Molecular biology and impact on modern therapeutical approaches of cutaneous sarcomas

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1. Introduction

This presentation covers a spectrum of highly divergent soft tissue malignancies from locally aggressive with frequent recurrences, only rare metastases and a good long term survival to highly aggressive, rapidly metastatic lesions with short term survival. Lesions have in common to be rare, are clinically frequently misdiagnosed or often with delay, and have characteristic clinicopathologic correlations including immunophenotypic and molecular genetic findings. Besides karyotyping from tissue cultures more recently fluorescence in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR) have been particularly helpful to detect specific translocations, gain & loss of chromosomal material or fusion genes and their products. Most recently, further developments of designed drugs interacting with fusion genes and/or products such as imatinab (Glivec^R) have opened a fascinating new therapeutic field with dramatic impact and effects on tumour growth and regression.

Sarcomas are malignant mesenchymal neoplasias which usually arise in deep soft tissue, less commonly subcutis and rarely primarily in the dermis. The most common cutaneous entities in order of decreasing frequency are dermatofibrosarcoma protuberans (incidence: 2-3/100.000/year), leiomyosarcoma and angiosarcoma of face and scalp in elderly people. Deep location and early subtle clinical features cause delay in diagnosis, and a frequently large size (more than 5cm in diameter) at time of diagnosis. Criteria of malignancy derive from asymmetry in silhouette, inhomogeneous appearance in consistency, demarcation and colour, destructive growth with exulceration and deletion of preexisting tissue, infiltration of nerves with corresponding hypo- or paraesthesia and erosion of cartilaginous to osseous and vascular structures. Besides differentiation criteria many entities frequently have a stereotypical clinical presentation (1-2). Yet, in many instances the clinical experience with these rare disorders is fgrequently too low to allow exact clinical diagnosis. Definitive diagnosis is confirmed by (excisional) biopsy with histology supplemented by immunhistochemistry, occasionally electron microscopy (particularly in neural lesions and pleomorphic sarcomas) as well as in some cases molecular methods (PCR, RT-PCR, sequencing, FISH) (3,4); the latter methods can at present successfully be applied in rather one third of all sarcomas (Table 1).

Table 1: Translocations and fusion products in sarcomas

Sarcoma	Translocation	Fusion product
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	*COL1A1-PDGFB*
Congenital fibrosarcoma/mesoblastic nephroma	t(12;15)(p13;q25)	*ETV6-NTRK3*
Low grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS-CREB3L2*

(including hyalinizing spindle cell tumour with giant rosettes)	t(11;16)(p11;p11)	*FUS-CREB3L1* (rare)
Inflammatory myofibroblastic tumour	t(1;2)q(22;p23) t(2;19)(p23;p13) t(2;17)(p23;q23) t(2;2)(p23;q13)	*TPM3-ALK* *TPM4-ALK* *CLTC-ALK* *RANBP2-ALK*
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) t(1;13)(p36;q14)	*PAX3-FOXO1A* *PAX7-FOXO1A*
Alveolar soft part sarcoma	t(X;17)(p11;q25)	*TFE3-ASPL*
Myxoid & round cell liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	*FUS-DDIT3* *EWS-DDIT3*
Ewing´s sarcoma/ primitive neuroectodermal tumour	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) t(2;22)(q33;q12)	*EWS-FLI1* *EWS-ERG* *EWS-ETV1* *EWS-E1AF* *EWS-FEV*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS-ATF1*
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	*FUS-ATF1*
Synovial sarcoma	t(X;18)(p11;q11)	*SS18-SSX1* *SS18-SSX2* *SS18-SSX4* (rare)
Extraseletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21)	*EWS-NR4A3* *RBP56-NR4A3* *TCF12-NR4A3*
Desmoplastic small round cell tumour	t(11;22)((p13;q12)	*EWS-WT1*

