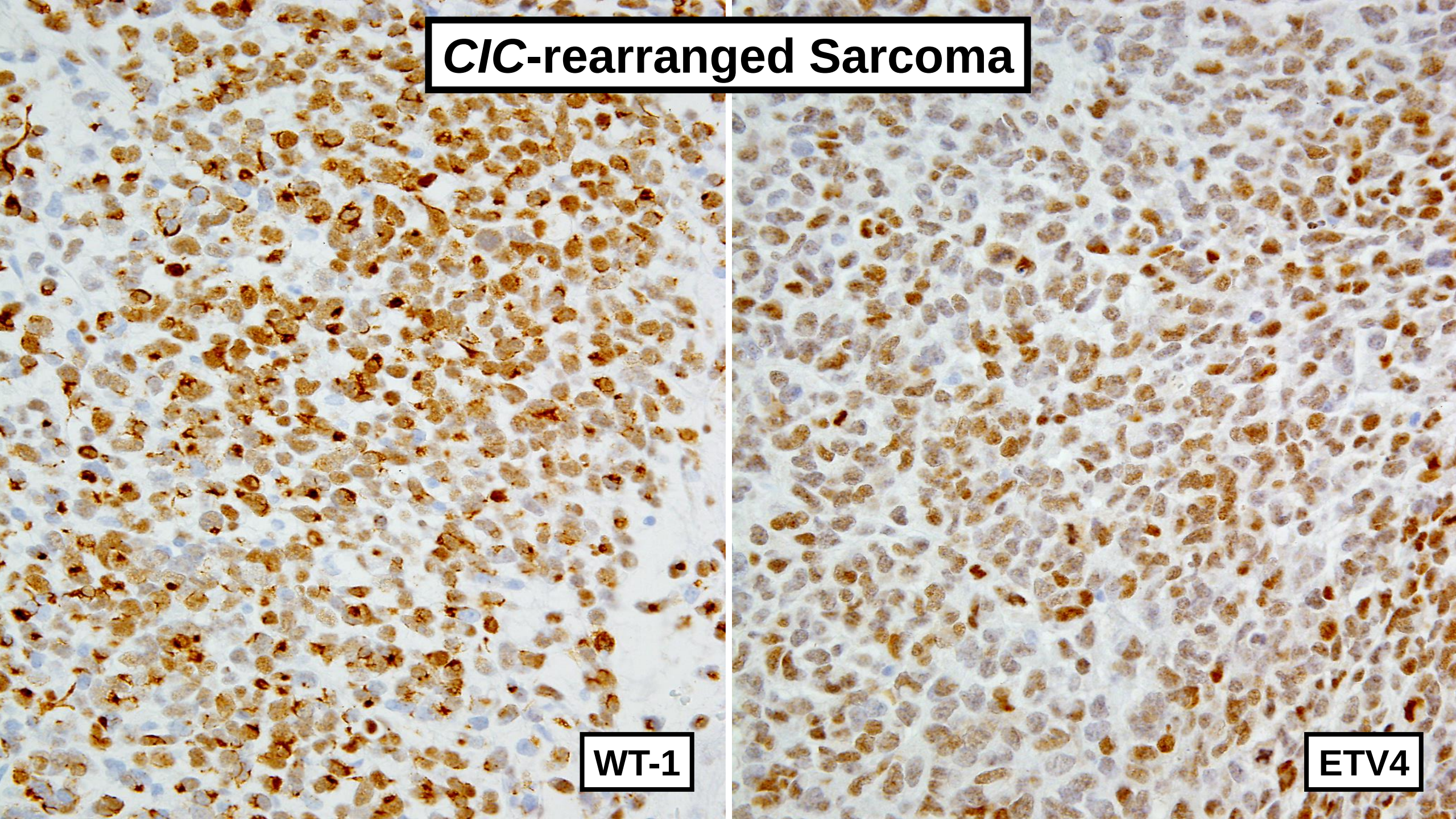
Marker	Positive	Comments
CD99	85%	Usually patchy; 20% diffuse
WT1	90%	Nuclear +/- cytoplasmic
ETV4	95%	Nuclear
Keratins	15%	Focal

Hung et al. *Mod Pathol* 2016

Le Guellec et al. *Mod Pathol* 2016

Antonescu et al. *Am J Surg Pathol* 2017


CIC-rearranged Sarcoma
CD99


CIC-rearranged Sarcoma
WT-1
ETV4

CIC-rearranged sarcoma: Genetics

Gene fusion	Frequency
CIC::DUX4	>95%
CIC::FOXO4	Rare
CIC::LEUTX	Rare
CIC::NUTM1	Rare
CIC::NUTM2A	Rare
CIC::AXL	Rare
CIC::CITED1	Rare
CIC::SYK	Rare

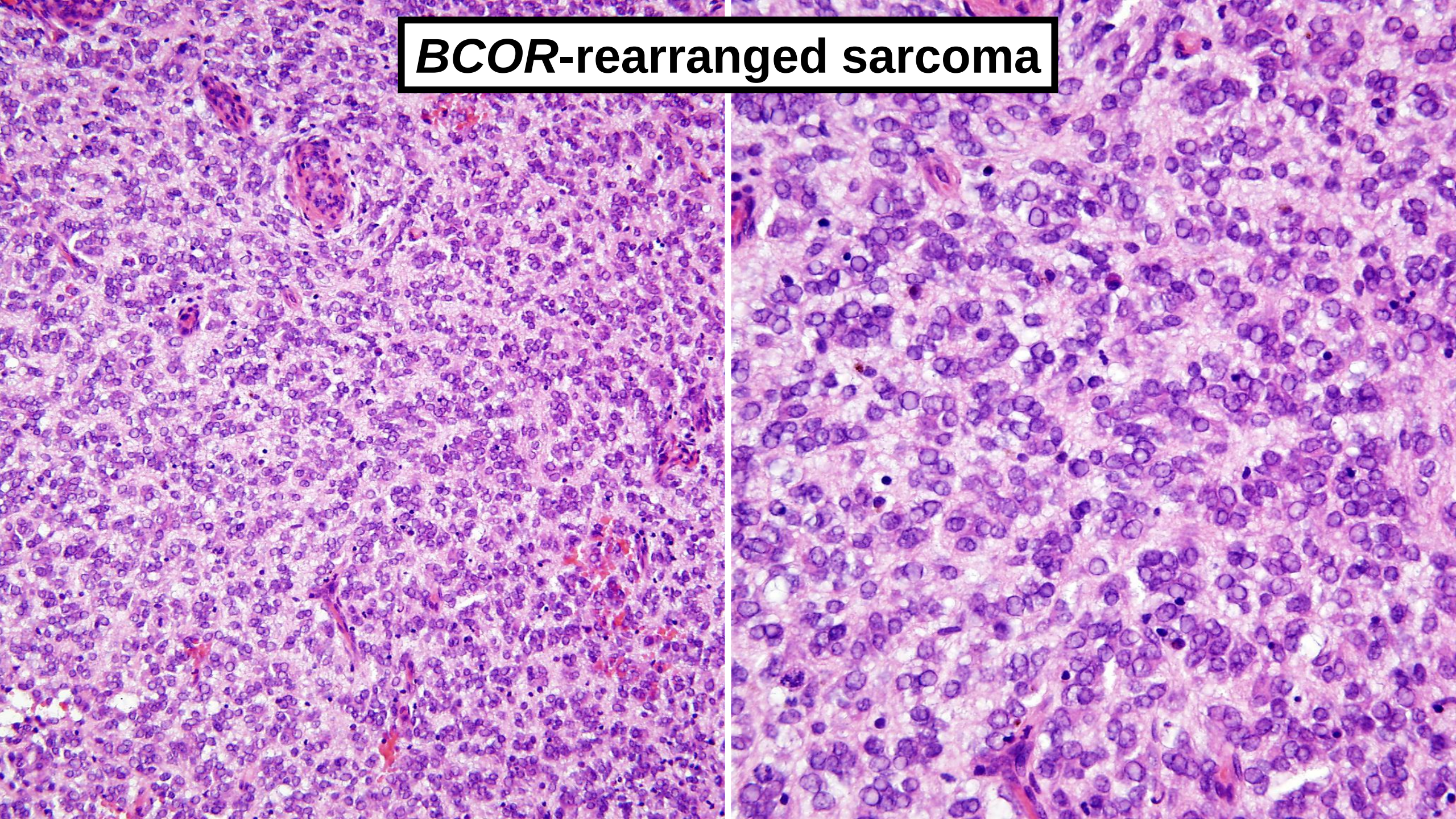
- FISH negative in 15% (cryptic rearrangement)
- Next-generation sequencing can also miss gene fusions
- IHC detection of ETV transcriptional upregulation more sensitive

Antonescu et al. *Am J Surg Pathol* 2017

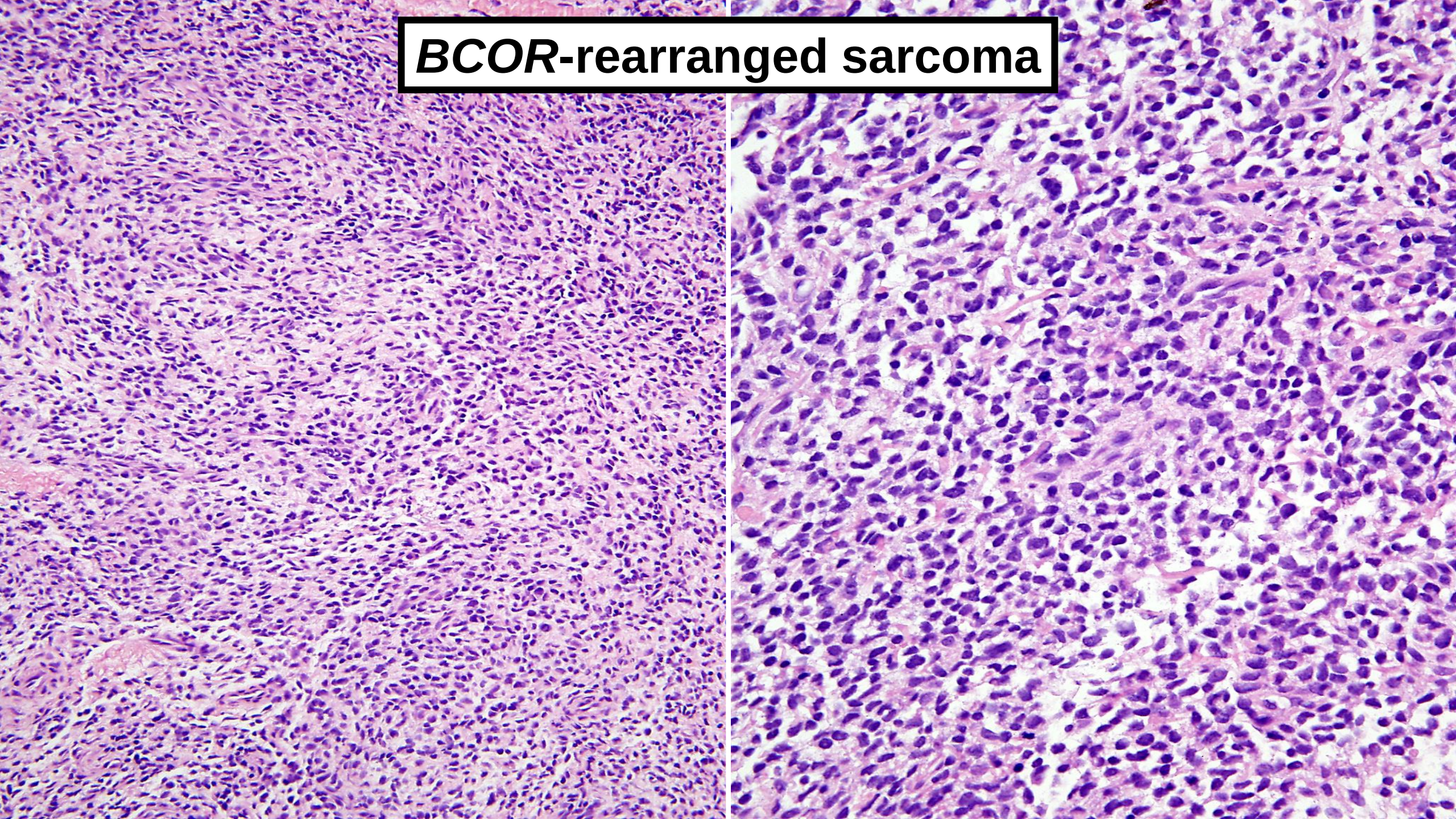
Linos et al. *Mod Pathol* 2023

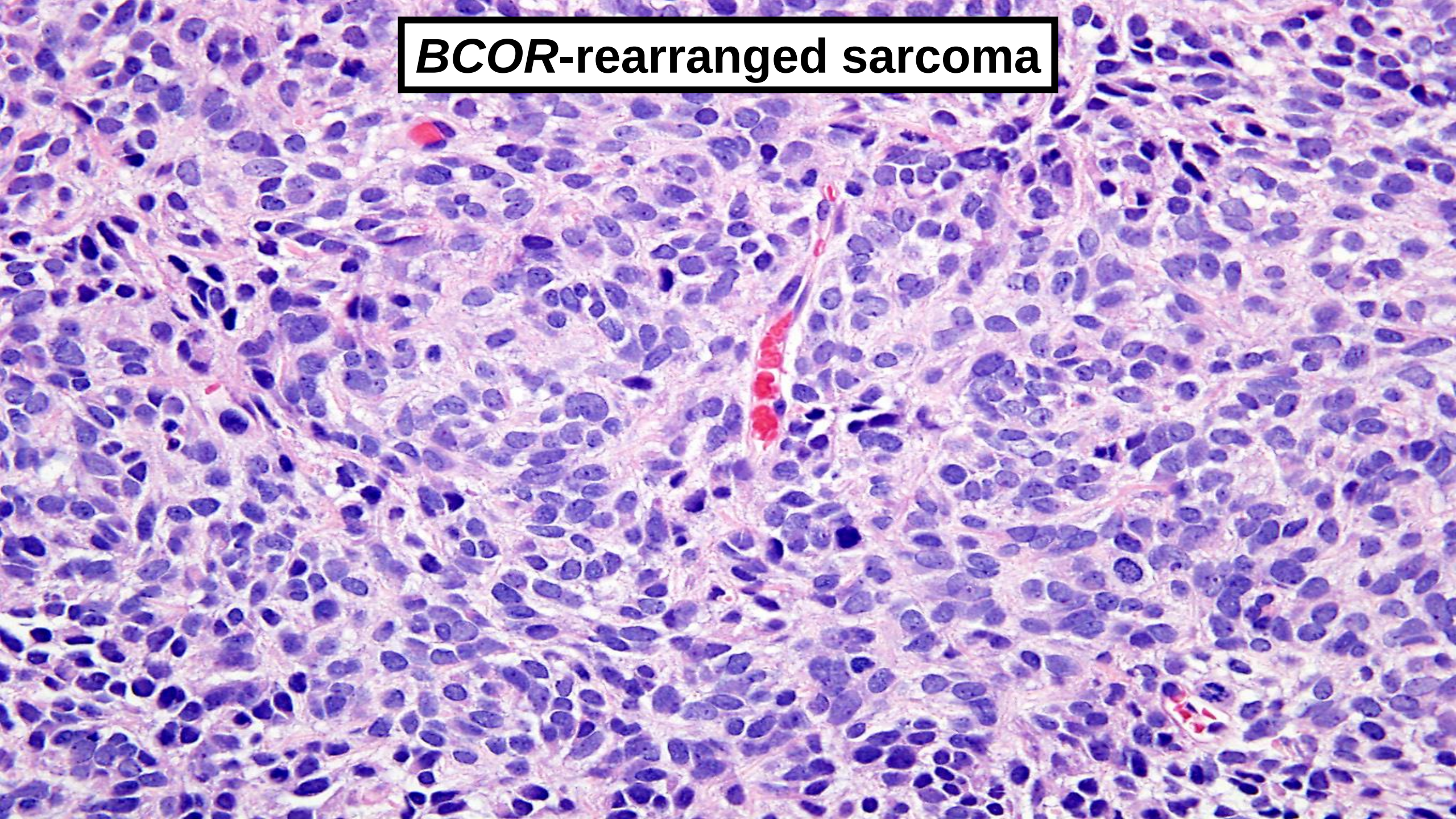
BCOR-rearranged sarcoma

- **Around 5% of undifferentiated round cell sarcomas that lack *EWSR1* gene rearrangements**
- **Most with *BCOR::CCNB3* (90%)**
- **Peak in adolescents and young adults (mean 15 yrs)**
- **Marked male predominance (M:F >30:1)**
- **Bone in 60%; soft tissue in 40% (trunk, extremities > head and neck)**
- **Similar prognosis as Ewing sarcoma**


BCOR-rearranged sarcoma

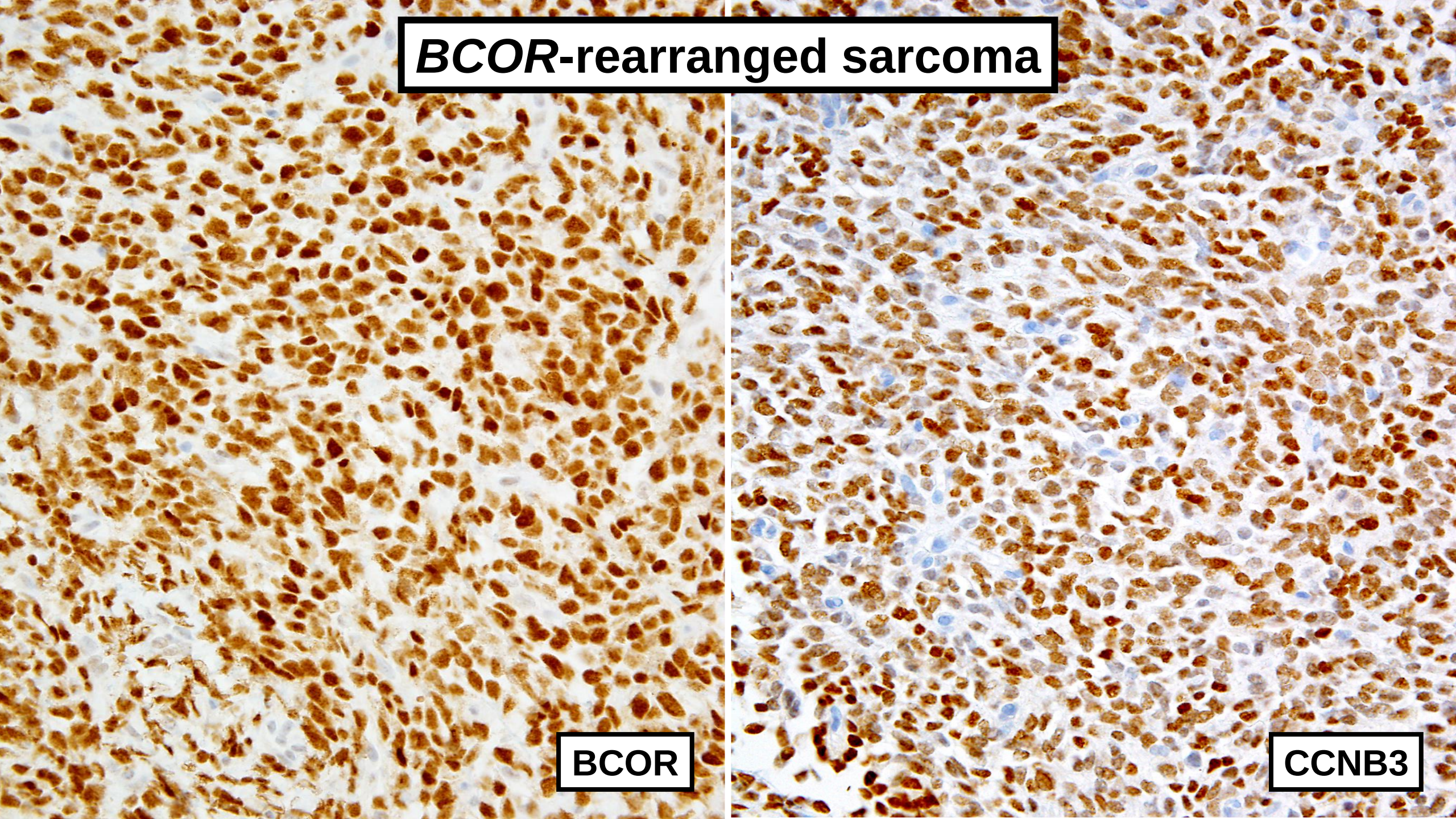
BCOR-rearranged sarcoma

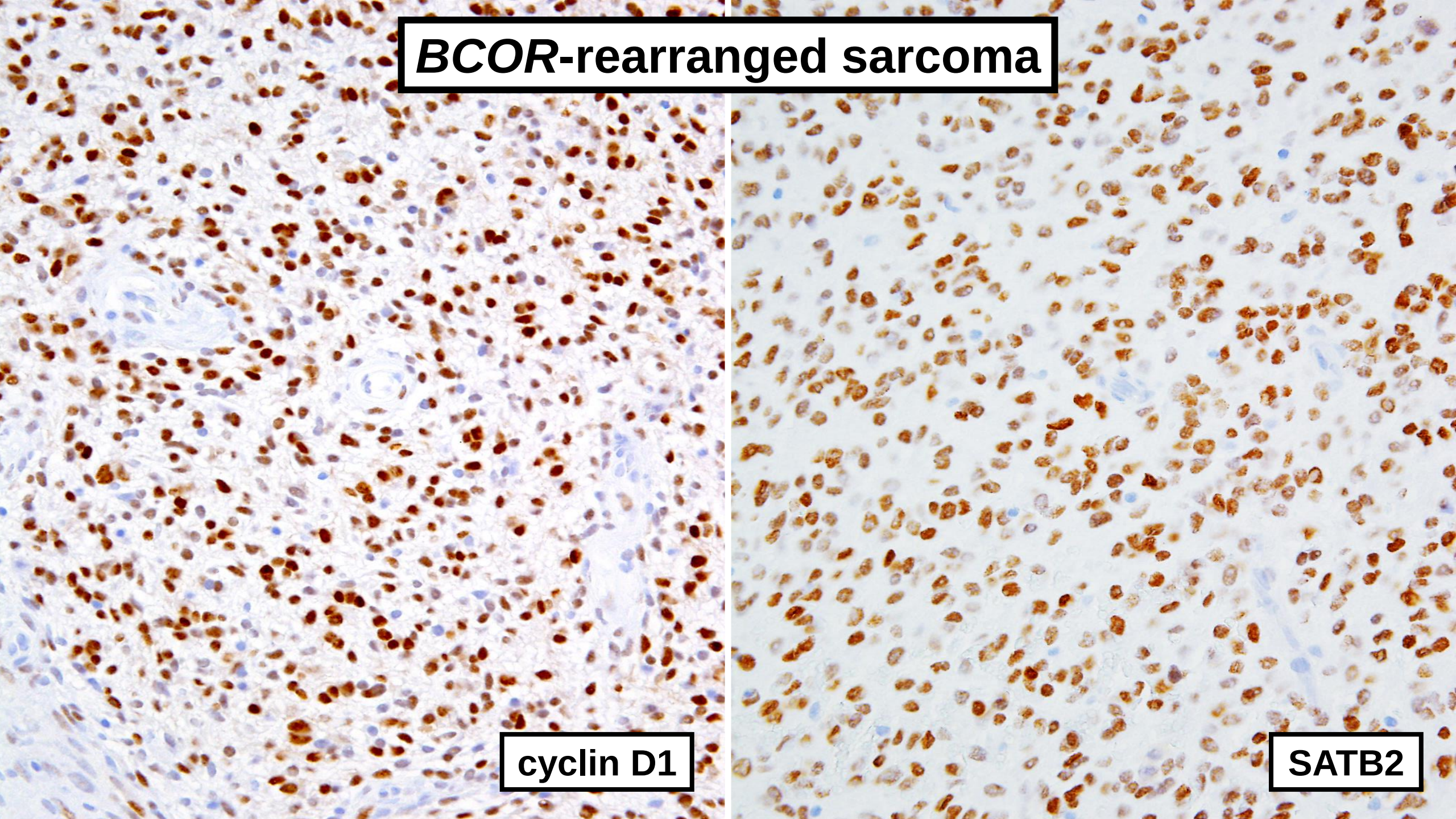



BCOR-rearranged sarcoma

BCOR-rearranged sarcoma: IHC

Marker	Positive	Comments
BCOR	~100%	Strong, diffuse, nuclear
CCNB3	90%	Nuclear
Cyclin D1	90%	Nuclear
SATB2	80%	Variable extent and intensity
CD99	50%	Variable extent and intensity

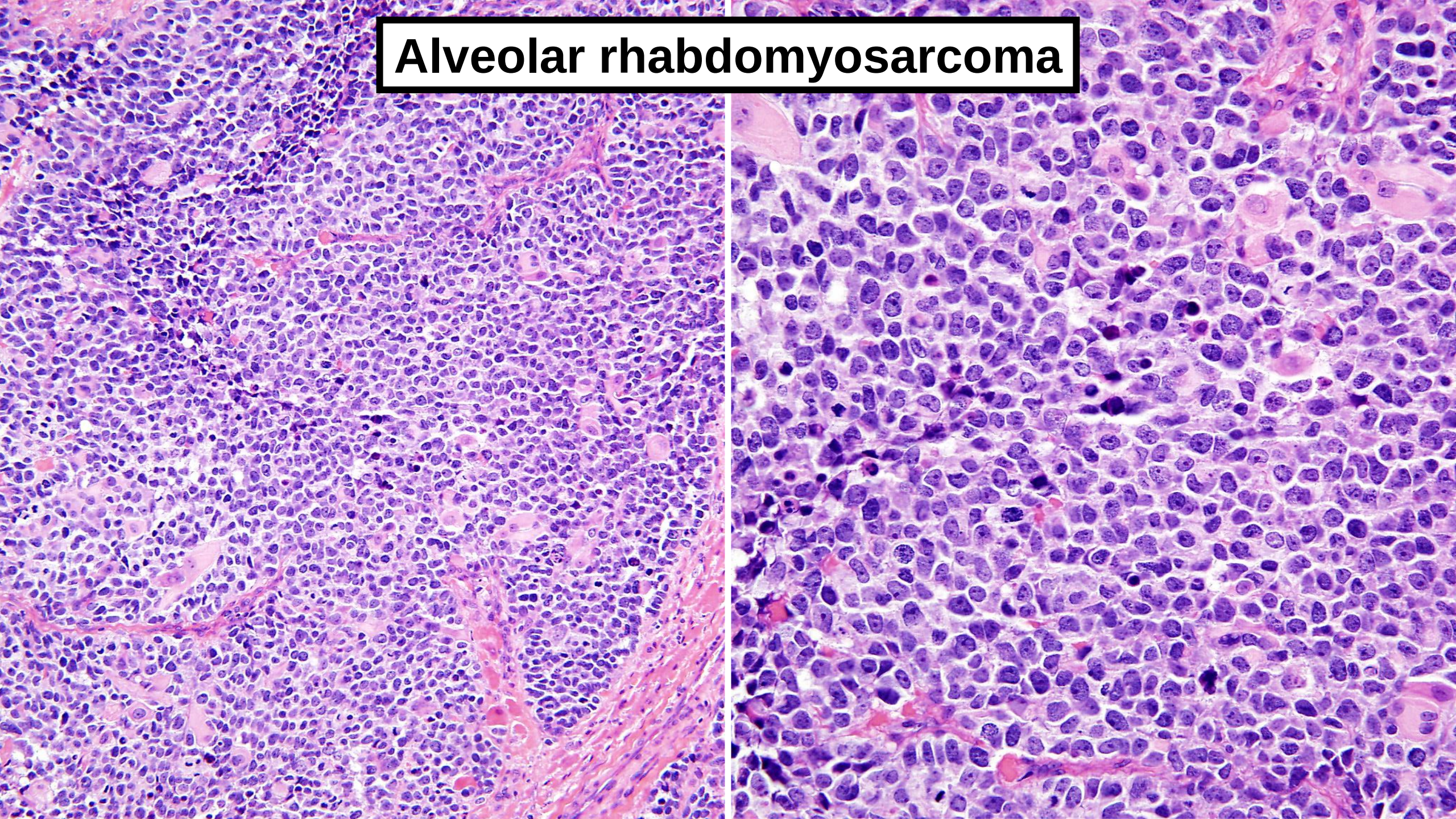

BCOR-rearranged sarcoma
BCOR
CCNB3


BCOR-rearranged sarcoma
cyclin D1
SATB2

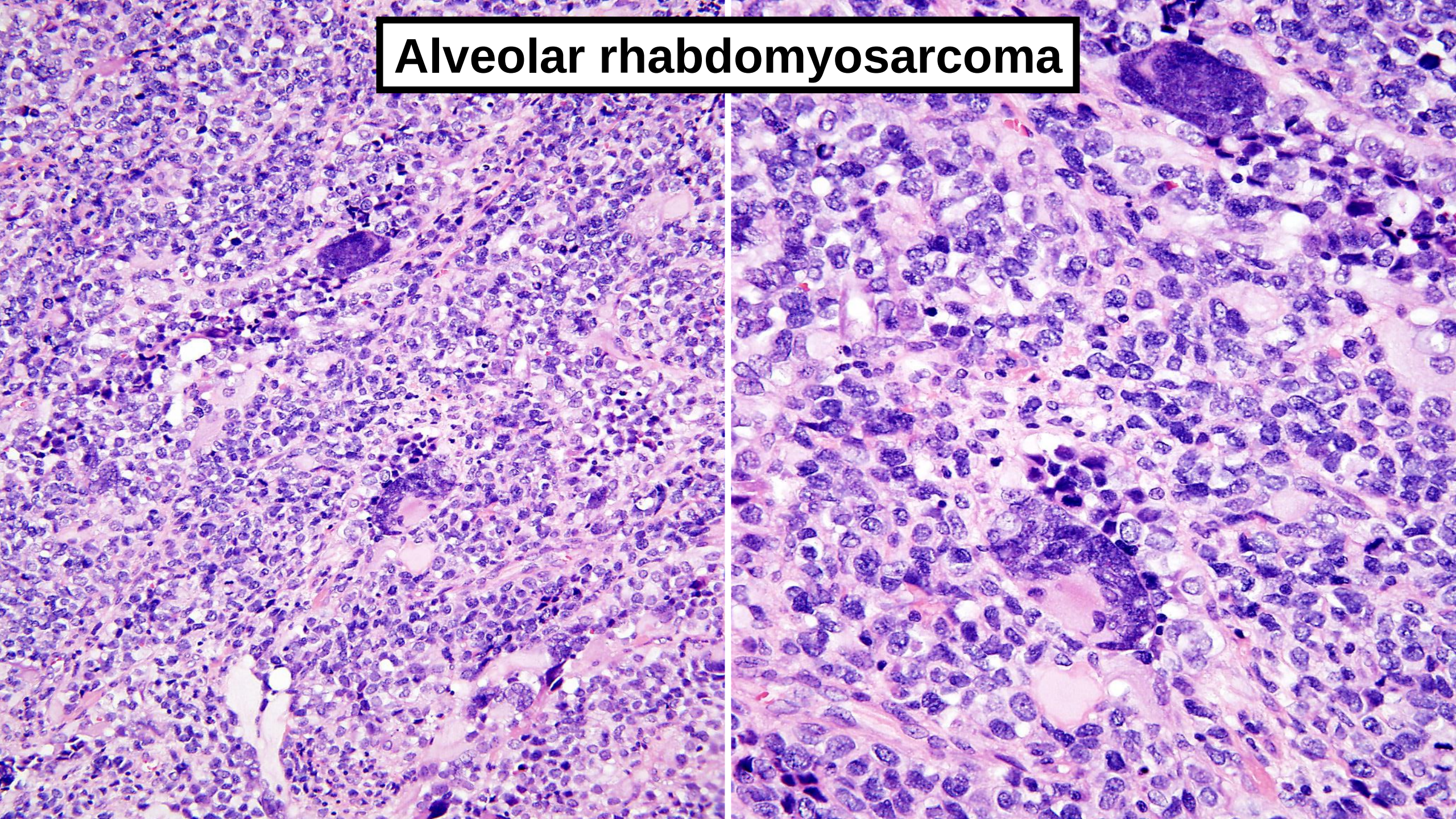
Alveolar rhabdomyosarcoma

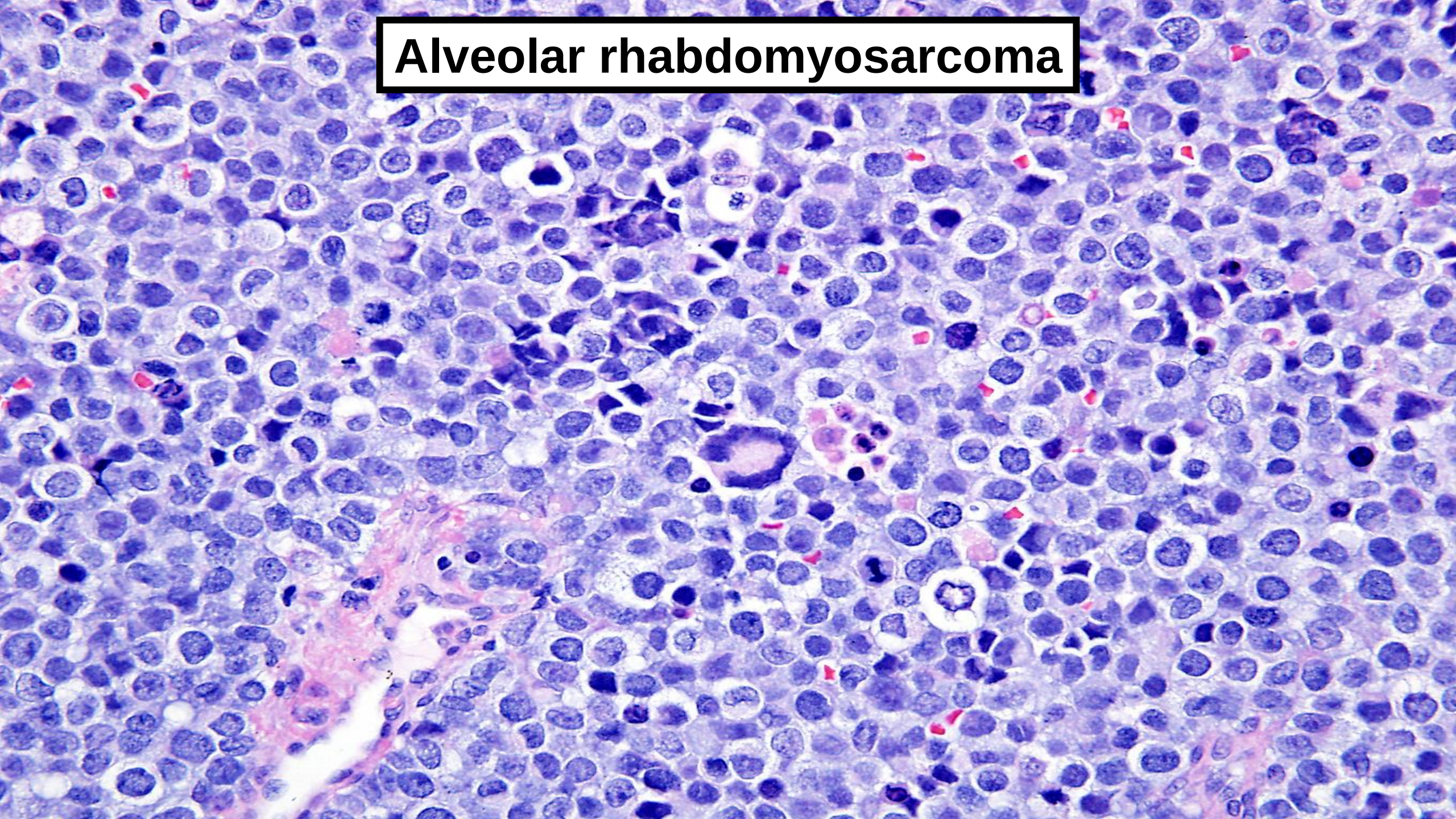
- Alveolar RMS less common than embryonal RMS (1:3)
- Peak in adolescents and young adults
- Predilection for head and neck (especially sinonasal), extremities, trunk, pelvis
- Small subset affects older adults
- Poor prognosis; high risk of metastasis
- Lymph nodes and lung common metastatic sites