2. Lesions with fibrocytic differentiation: Dermatofibrosarcoma protuberans

In dermatofibrosarcoma protuberans (DFSP) and its juvenile variant, giant cell fibroblastoma, translocations between chromosome 17 and 22 with or without a ring chromosome 17 have led to a fusion gene between COL1A1 and PDGFB. The fusion product of translocations between chromosome 17 and 22 activates a tyrosine-kinase and thereby stimulates proliferation which may be inhibited by a synthetic designer drug imatinib (Glivec[R]). Imatinib originally was developed as an inhibitor of CD117 or c-kit, the ligand of a tyrosine-kinase receptor in gastrointestinal stromal tumor, a frequent (malignant) neoplasm deriving from interstitial cells of Cajal which usually regulate the gastrointestinal motility. Arrest of tumor growth as well as regression were observed in primarily unresectable tumor loads, metastases and other fibrocytic lesions such as desmoids (5-7). The latter entity shows mutations of APC gene causing activation of β-katenin. According to grading heterogeneous genetic changes are seen in myxofibrosarcoma.

3. Lesions with myogenic differentiation: Leio- & rhabdomyosarcoma

No characteristic translocations are seen in leiomyosarcomas which are cytogenetically complex and heterogeneous. Similarly, embryonal and spindle cell rhabdomyosarcomas of childhood have no specific molecular findings. In contrast in alveolar rhabdomyosarcoma, more common in early adults, a discohesive "alveolar" pattern correlates with characteristic translocations t(2;13)(q35;q14) and t(1;13)(p36;q14) corresponding to fusion products *PAX3-FOXO1A and PAX7-FOXO1A*, respectively (Table 1), not seen in embryonal forms, while pleomorphic rhabdomyosarcomas of adults in their fifties are cytogenetically complex and heterogeneous.

4. Lesions with lipogenic differentiation: Liposarcoma

Cytogenetics reveals a ring or giant chromosome 12q13-15 in well-differentiated liposarcoma as well as its dedifferentiated manifestation. Thereby, FISH will reveal an increase of genes coding mdm2 or CK4, whose gene products sometimes can even be detected by immunohistochemistry. Both, myxoid and round cell liposarcomas have a common translocation t(12;16)(q13;p11). Beside overlapping features in histology these molecular findings proved the common nature of these entities which originally were described as different neoplasms. In contrast pleomorphic liposarcomas are cytogenetically very different and inhomogenous.

5. Lesions with vascular differentiation: Angiosarcoma

So far there is no specific finding in cytogenetics which would reliably classify angiosarcomas. Modern biologicals (bevacicumab, an anti-VEGF antibody; Avastin[R]) have so far been of limited value. While nowadays the evidence, that *Morbus Kaposi ("Kaposi sarcoma")* is a reactive-inflammatory response to HHV8 and not a sarcoma, seems convincing (8), there are numerous other vascular lesions which have been considered as low grade vascular neoplasms or *"hemangioendotheliomas"*.

6. Lesions with neural differentiation: Malignant peripheral nerve sheath tumor

This group includes soft tissue neoplasias with schwannian or, rarely, perineural differentiation. The former are best termed neurogenic, the latter perineurial sarcomas. Cytogenetically, one frequently sees a deletion of NF1 gene on chromosome 17, responsible for a tumor suppressor gene, together with a complex and genetically very instable karyotype.

7. Lesions of uncertain differentiation

This is a heterogeneous group of well-defined clinicopathologic entities, whose differentiation is unclear or controversial. One sarcoma which is most relevant to dermatopathologists is:

Clear cell sarcoma

This lesion, also known as *malignant melanoma of soft parts*, shows all characteristics of a melanoma except a highly characteristic chromosomal translocation t(12;22)(q13;q12) so far never seen in melanoma. Usually there is no connection to the epidermis.

8. Conclusions

Novel therapeutic approaches aim at targeting vital molecular pathways of neoplastic cell survival. Entities sometimes show very characteristic alterations of distinct pathways, therefore one can expect that these novel therapies might elicit a more tumor specific hence less toxic effect. Whereas in other solid neoplasms, molecular therapies are more and more applied in the clinic, they are still in their infancy in soft tissue sarcomas. A rational for using molecular therapy in malignancies is the dependency of a given neoplastic cell for a certain altered molecular pathway. Data showing

over-expression of the epidermal growth factor receptor in soft tissue sarcoma and the association of vascular endothelia growth factor expression with a poorer prognosis provide a rational for molecular therapy (8, 9). Trials in which tumor angiogenesis is tackled with bevacizumab or with pan tyrosin kinase inhibitors are on their way. The serine-threonine kinase mTOR plays a crucial role in intracellular signal transduction. Its inhibition has shown promising anti-tumor activity in heavily pretreated patients with soft tissue sarcomas where stable disease or a partial response was achieved in approximately 30% of patients resulting in a median overall survival of 40 weeks.