Alveolar rhabdomyosarcoma



Alveolar rhabdomyosarcoma

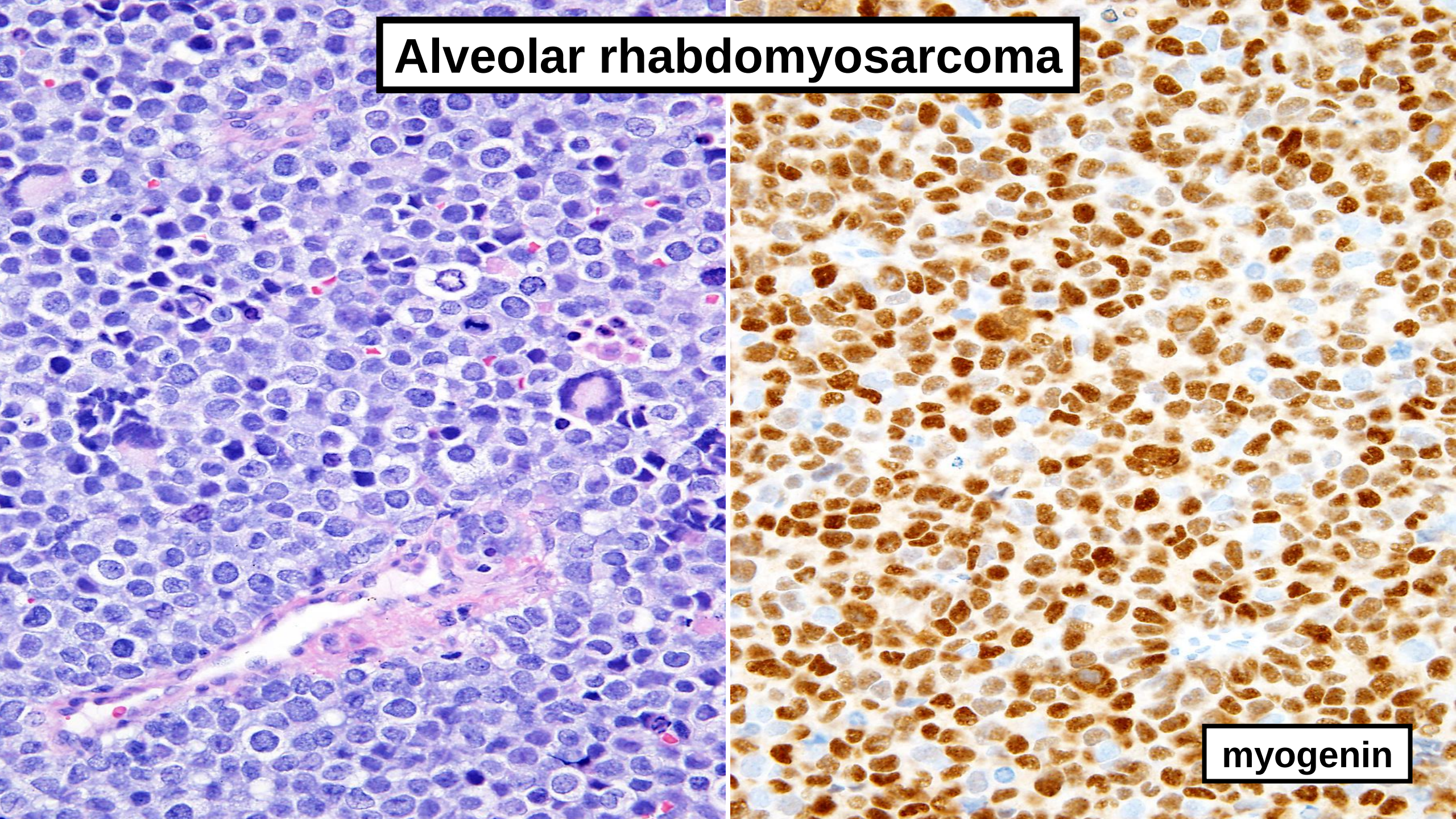



Alveolar rhabdomyosarcoma

Alveolar rhabdomyosarcoma: ancillary studies

- **IHC:**
  - **desmin: positive**
  - **Myogenin, MyoD1: strong, diffuse nuclear**
  - **Keratins: may be focally positive**
  - **Synaptophysin: may be focally positive**
- **Molecular genetics:**
  - **t(2;13) with *PAX3::FOXO1* in 80%**
  - **t(1;13) with *PAX7::FOXO1* in 20%**
  - **Other *PAX3* fusions rare**

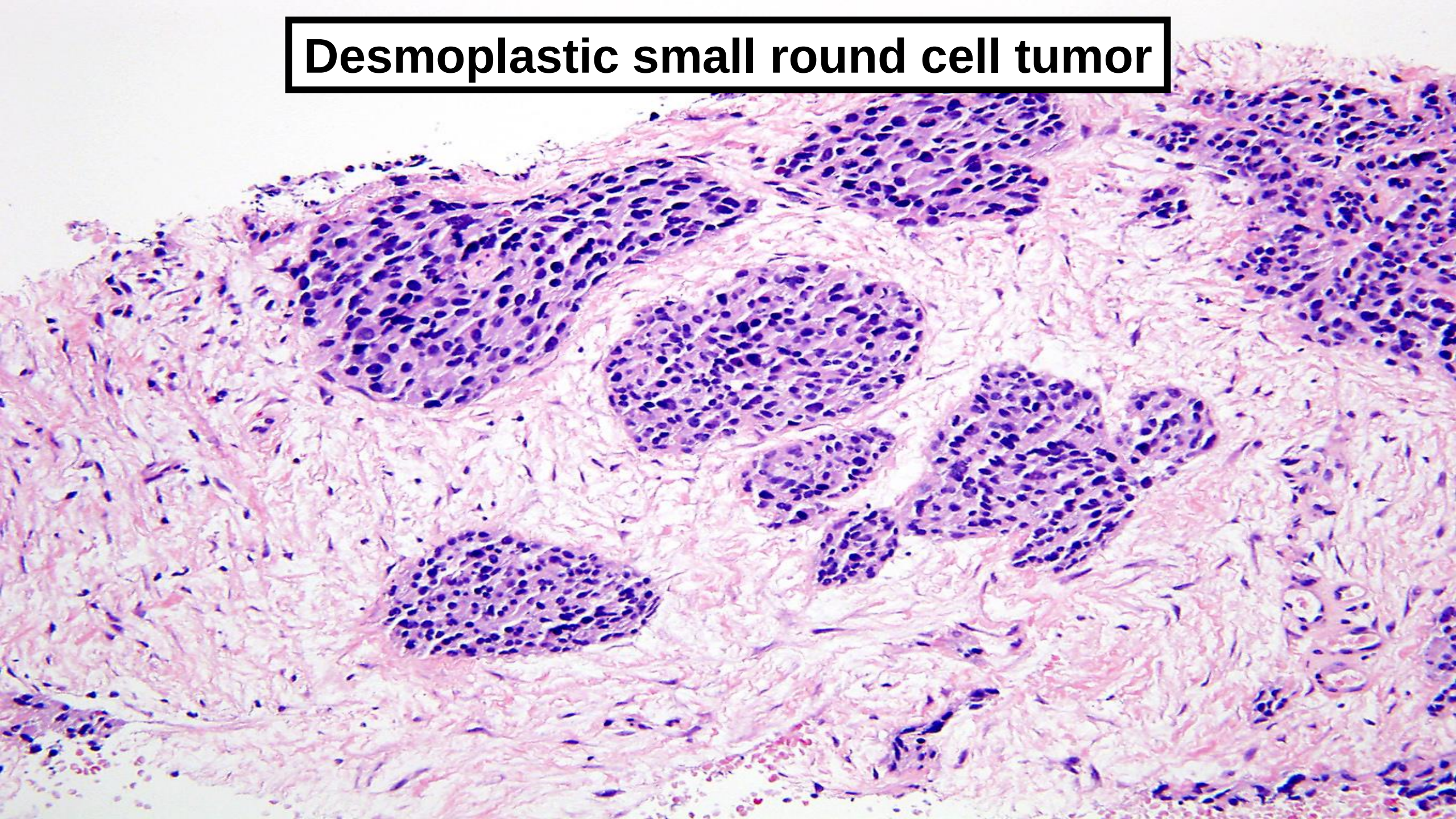
Alveolar rhabdomyosarcoma



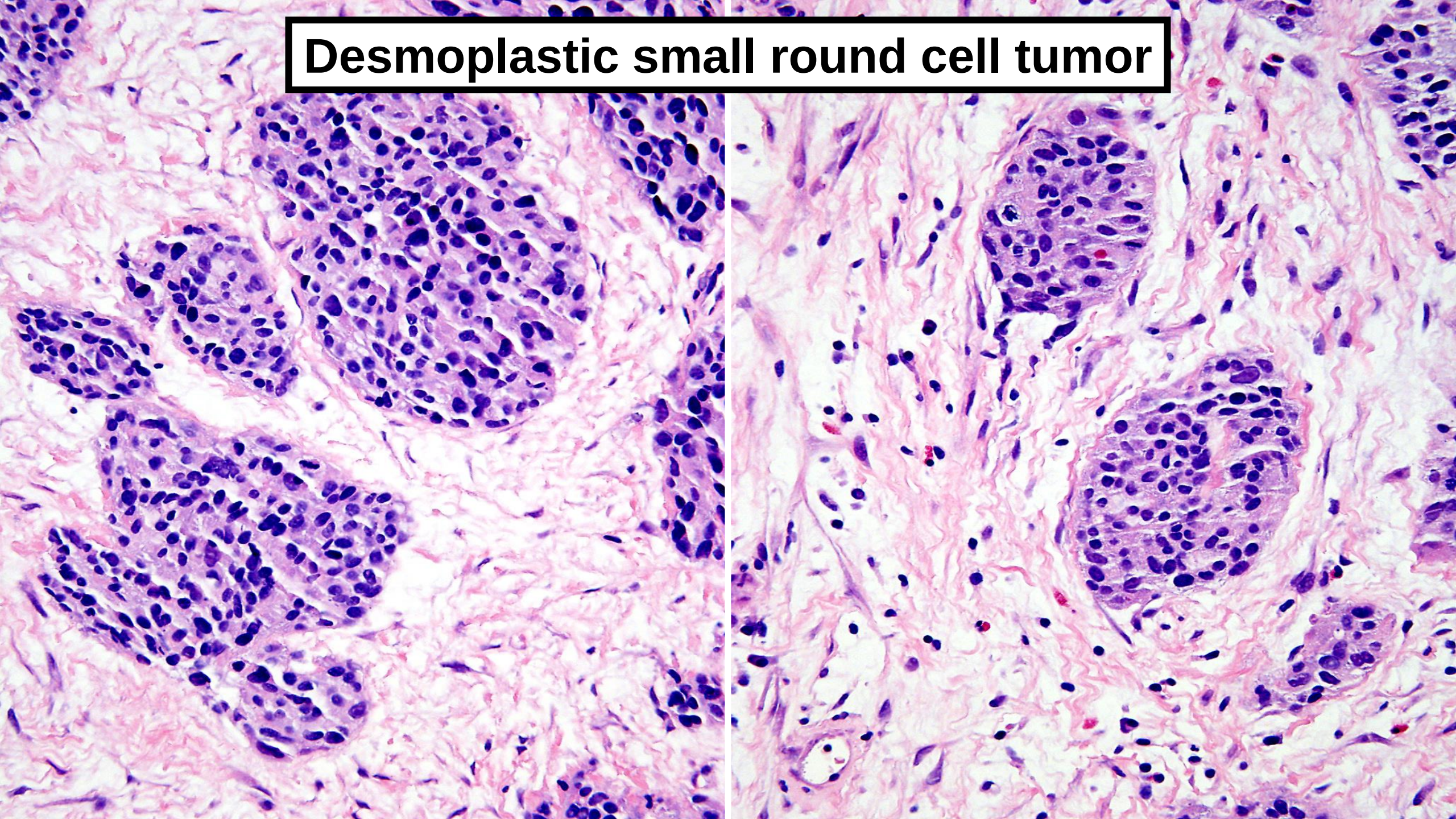
Desmoplastic small round cell tumor

- **Marked predilection for abdominal cavity**
- **Marked male predominance**
- **Adolescents and young adults**
- **Most patient present with disseminated intra-abdominal disease involving peritoneum (“sarcomatosis”)**
- **Despite aggressive treatment, prognosis very poor**

Desmoplastic small round cell tumor



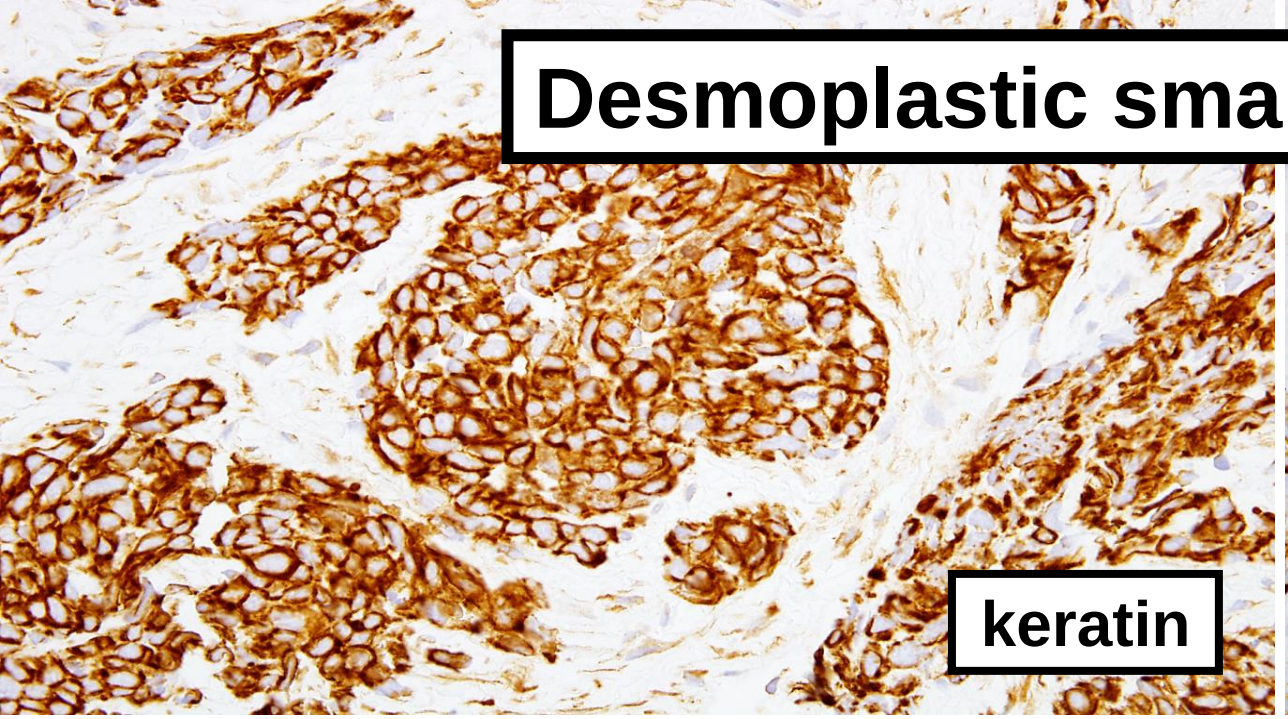
Desmoplastic small round cell tumor

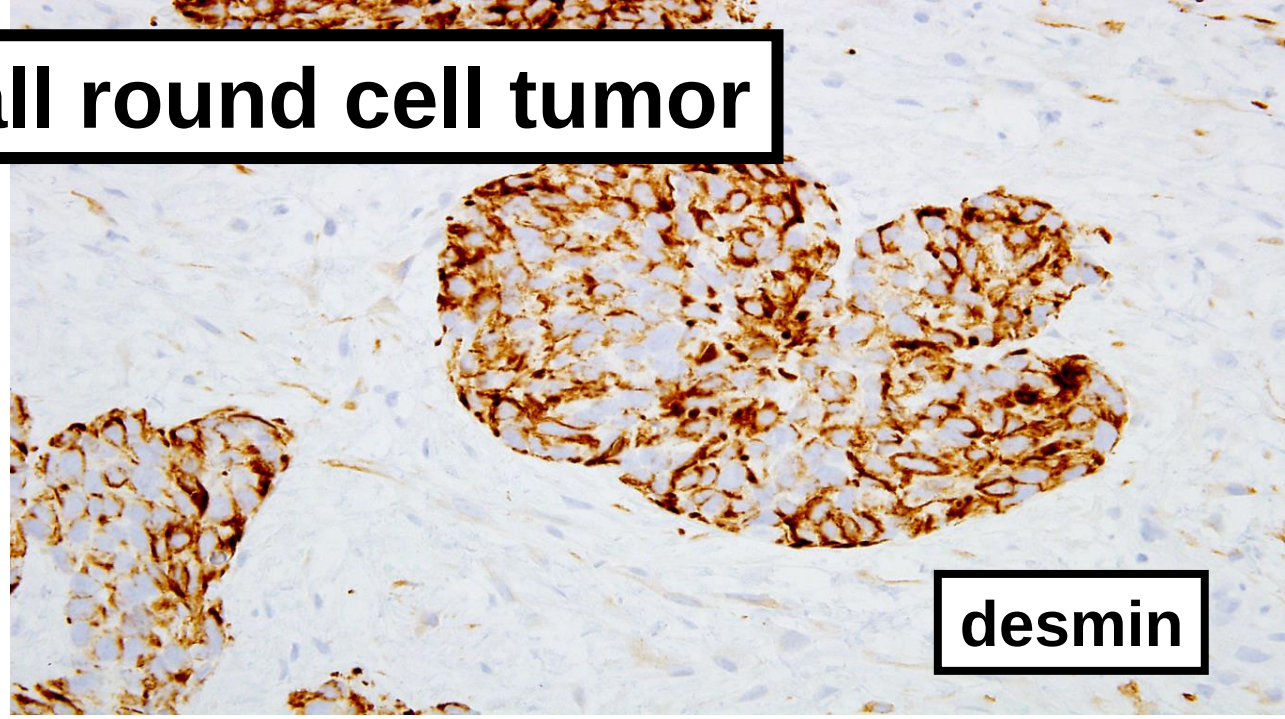


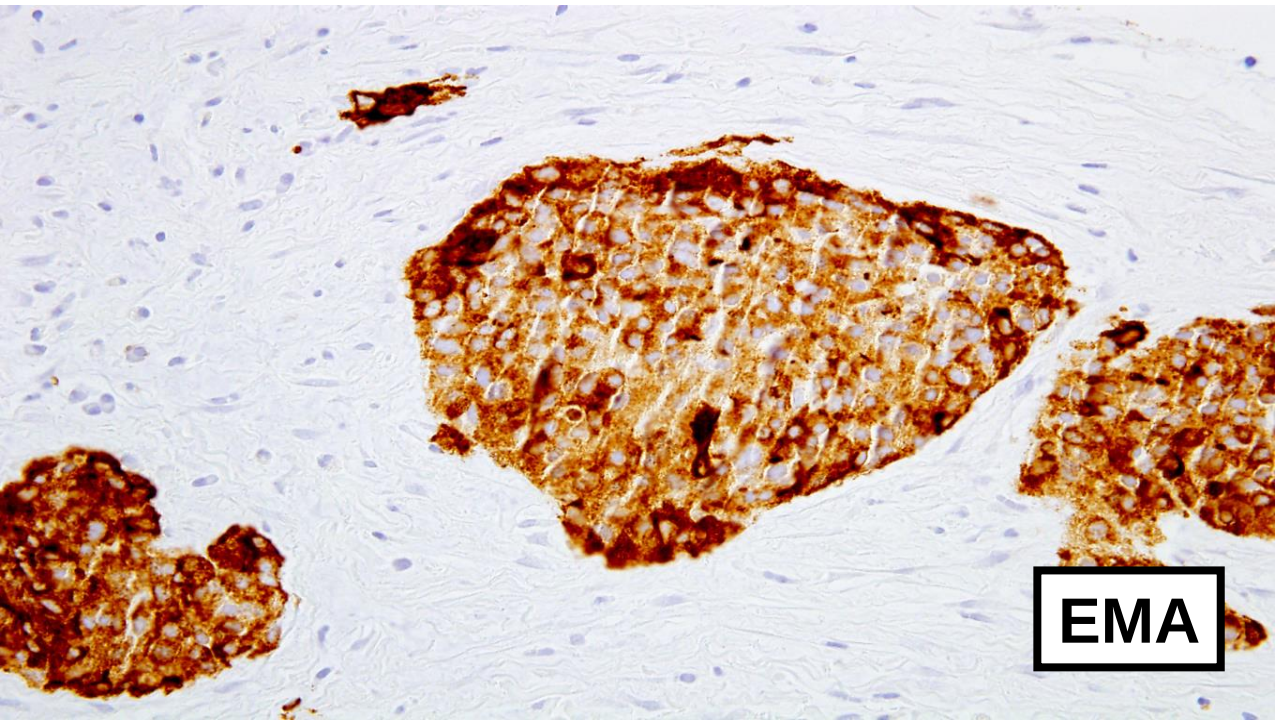
Desmoplastic small round cell tumor: ancillary studies

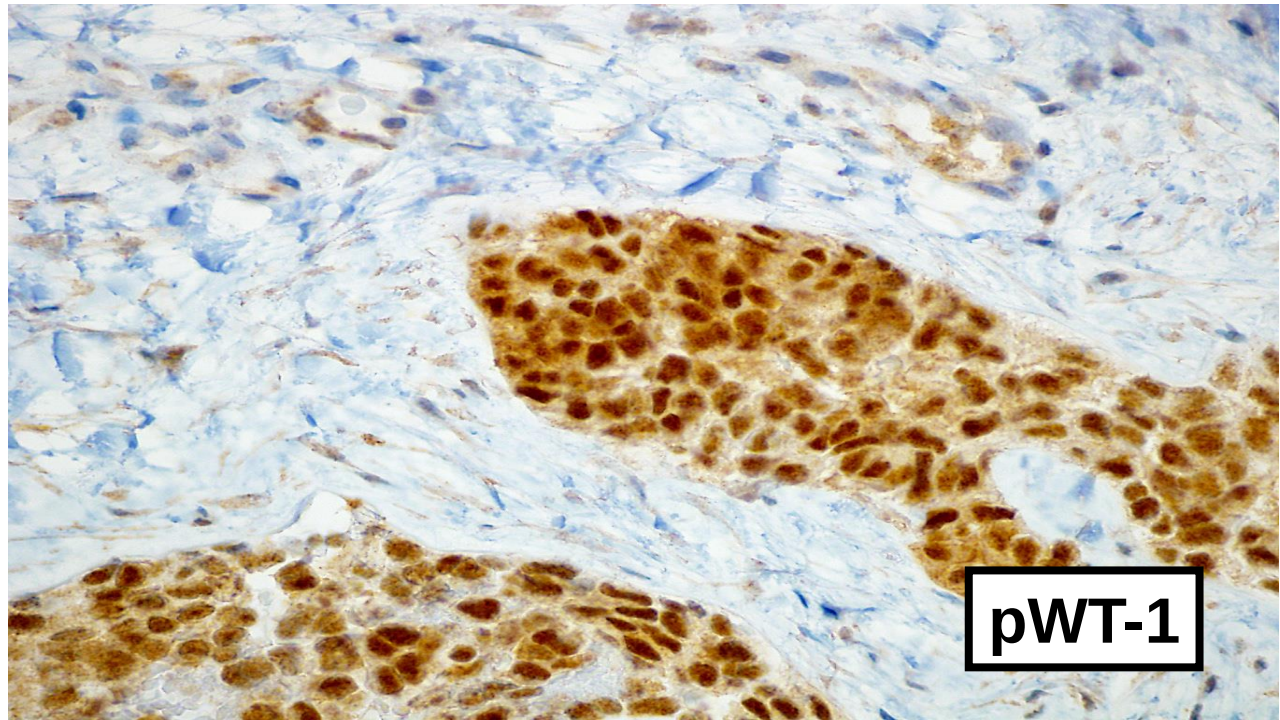
- **IHC – polyphenotypic (each 85-90%):**
  - **Keratins**
  - **EMA**
  - **Desmin**
  - **polyclonal WT-1 (C-terminus)**
- **Molecular genetics:**
  - **t(11;22) with *EWSR1::WT1* pathognomonic**

Desmoplastic small round cell tumor



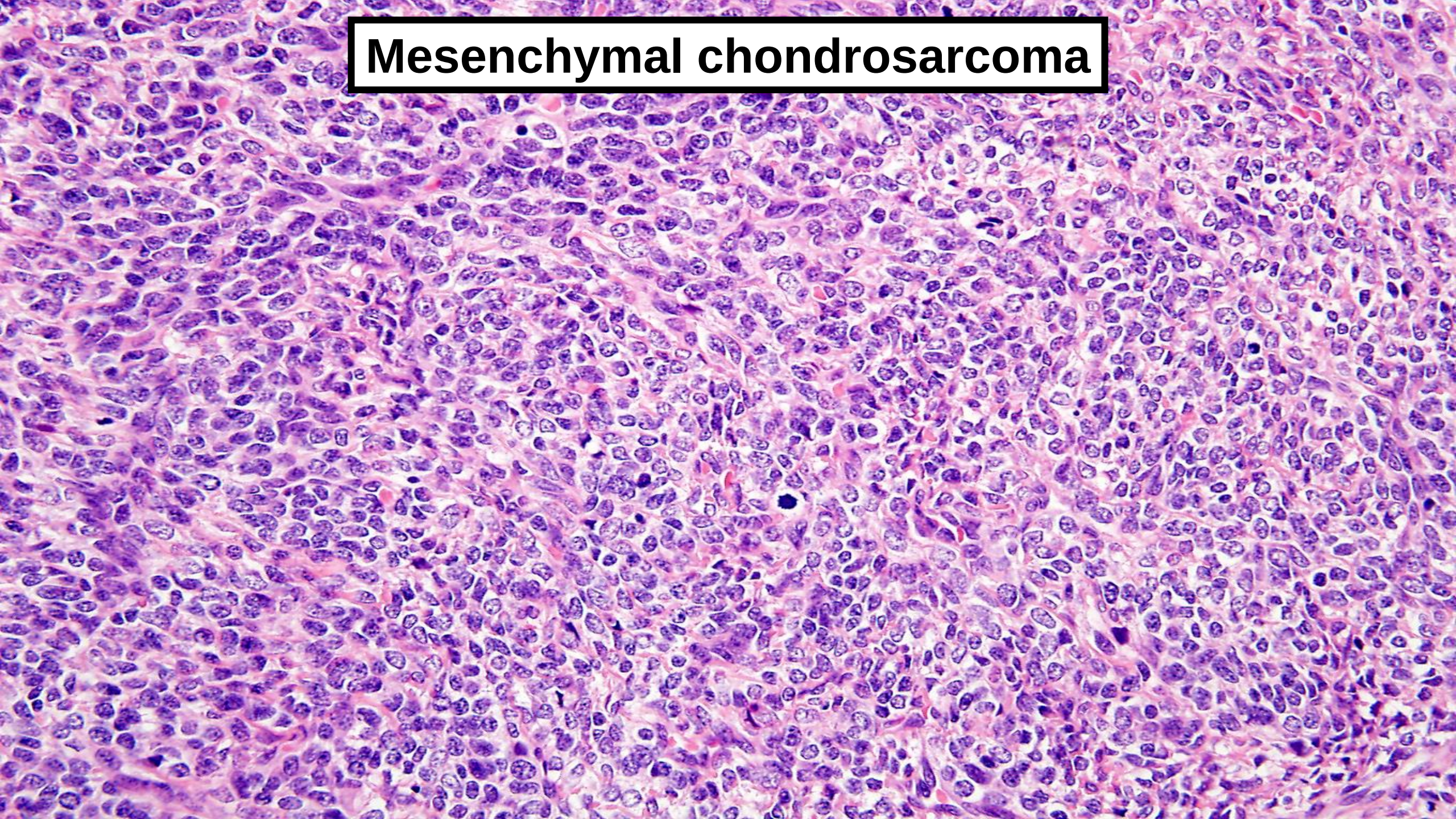




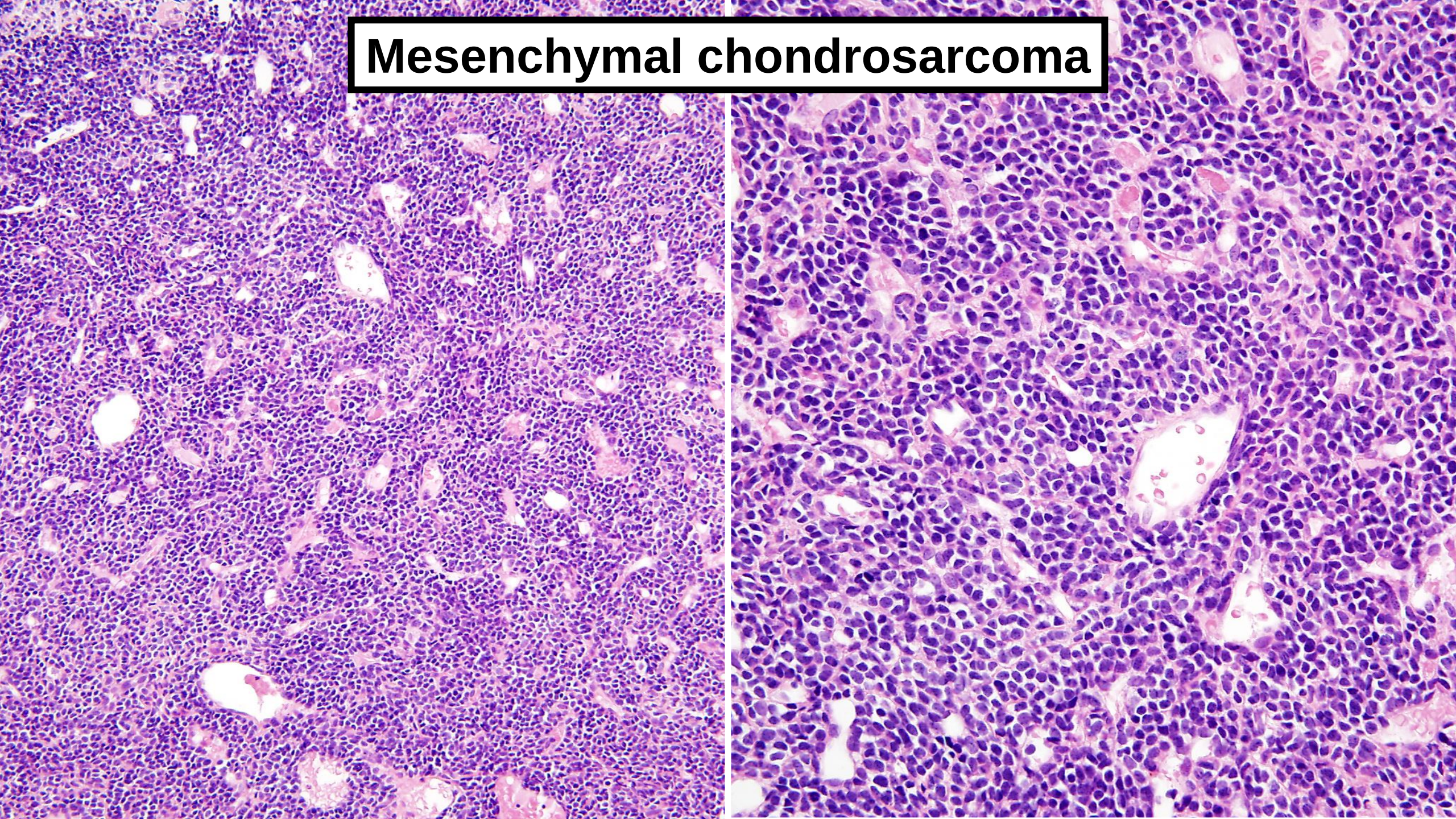


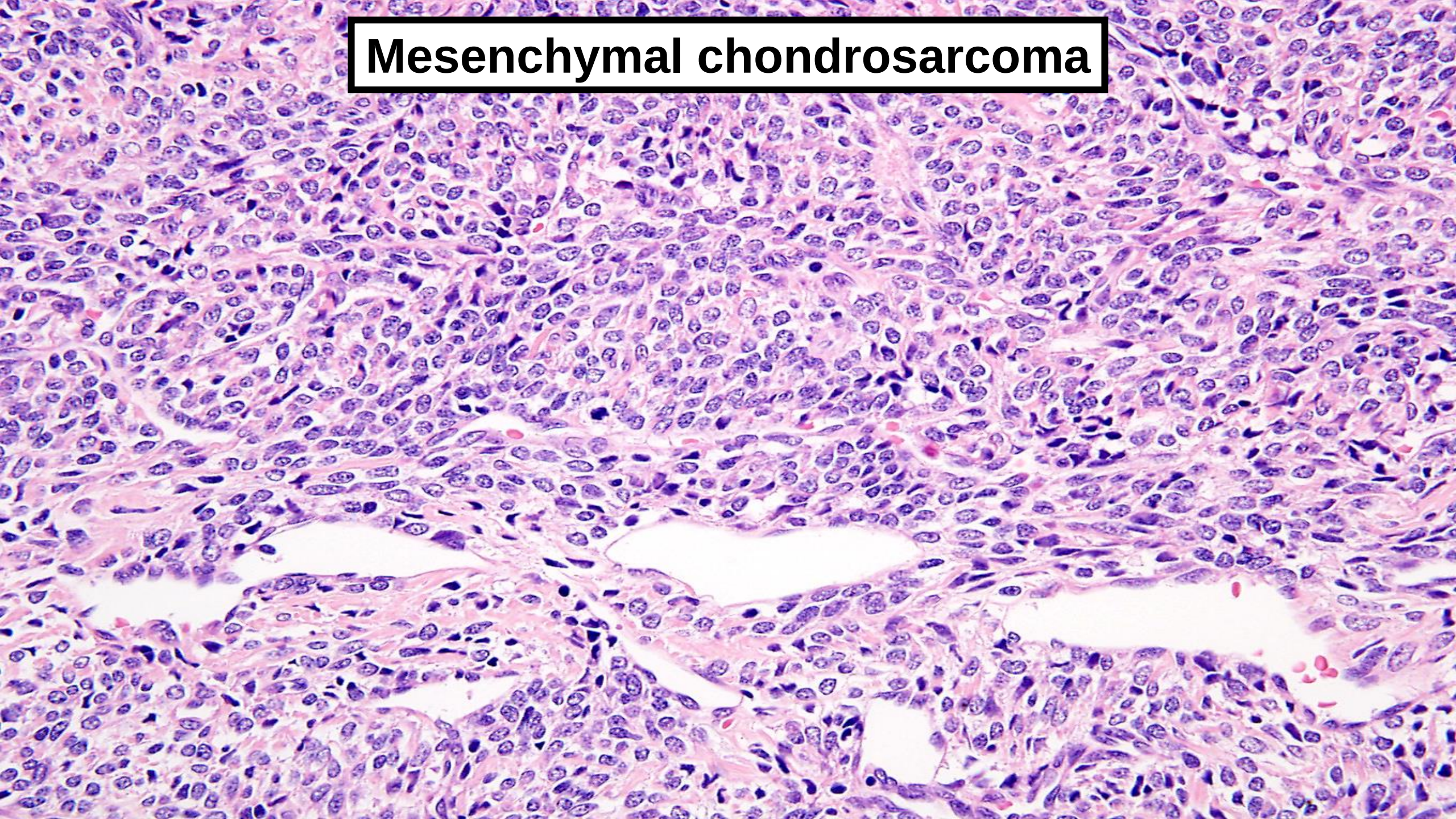
Mesenchymal chondrosarcoma

- Rare sarcoma may arise in soft tissue > bone
- Extraskeletal: predilection for thigh and head and neck
- Female predominance
- May be aggressive, although variable clinical course:
  - Some patients develop early metastases (especially lungs)
  - Other patients with prolonged disease-free intervals
- Systemic therapy limited success