The great challenge in the future will be to incorporate molecular targeted therapies into conventional treatment regimens. Given the plethora of molecular pathways and the variety of different drugs available today, this task will be substantial. The conduction of hypothesis driven clinical trials based on a strong molecular rational will be required in order to significantly improve systemic treatment options in patients with (cutaneous) sarcomas.

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Classification and Diagnosis of Primary Cutaneous Lymphomas

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Introduction

It is widely accepted now that primary cutaneous lymphomas represent distinct clinical and histopathologic subtypes of extranodal lymphomas. Primary cutaneous lymphomas can be defined as neoplasms of the immune system, characterized by a proliferation of either T or B lymphocytes which show a particular tropism for the skin, without extracutaneous manifestation of the disease at presentation. Extracutaneous spread with lymph nodes involvement can be observed during the course of the disease. Primary cutaneous lymphomas should be separated from secondary skin manifestations of extracutaneous (usually nodal) lymphomas and leukemias, which represent metastatic disease characterized by a worse prognosis and require different treatment. Because the histopathology of primary and secondary cutaneous lymphomas may be similar or identical, in most cases complete staging investigations are needed to establish this distinction.

Thanks to the efforts of the lymphoma groups of both the World Health Organisation (WHO) and European Organisation of Research and Treatment of Cancer (EORTC), in 2005 a joint WHO-EORTC classification for primary cutaneous lymphomas has been proposed. This classification has been absorbed virtually unchanged into the new WHO classification of tumors of hematopoietic and lymphoid tissues published in 2008 (see table at page 8).

PRIMARY CUTANEOUS T CELL LYMPHOMAS

T-cell lymphomas are the most frequent group of malignant lymphomas in the skin, and mycosis fungoides is by far the most frequent single entity, alone representing approximately half of all primary cutaneous lymphomas.

Mycosis fungoides (MF)

Mycosis fungoides is traditionally divided into three clinical phases: patch (early), plaque, and tumor stage. It is characterized by a typical slow evolution, usually over years or decades, and protracted course. It is estimated that over 90% of patients with early mycosis fungoides neither progress to tumor stage nor show extracutaneous manifestations of the disease. The incidence is around 6-7 cases per million people. A genetic predisposition may play a role in some cases but the existence of environmental factors such as viral agents, long-term exposure to various allergens and association with chronic skin disease has been pointed out. Staging investigations are not necessary in early MF(patch stage), and only clinical examination is performed (assessment of percentage of body involvement and of superficial lymph nodes). Patients with plaques, tumors or erythroderma should be screened for extracutaneous involvement (laboratory investigations, sonography of lymph nodes, CT scan of chest, abdomen and pelvis, bone marrow biopsy, examination of the peripheral blood). A monoclonal population of T lymphocytes within the peripheral blood has been observed in some patients with early MF, in many of the cases the clone was different from the one detected in the skin lesions.

Clinical and histologic features

Itching is often a syntom. Erythroderma may develop in the course of the disease rending distinction from Sezary syndrome difficult.

Patch stage

Variably large, erythematous, finely scaling lesions with a predilection for the buttocks and other sun-protected areas. These patches may also have different aspects such as a wrinkled appearance (loss of the elastic fibers and atrophy of the epidermis), "xanthomatous"-like aspect (xanthoerythroderma perstans) characterized by a yellowish hue, and a digitate pattern.
The histopathology of early lesions of MF reveal a patchy lichenoid or band-like infiltrate in the papillary dermis. Small lymphocytes predominate and atypical cells can be seen only in a minority of cases. Epidermotropism of solitary lymphocytes with nuclei slightly larger than those of lymphocytes within the upper dermis is usually found but Darier's nests (Pautrier's microabscesses) are rare.