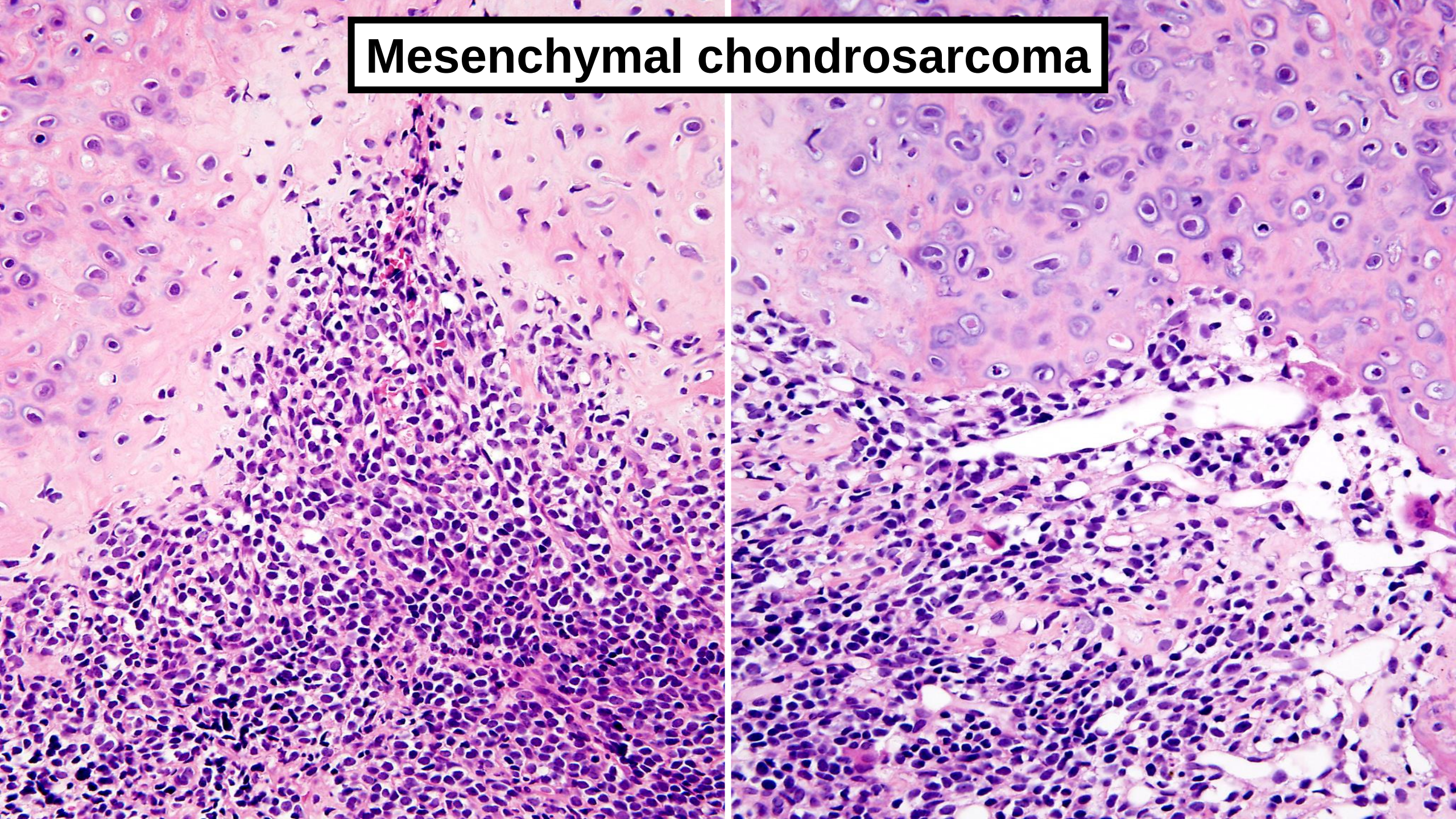

Mesenchymal chondrosarcoma

Mesenchymal chondrosarcoma




Mesenchymal chondrosarcoma

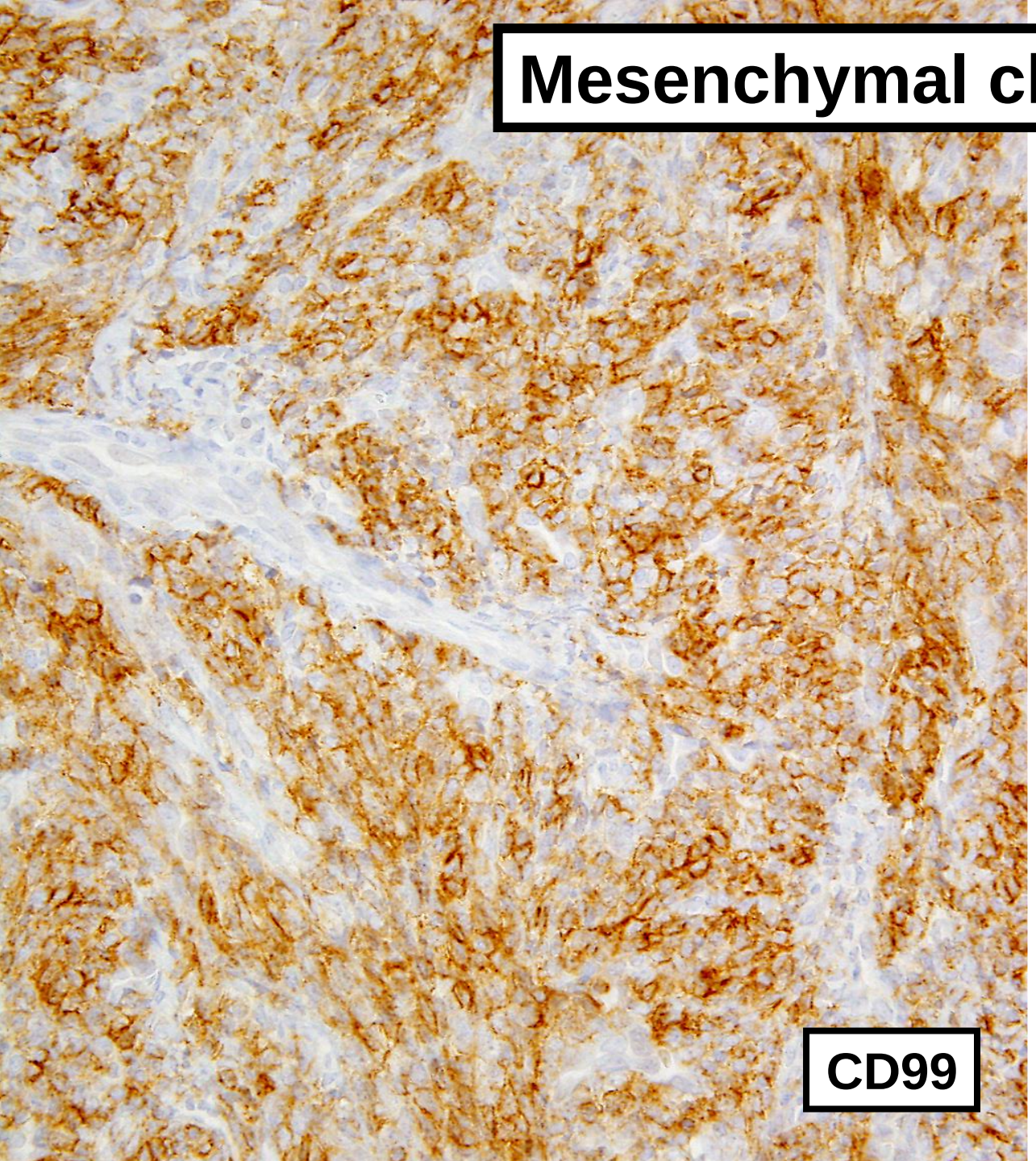
Mesenchymal chondrosarcoma

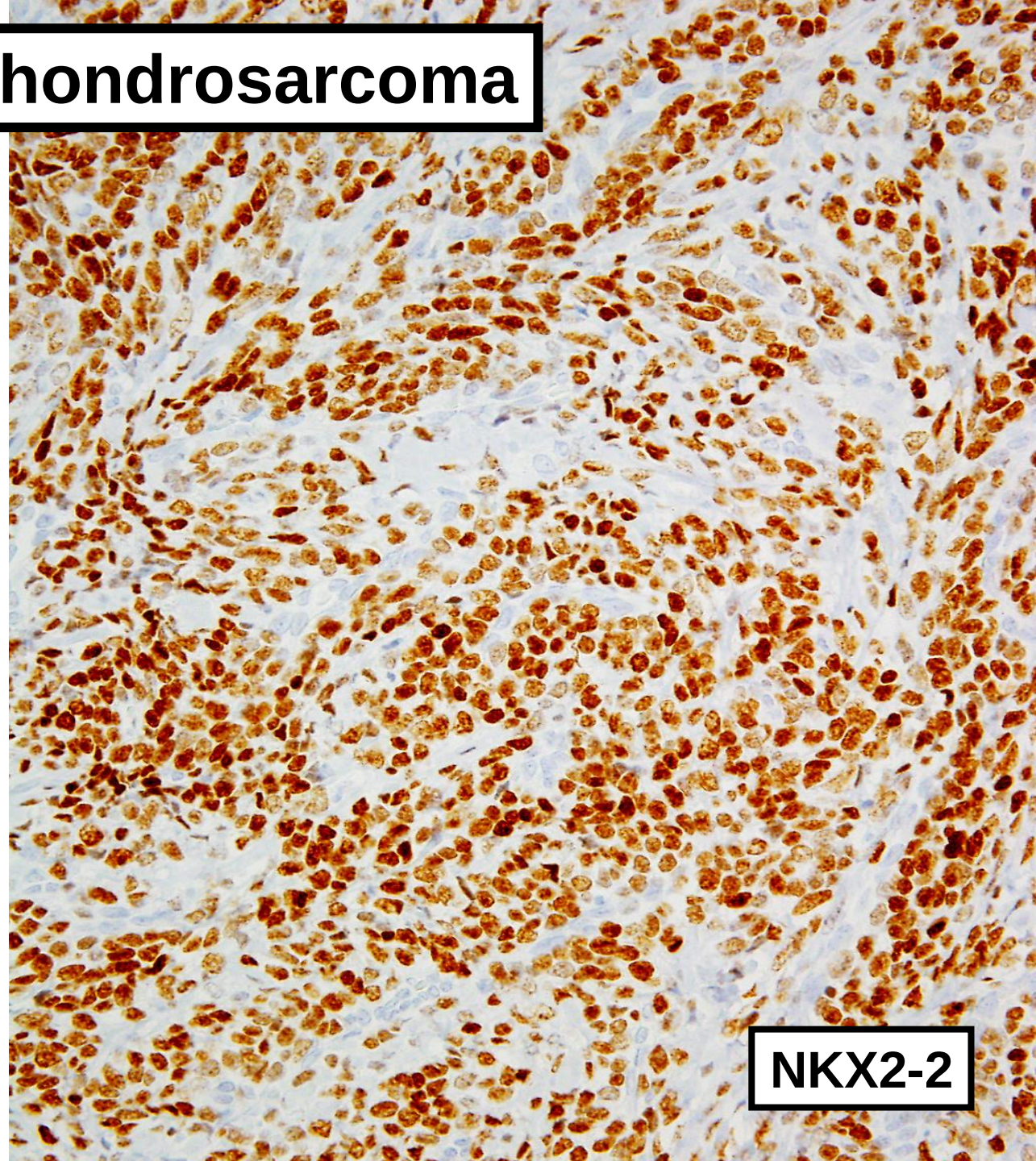


Mesenchymal chondrosarcoma: ancillary studies

- **IHC – no specific markers**
  - **CD99: often positive, may be diffuse/membranous**
  - **NKX2-2: strong, diffuse nuclear in 75%**
  - **STAT6, SS18::SSX, keratins: negative**
- **Molecular genetics:**
  - ***HEY1::NCOA2* pathognomonic**
  - **Molecular diagnostics rarely needed**

Mesenchymal chondrosarcoma

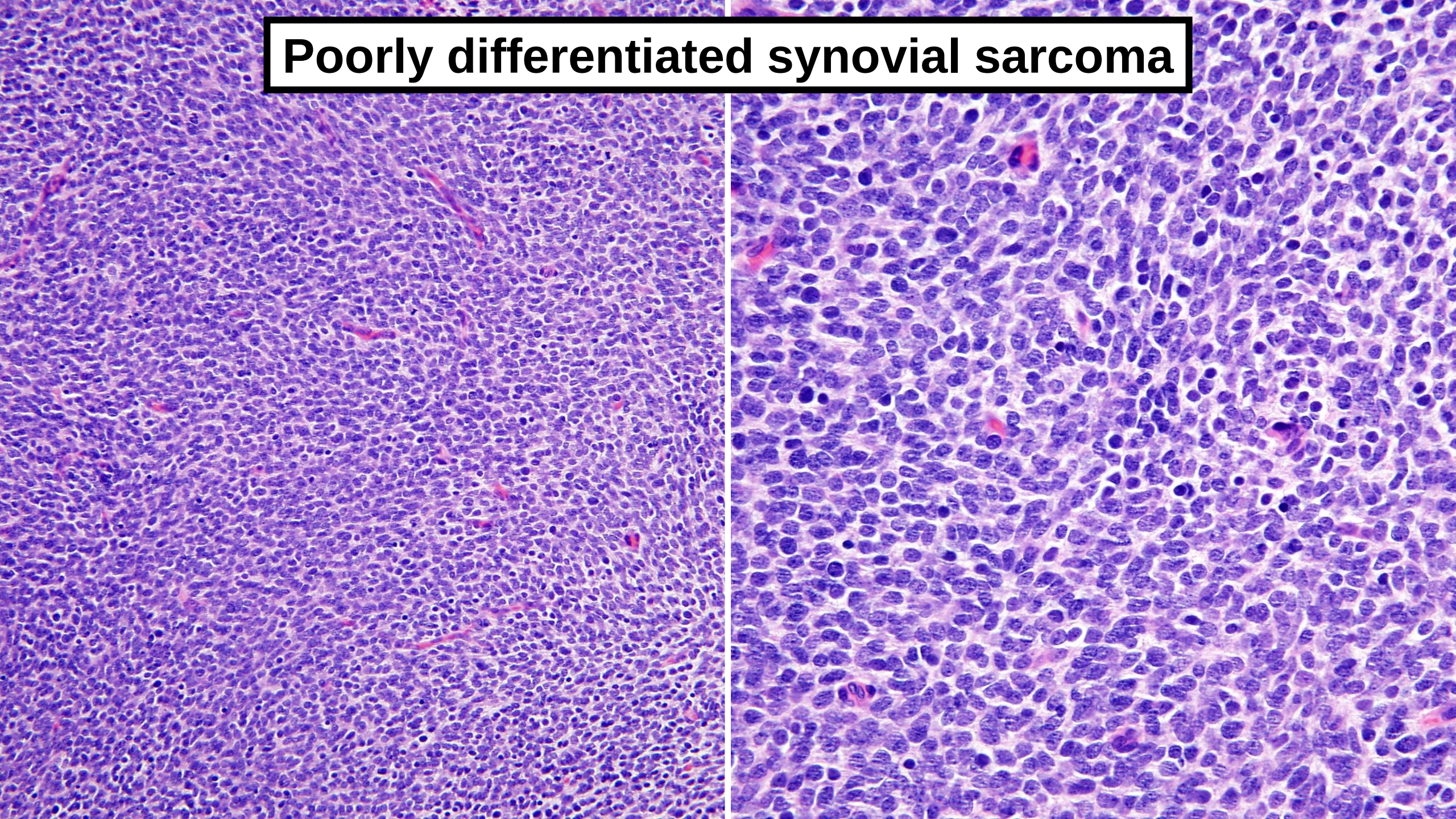


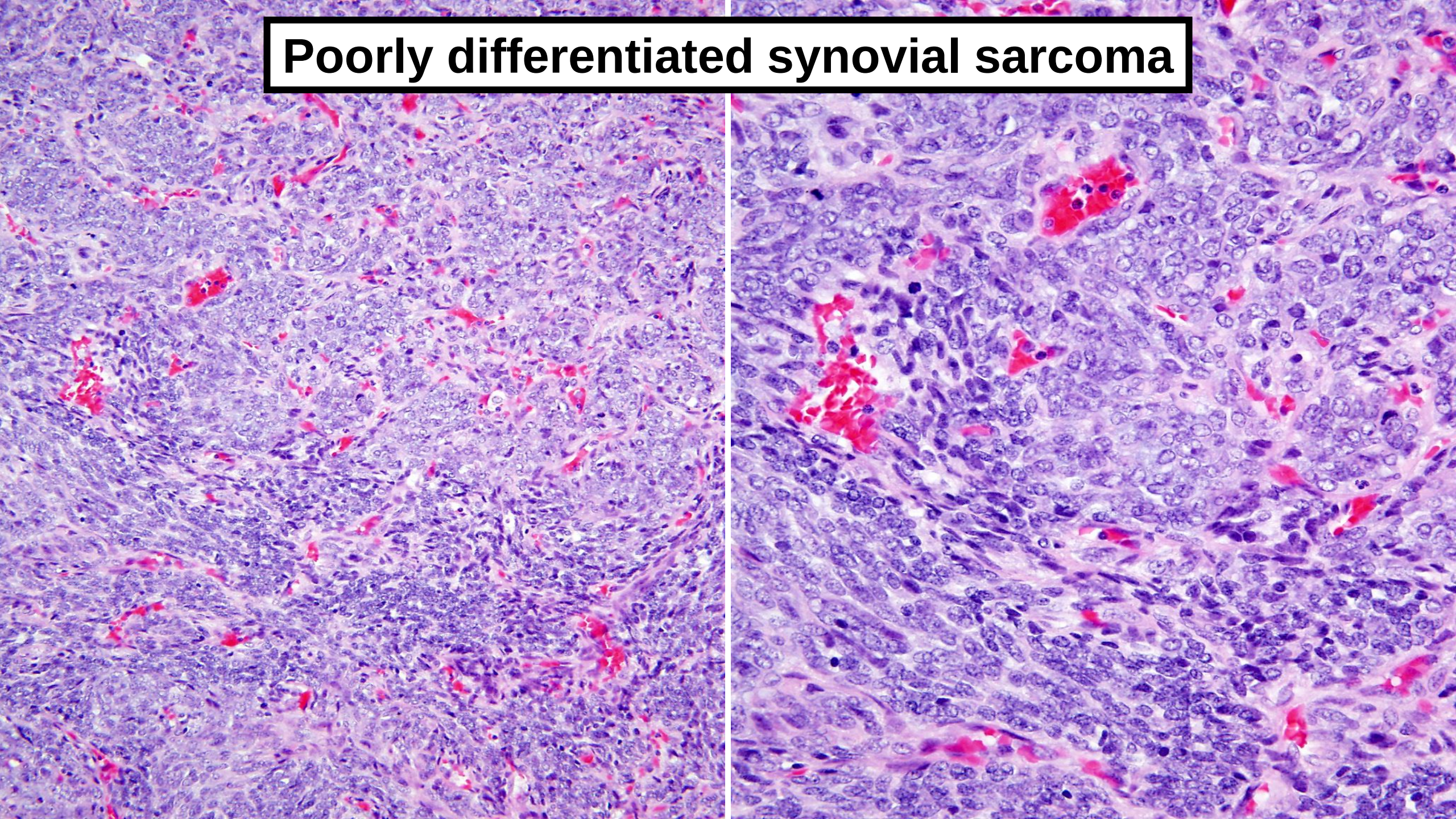


Poorly differentiated synovial sarcoma

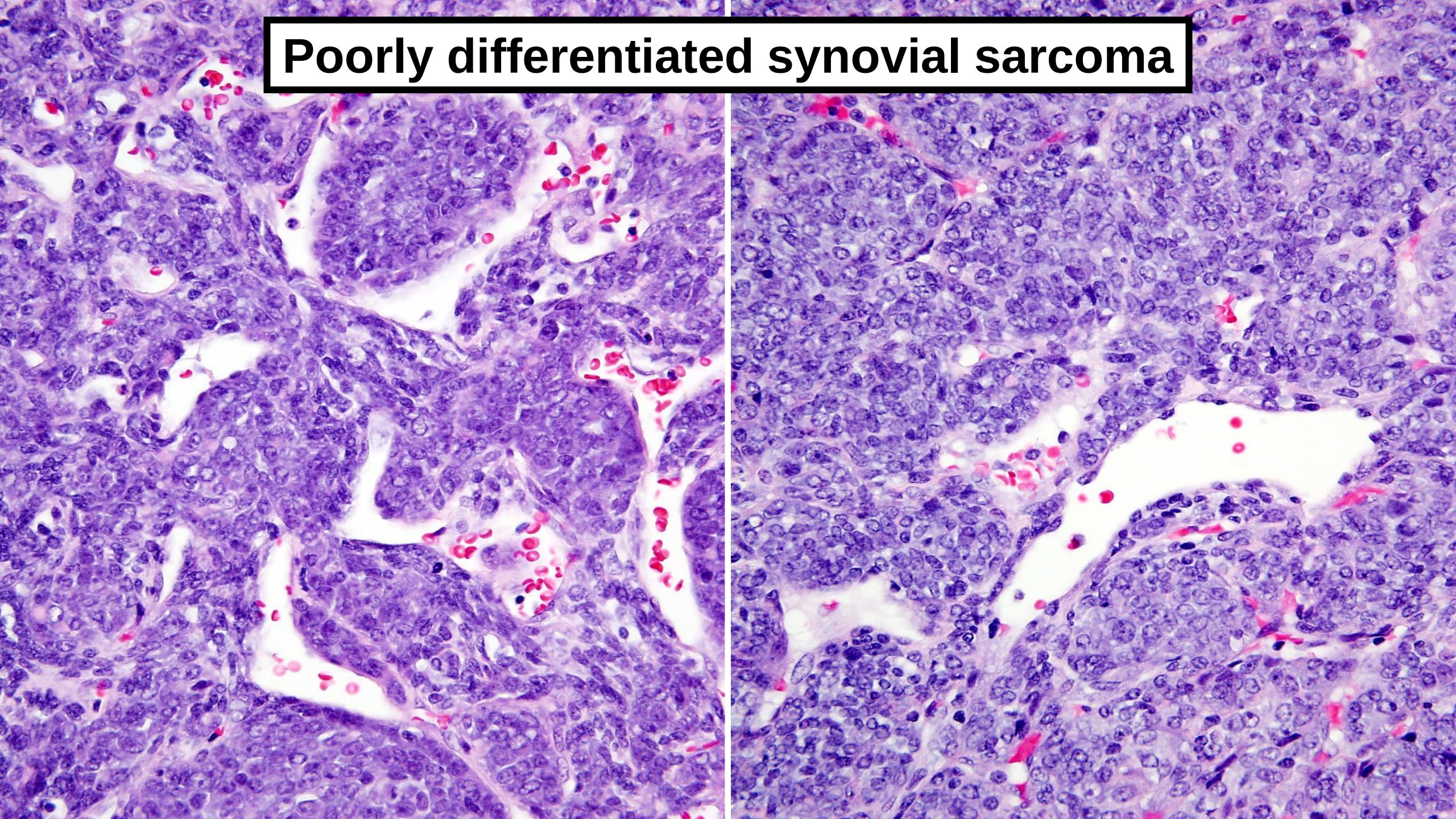
- Monophasic, biphasic and poorly differentiated variants
- Poorly differentiated (round cell) often arises in deep soft tissue of extremities, although distribution is wide
- Young adults most often affected
- Male predominance
- Aggressive, with higher metastatic potential than conventional monophasic or biphasic

Poorly differentiated synovial sarcoma




Poorly differentiated synovial sarcoma

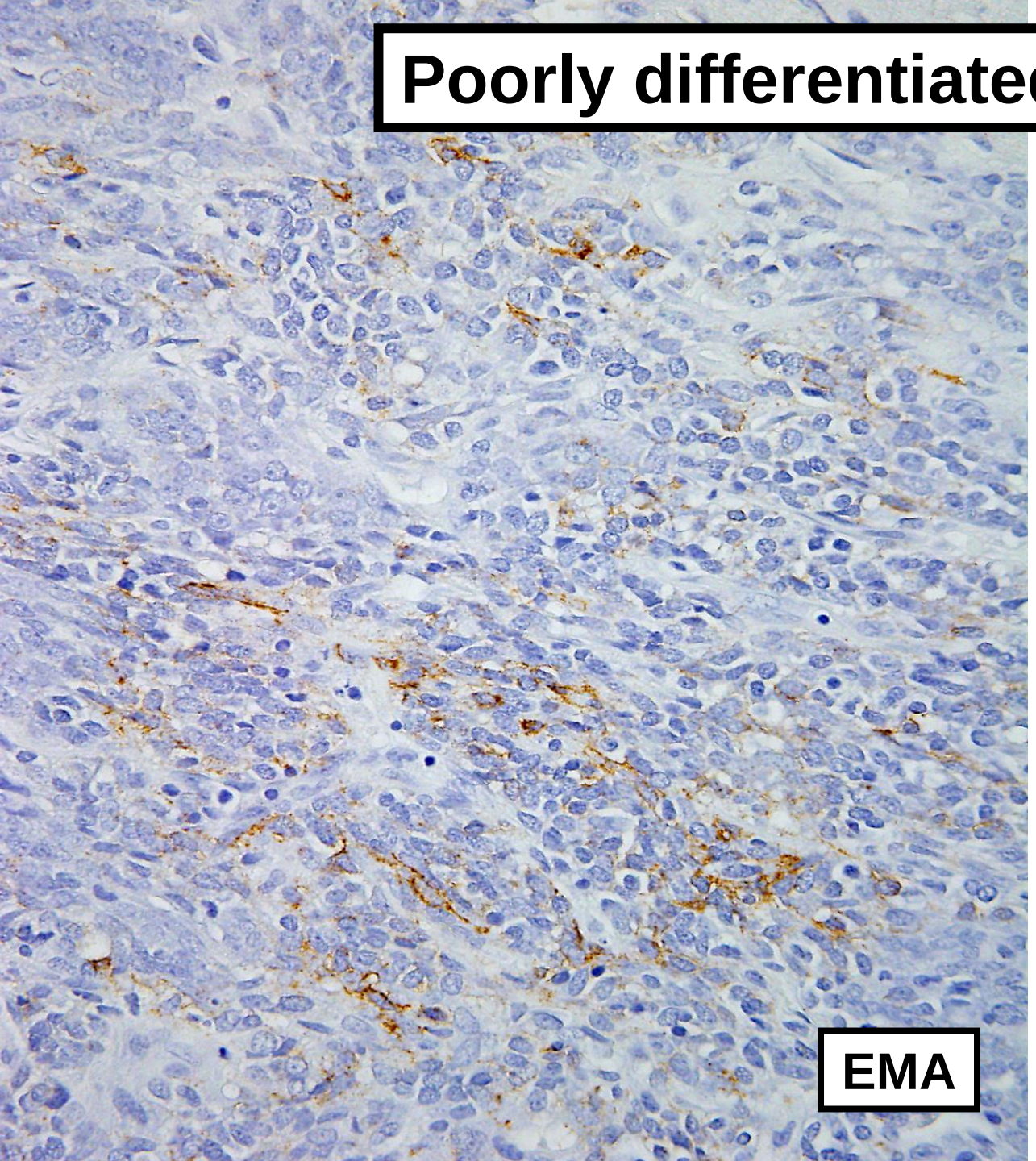
Poorly differentiated synovial sarcoma

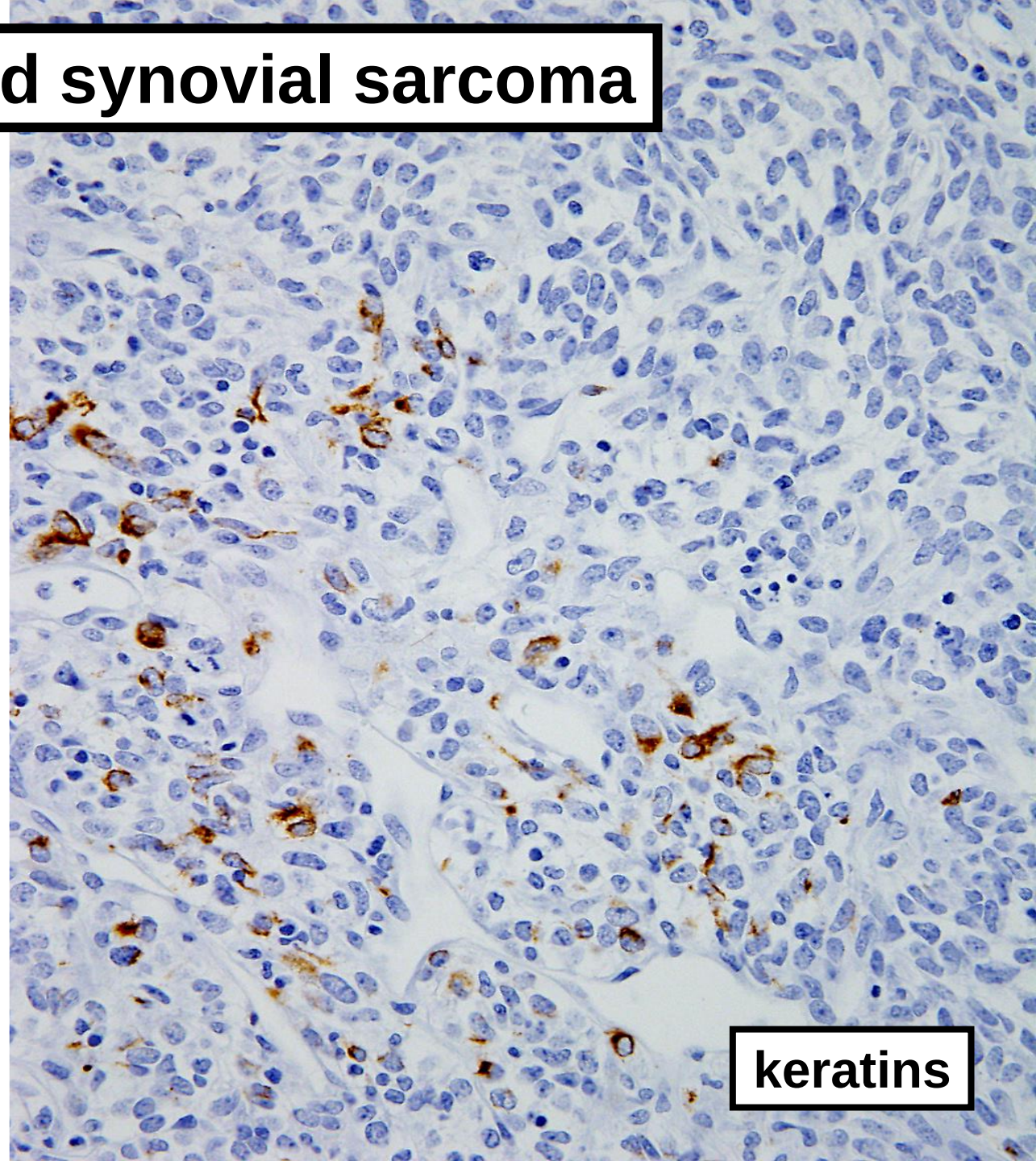


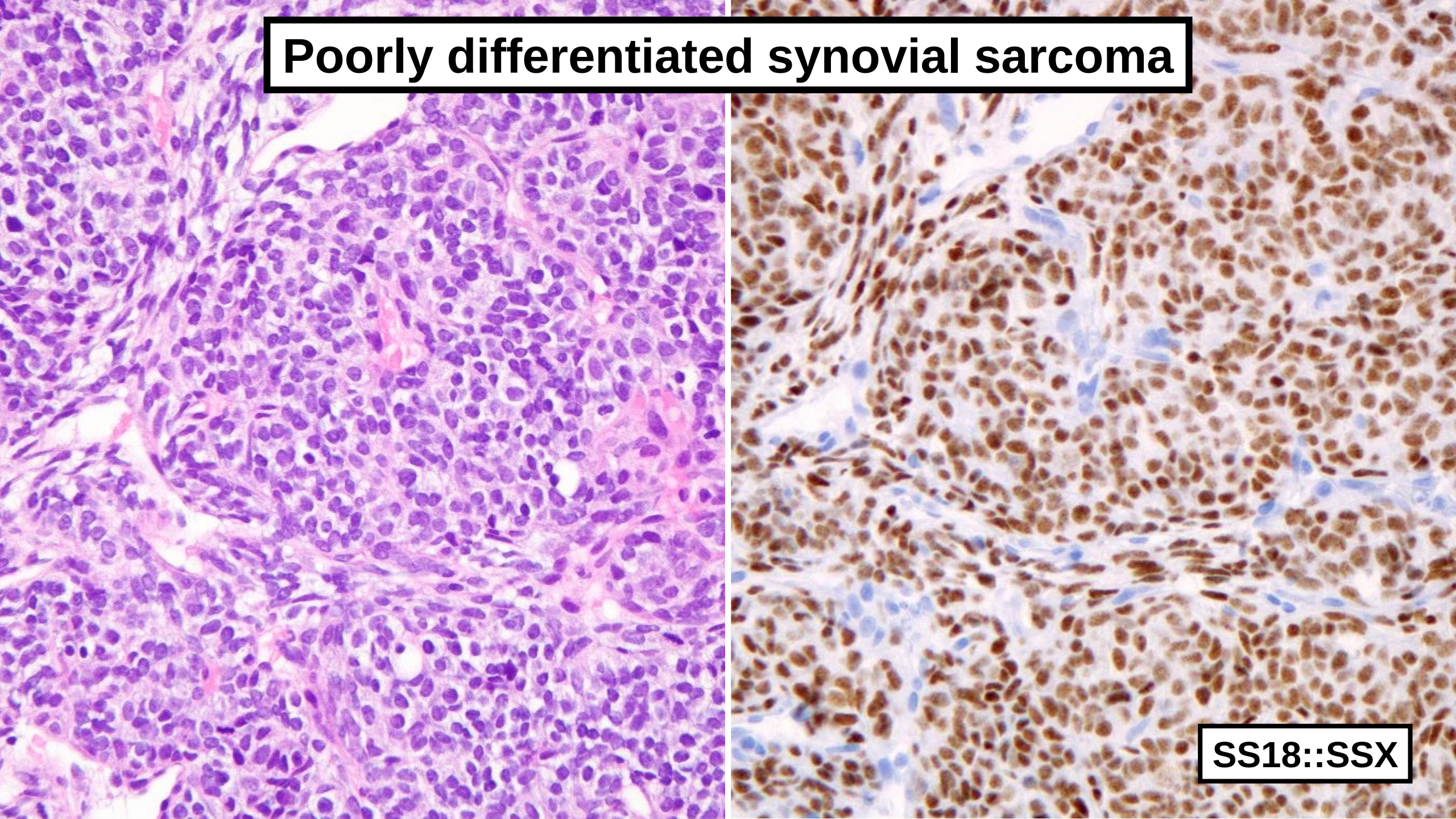
Poorly differentiated synovial sarcoma: ancillary studies

- **IHC:**
  - **CD99: often positive; usually patchy and cytoplasmic**
  - **EMA: patchy in most cases**
  - **Keratins: occasional cells in most cases**
  - **TLE1: strong, diffuse in 95% but only moderate specificity (I do not use any longer)**
  - **SS18::SSX fusion-specific antibody now available**
- **Molecular genetics:**
  - **t(X;18) with *SS18::SSX1* or *SS18::SSX2* pathognomonic**
  - **Confirmation straightforward by IHC, FISH, NGS**