Plaque stage

Infiltrated, variably scaling, reddish-brown lesions. Typical patches are usually observed contiguous to plaques or at other sites of the body.
Histology shows a dense, band-like infiltrate within the upper dermis. Intraepidermal lymphocytes arranged in Darier's nests are a common finding. Cytomorphologically, small pleomorphic (cerebriform) cells predominate. In some cases plaques of MF may present with a predominantly interstitial infiltrate (interstitial MF).

Tumor stage

In this stage a combination of patches, plaques and tumors is usually found, but tumors may be observed solitary, localized or generalized in the absence of other lesions. Ulceration is common. Onychodystrophy may be prominent. Unusual sites may be involved, such as the mucosal regions (differential diagnosis with cytotoxic NK/T-cell lymphomas).
A dense, nodular or diffuse infiltrate is found within the entire dermis, usually involving the subcutaneous fat. Epidermotropism may be lost. Angiocentricity and/or angiodestruction may be observed in some cases. As in plaque stage, flat tumors may present a predominantly interstitial infiltrate (interstitial MF).
In more than 50% of patients with tumor-stage MF large cell transformation has been detected, consisting of large cells (immunoblasts, pleomorphic cells or large anaplastic cells) exceeding 25% of the infiltrate or large cells forming microscopic nodules. Tumors with large cell morphology may or may not express CD30 without any prognostic significance. Large cells transformation of MF bears a poor prognosis and usually heralds the terminal stage of the disease.

Immunophenotype

MF is characterized by an infiltrate of α/β T-helper memory lymphocytes (β F1$^+$, CD3$^+$, CD4$^+$, CD5$^+$, CD8$^-$, CD45Ro$^+$). Only a minority of cases exhibit a T-cytotoxic (β F1$^+$, CD3$^+$, CD4$^-$, CD5$^+$, CD8$^+$) or γ/δ (β F1$^-$, CD3$^+$, CD4$^-$, CD5$^+$, CD8$^+$) lineage which show no clinical and/or prognostic differences. In these cases correlation with the clinical features is crucial in order to rule out skin involvement by aggressive cytotoxic lymphomas. In late stages there may be a partial loss of pan-

T-cell antigen expression. Cytotoxic-associated markers such as TIA-1, granzyme B and perforin are negative in MF, although occasionally in late stages of the disease positivity may be observed.

Clinical and histopathologic variants

Several clinical and/or histopathologic variants have been described. Some of them are peculiar clinical or histopathologic presentations observed only in a few patients, but others are more frequent.

- *MF with follicular mucinosis/follicular (pilotropic) MF*: follicular papules and plaques characterized histopathologically by abundant deposition of mucin within hair follicles surrounded by a more or less dense infiltrate of T-lymphocytes. The hair follicles are infiltrated by the lymphocytes (pilotropism). The epidermis between affected follicles may be spared or involved by the disease. Alopecia due to destruction of the follicles is common (alopecia mucinosa), either generalized or localized. Itching is severe. A variant of MF with marked involvement of the hair follicles but without deposition of mucin has also been described (pilotropic MF).

- *Localized pagetoid reticulosis (Woringer-Kolopp type)/unilesional (solitary) MF*: solitary, psoriasiform, scaly erythematous patches or plaques, usually located on the extremities. The histologic picture shows a hyperplastic epidermis with striking epidermotropism of T-lymphocytes usually characterized by medium-sized pleomorphic nuclei. Patients with the generalized form of pagetoid reticulosis (Ketron-Goodman type) probably have either classic MF or, more frequently, one of primary cutaneous cytotoxic NK/T-cell lymphoma. The prognosis of patients with solitary pagetoid reticulosis is excellent.

- *Granulomatous MF/granulomatous slack skin*: a granulomatous pattern can be observed in different lesions of MF and in some cases within affected lymph nodes. Granulomatous lesions may either precede, be concomitant with or follow "classic MF". A rare variant of granulomatous MF is represented by so-called granulomatous slack skin, which is characterized clinically by the occurrence of bulky, pendulous skinfolds, usually located in flexural areas.