Poorly differentiated synovial sarcoma

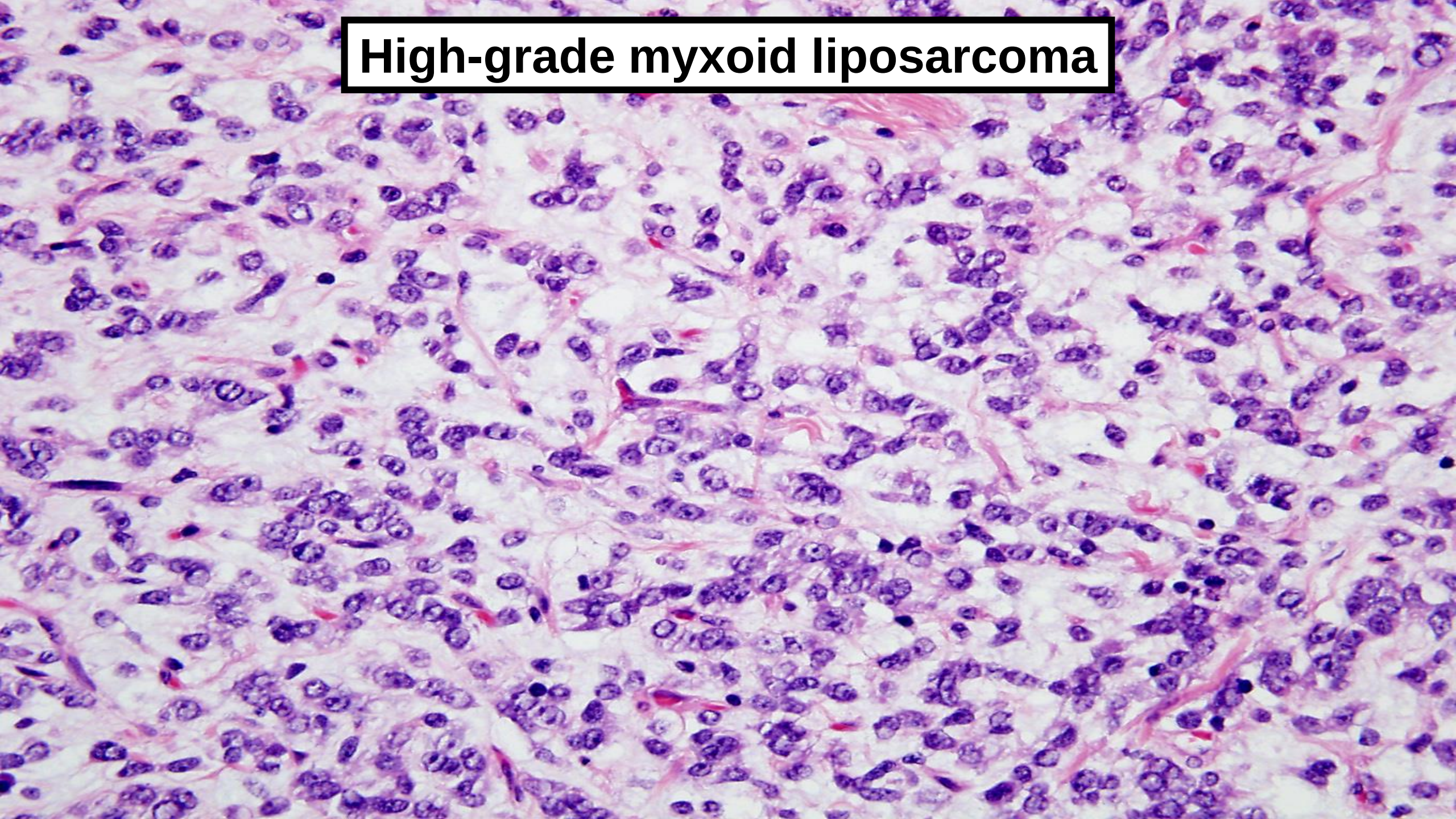




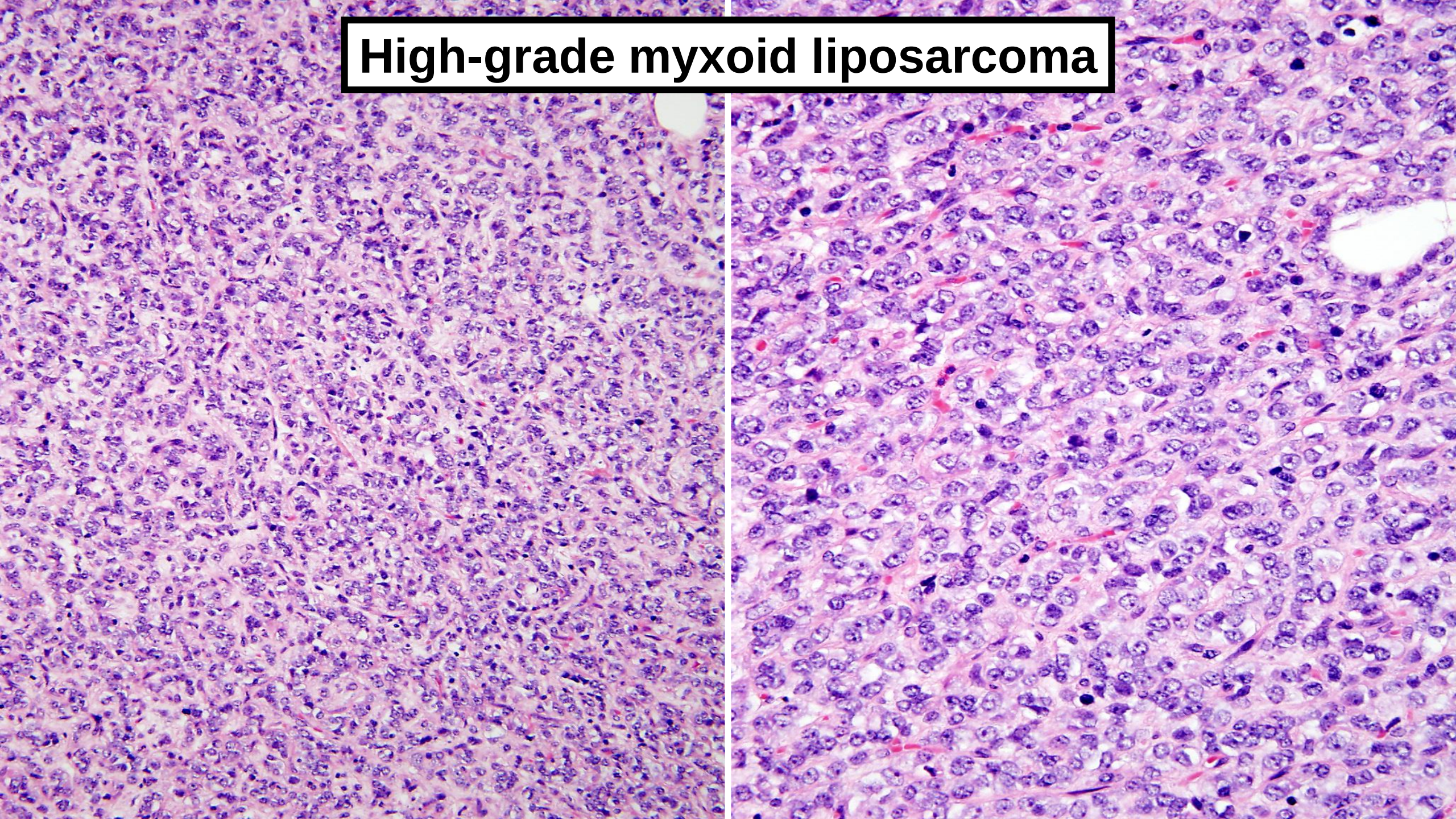

Poorly differentiated synovial sarcoma
SS18::SSX

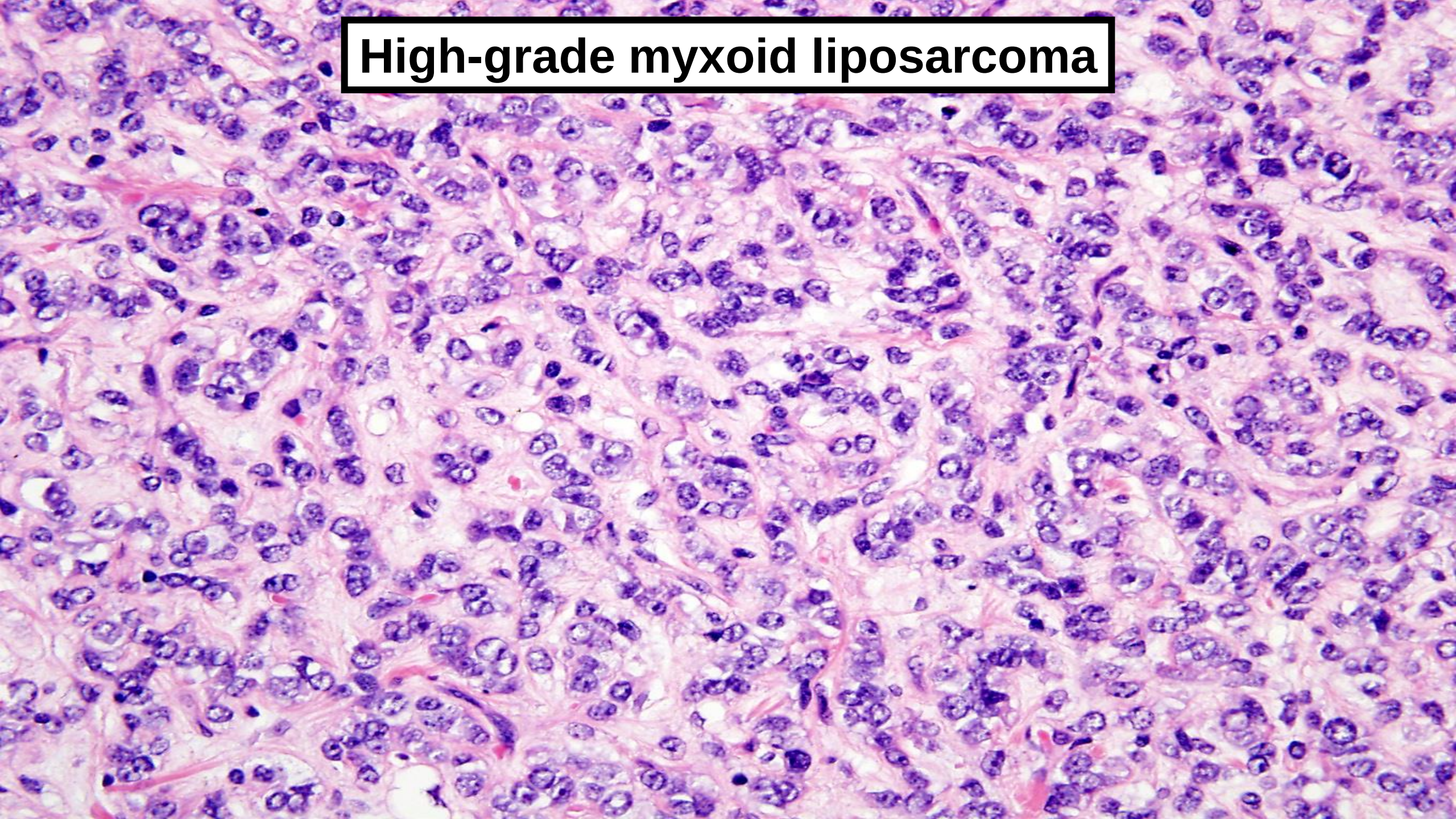
“Round cell” liposarcoma

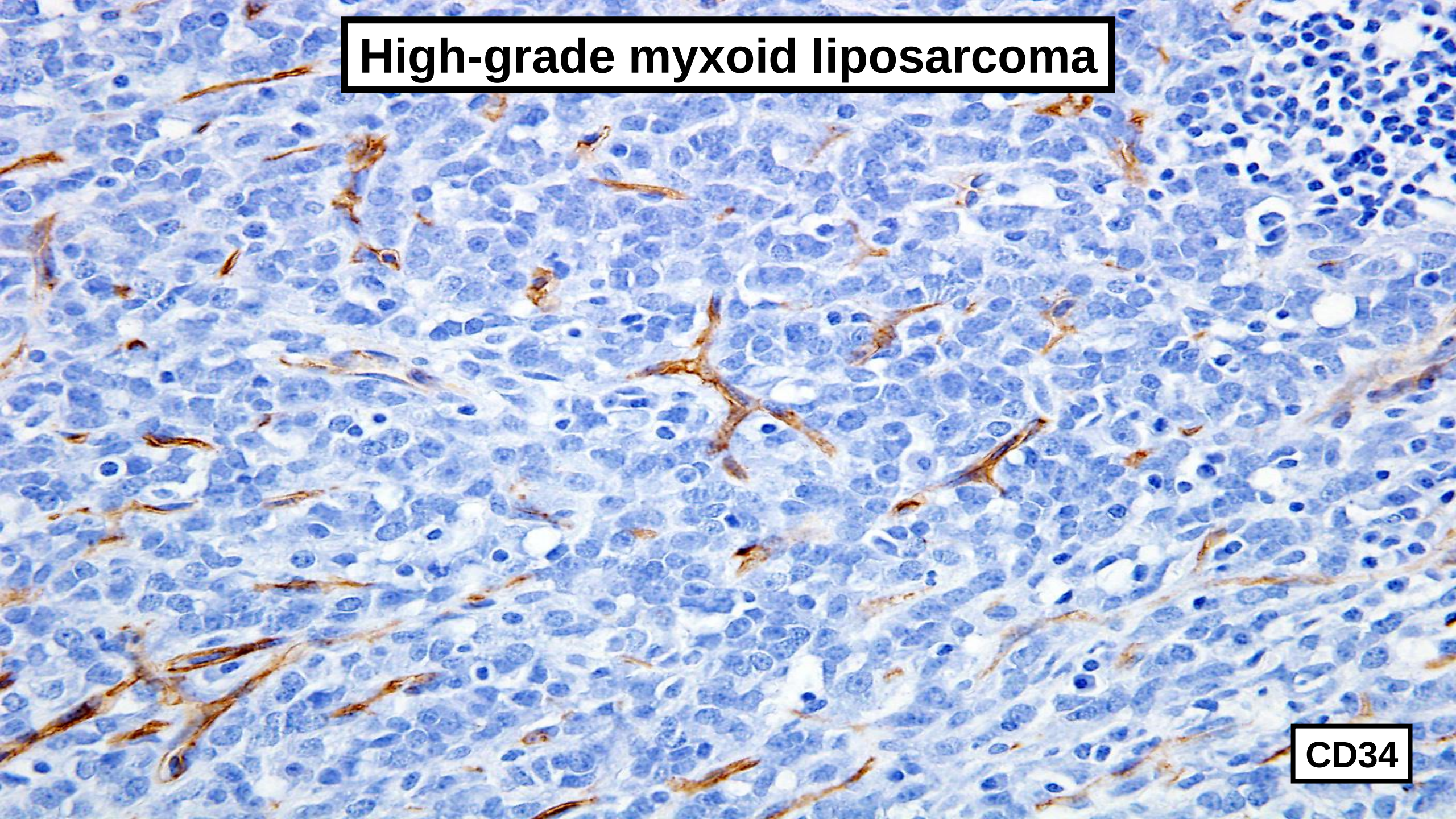
- **Historical term for high grade myxoid liposarcomas composed predominantly of round cells (>80%)**
- **Term no longer used in WHO classification**
- **Marked predilection for extremities, especially thigh**
- **Peak in young adults**
- **High metastatic risk (>50%)**
- **Unusual tendency to metastasize to soft tissues (including serosal surfaces and retroperitoneum) and bone (especially spine)**


High-grade myxoid liposarcoma

High-grade myxoid liposarcoma

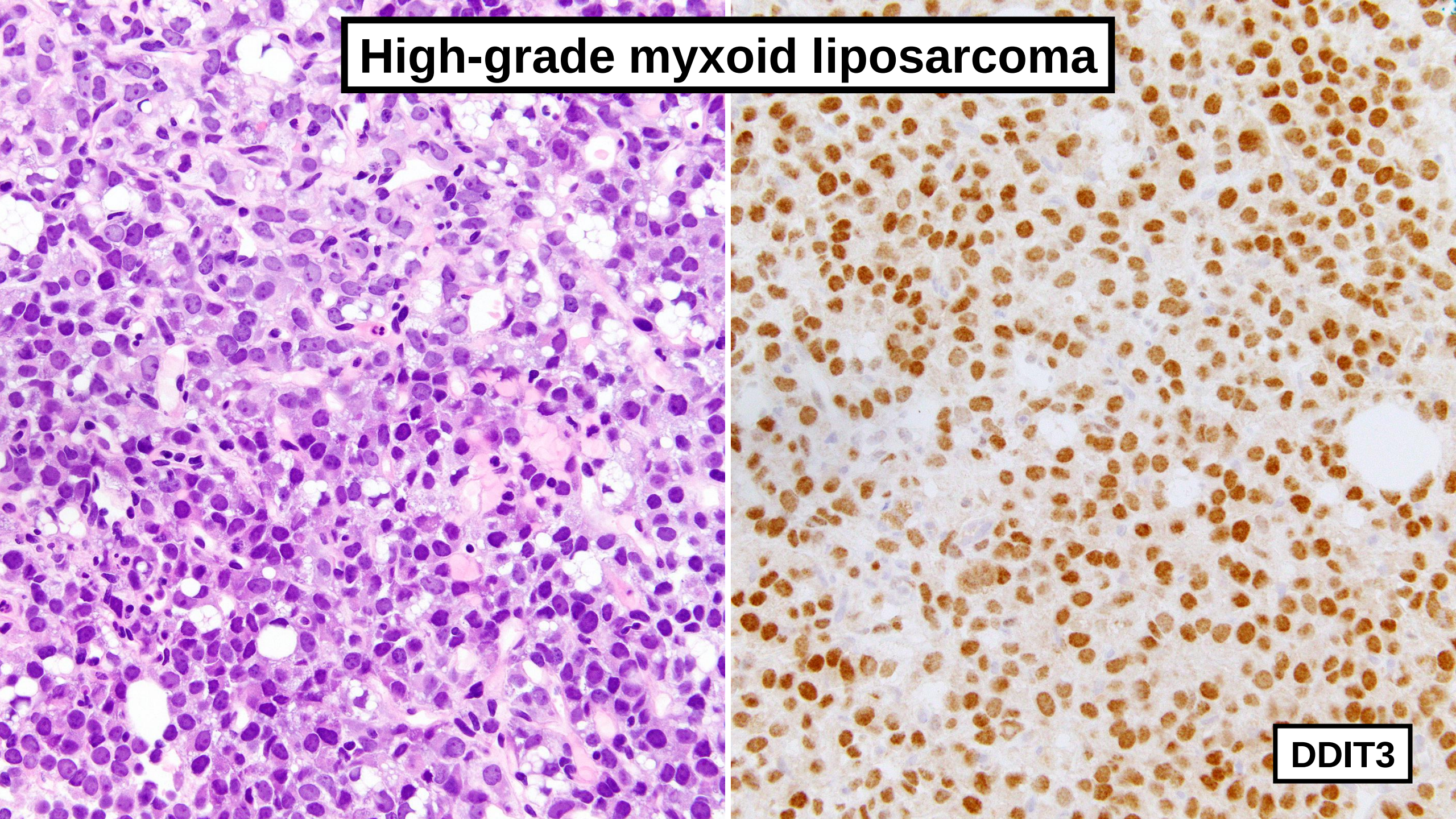



High-grade myxoid liposarcoma


High-grade myxoid liposarcoma
CD34

Round cell liposarcoma: ancillary studies

- **IHC helpful to confirm diagnosis**
  - **DDIT3 highly sensitive and specific**
  - **S100 protein often positive**
- **Molecular genetics:**
  - **t(12;16) with *FUS::DDIT3* in 95%**
  - **t(12;22) with *EWSR1::DDIT3* in 5%**
  - **Confirmation by FISH or NGS straightforward**
  - **FISH for *DDIT3* sensitive and specific (IHC can now be used instead)**


High-grade myxoid liposarcoma
DDIT3

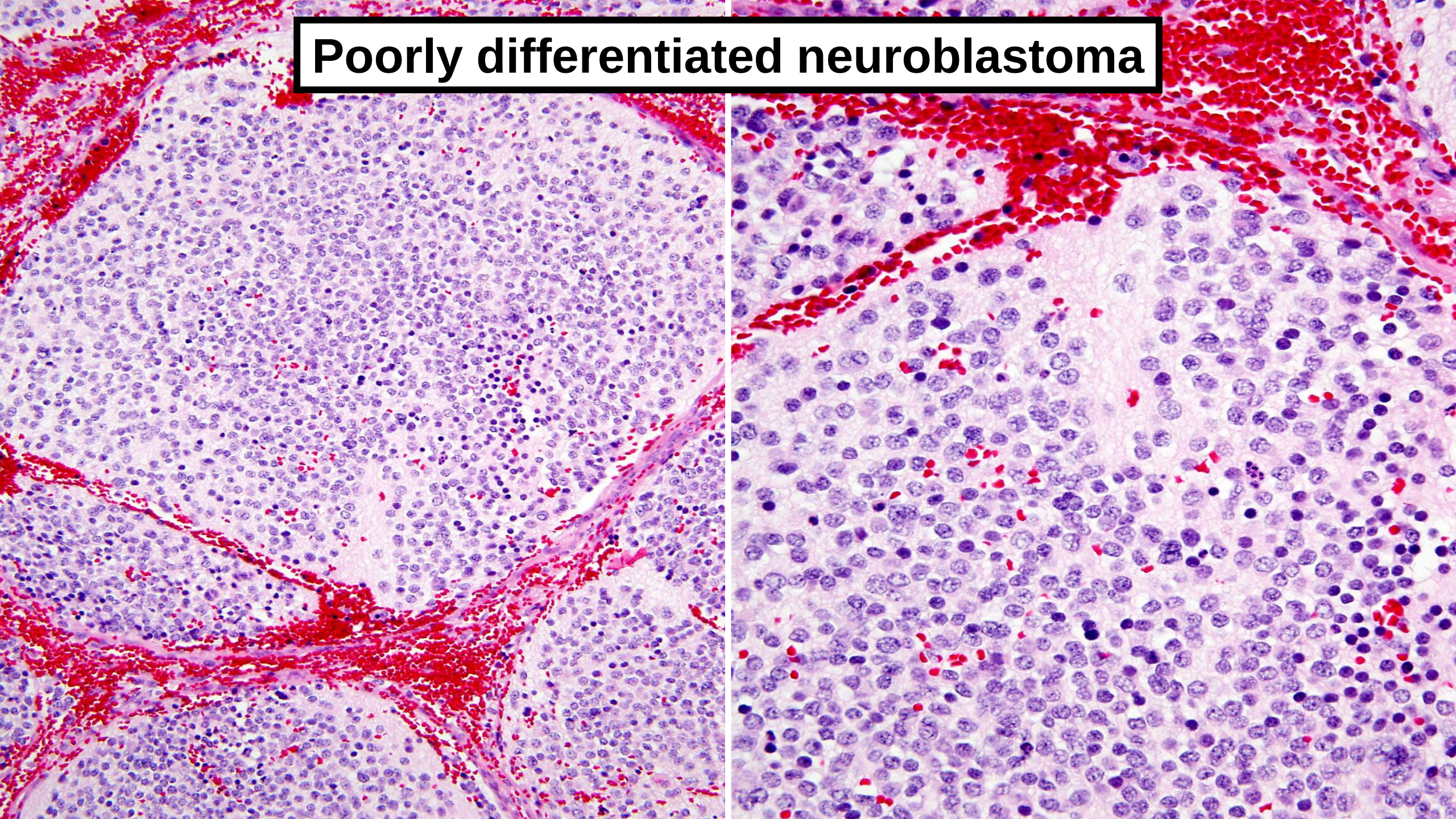
Neuroblastoma

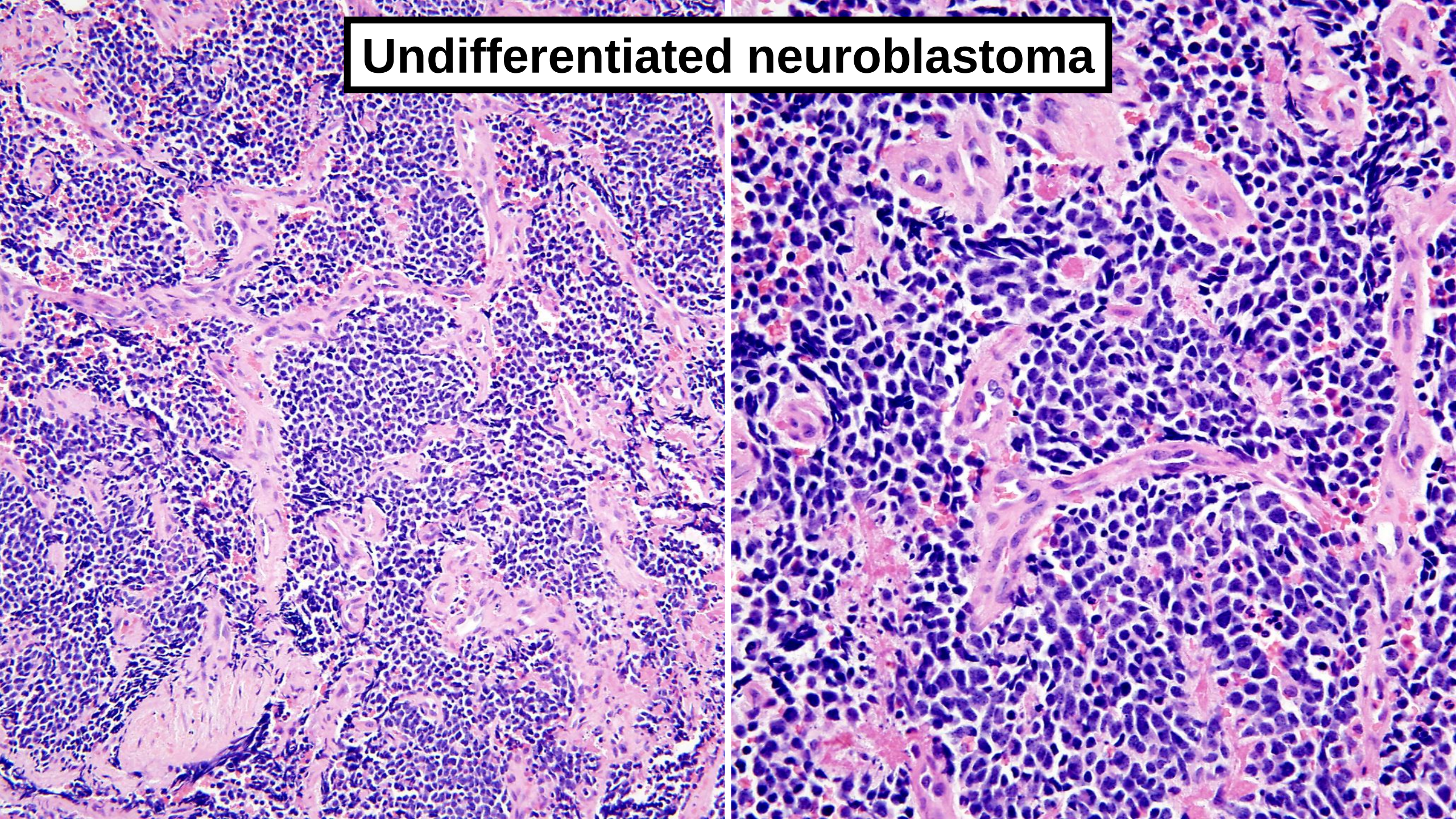
- **Most common extracranial pediatric solid tumor**
- **Most patients present under 5 yrs; median 18 mos**
- **Most common locations: adrenal gland, retroperitoneum, thoracic cavity, abdominal cavity, head and neck**
- **Highly variable clinical course, ranging from indolent (even with metastases) to highly aggressive**
- **May first present with bone metastases**

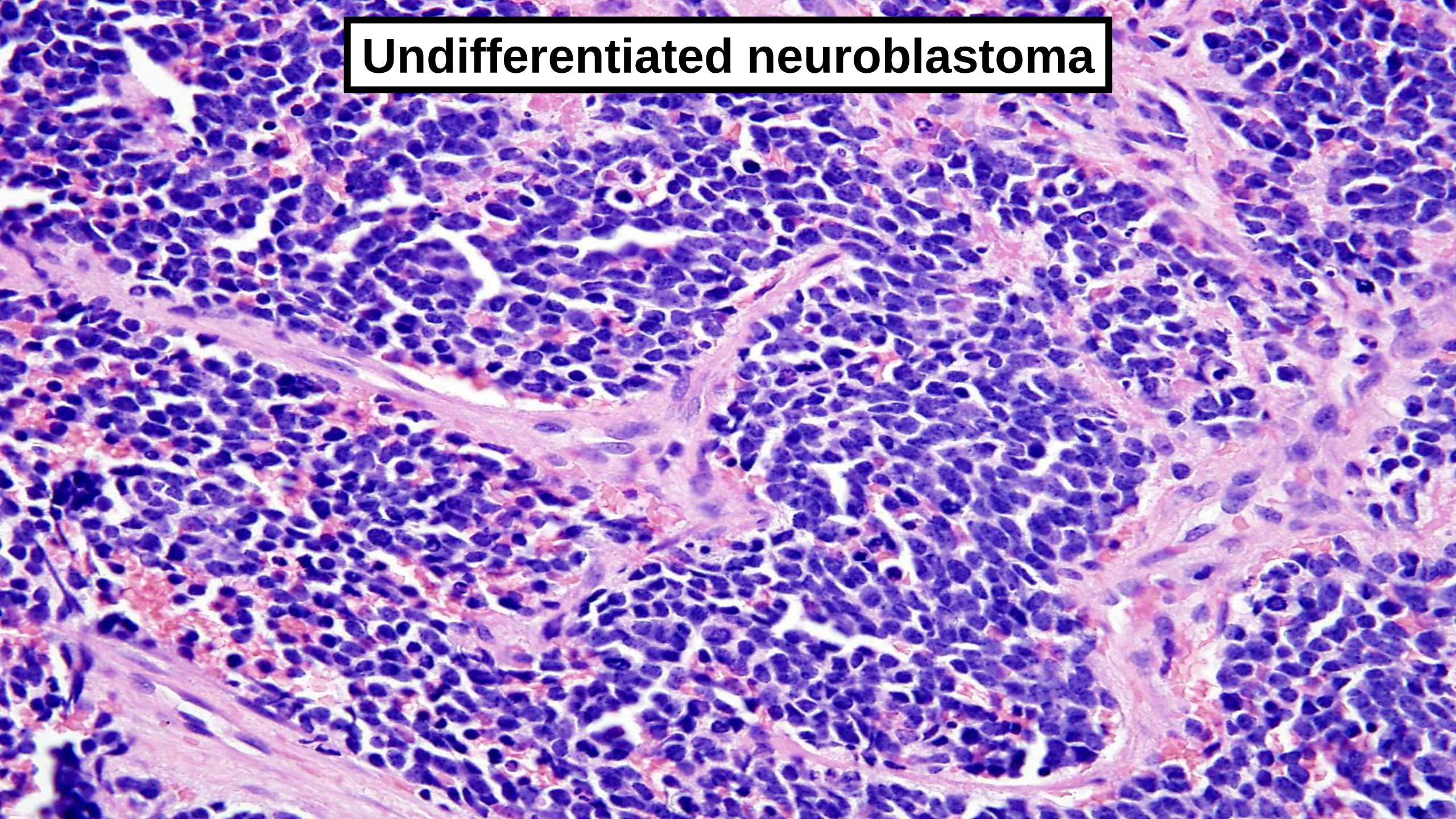
Neuroblastoma: histology

- **Undifferentiated and some poorly differentiated neuroblastomas may be mistaken for round cell sarcomas**
- **Undifferentiated: sheets of round cells with fine chromatin, variably prominent nucleoli, scant cytoplasm**
- **Poorly differentiated: neurofibrillary stroma**

Poorly differentiated neuroblastoma

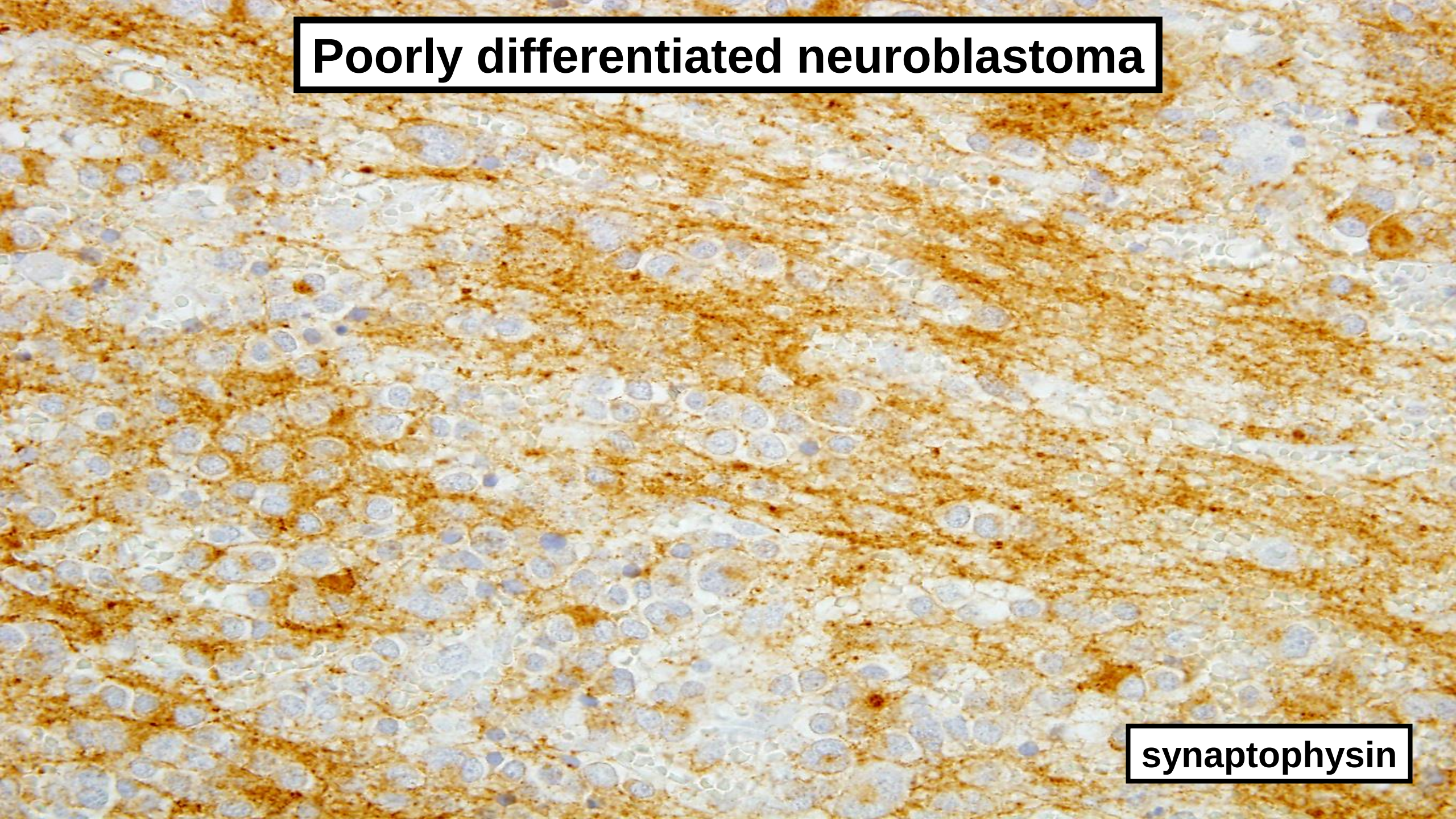


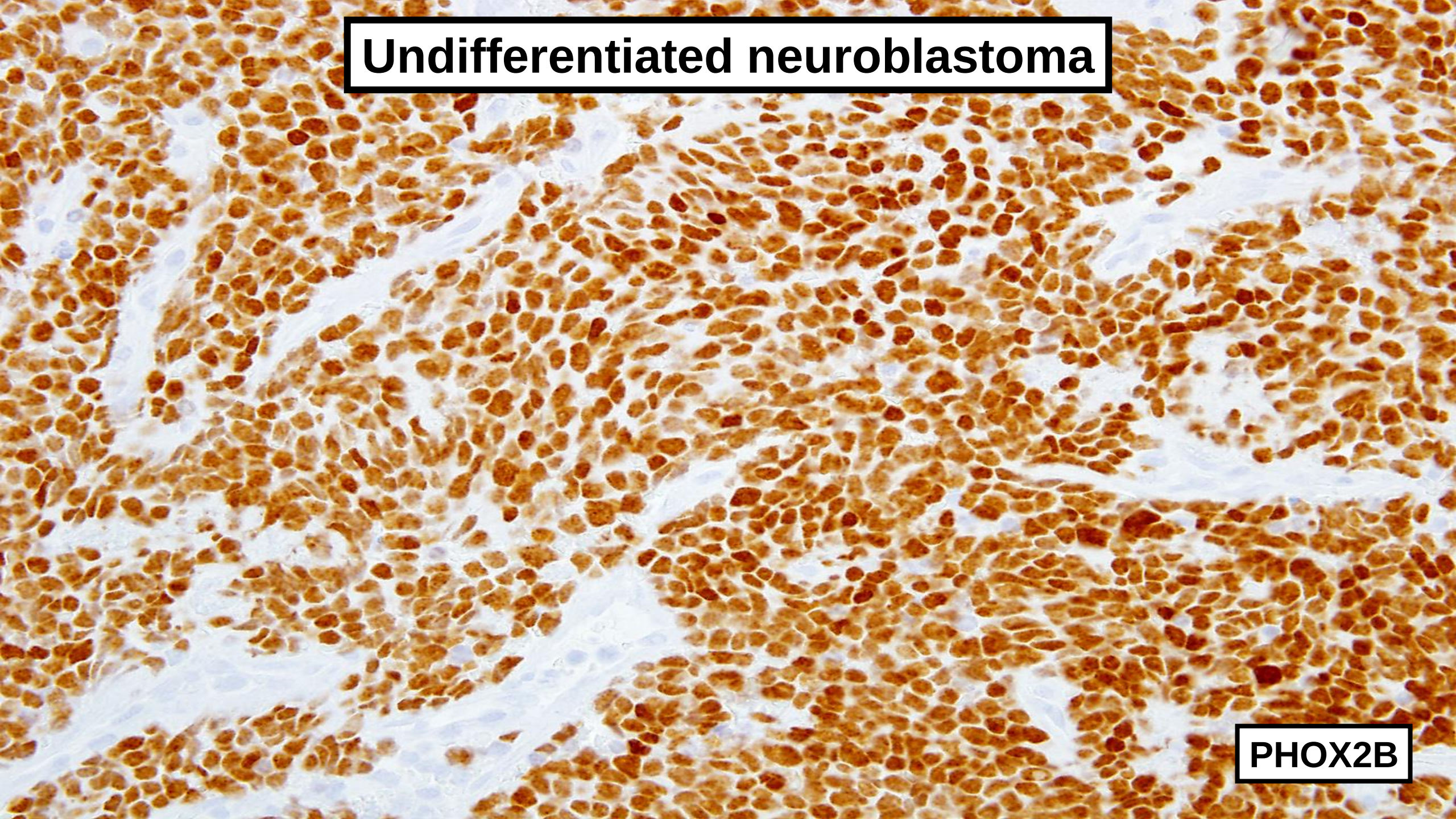

Undifferentiated neuroblastoma


Undifferentiated neuroblastoma

Neuroblastoma: immunohistochemistry

- **Synaptophysin: usually positive (highlights neurofibrillary matrix, when present)**
- **PHOX2B: diffuse, strong nuclear staining highly sensitive and specific**


Poorly differentiated neuroblastoma
synaptophysin


Undifferentiated neuroblastoma
PHOX2B

Practice points

- **Subclassification of round cell sarcomas critical for proper treatment and prognostication**
- **Vascular pattern, stroma, nuclear uniformity or variability helpful histologic clues**
- **Immunohistochemistry plays a central role – many new highly specific diagnostic markers**
- **Molecular genetics (especially FISH) can resolve differential diagnosis in challenging cases**

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THANK YOU!