- *Other variants*. Syringotropic MF, erythrodermic MF, poikilodermatous MF, hypopigmented/hyperpigmented MF, pigmented purpura-like MF, bullous (vesciculobullous) MF/disidrotic MF, papular MF, "invisible" MF, anetodermic MF.

Sezary's syndrome

Sézary syndrome is characterized clinically by pruritic erythroderma, generalized lymphadenopathy, and the presence of circulating malignant T lymphocytes. Other typical cutaneous changes include palmoplantar hyperkeratosis, alopecia and onychodystrophy. The demonstration of a monoclonal population of T lymphocytes within the peripheral blood and the presence of the same T-cell clone in the skin has been proposed as an important criterion for the diagnosis. Other useful criteria include the presence of at least 1000 circulating Sézary cells/mm^3, an expanded CD4$^+$ population in the peripheral blood, resulting in a markedly increased CD4$^+$/CD8$^+$ ratio (>10), an increased population of CD4$^+$/CD7$^-$ cells in the peripheral blood, Sézary cells larger than 14 μm in diameter, Sézary cells representing more than 20% of circulating lymphocytes, and the loss of T-cell antigens such as CD2, CD3, CD4, and CD5.Involvment of the bone marrow is rare in early phases but may be found at later stage. The histopathologic features of Sézary syndrome are indistinguishable from those of MF. Epidermotropism is usually less marked,

but typical Pautrier's microabscesses may be observed. There's a predominance of small- to medium-sized pleomorphic (cerebriform) lymphocytes. Differential diagnosis with MF is possible only by correlation of histopathologic features with clinical ones. Immunohistology reveals a predominance of T-helper lymphocytes (CD3$^+$, CD4$^+$, CD7$^-$, CD8$^-$). In the majority of cases a clonal rearrangement of the T-cell receptor (TCR) genes can be detected.

Primary cutaneous CD30$^+$ lymphoproliferative disorders

Lymphomatoid papulosis (LP)

Chronic, recurrent, self-healing eruption of papules and small nodules with the histopathologic features of a cutaneous T-cell lymphoma. In 10-20% of patients LP is preceded, concomitant with or followed by another type of lymphoma. In addition, patients with LP are also at higher risk of developing non lymphoid second malignancies. In the absence of any specific symptoms of other associated disease, complete staging investigations are not necessary in patients with LP. Young adults are usually affected. Clinically the disease presents as a generalized eruption of reddish-brown papules or small nodules on the trunk and proximal extremities, but in some cases only a few lesions may be present. Ulceration is common. Spontaneous resolution is observed within a few weeks or months. Large tumors with complete spontaneous resolution have also been observed. Three main histopathologic subtypes have been described: type A ("histiocytic" type) (most frequent) composed of large atypical CD30$^+$ cells admixed with small lymphocytes, eosinophils and neutrophils; type B (MF-like) (rare) characterized by a wedge-shaped or band-like infiltrate of small/medium-sized atypical cells with epidermotropism (CD30$^{+/-}$); type C (anaplastic large cell lymphoma-like) composed of sheets of large atypical cells admixed with a few small lymphocytes, neutrophils and eosinophils. Neoplastic cells are, in most of cases, CD30$^+$ and express the phenotypic markers of T-helper lymphocytes (CD3$^+$, CD4$^+$, CD8$^-$) in some cases and of T-cytotoxic cells (CD8$^+$) in others. Expression of CD56 is usually absent. Monoclonal rearrangement of the TCR is detected in the majority of cases.

Cutaneous anaplastic large cell lymphoma

It is defined as a CD30$^+$ large T-cell lymphoma presenting primary in the skin and characterized by a good prognosis and response to treatment. Complete staging investigations are mandatory before definitive diagnosis, in order to exclude secondary involvement from nodal disease. The disease occurs mostly in adults but cases in children have been reported. Clinically, patients present with solitary or localized, often ulcerated reddish-brown tumors. Partial regression can be observed. Clinical variants include the presence of satellite lesions around the primary tumor. The histology shows nodular or diffuse infiltrates characterized by cohesive sheets of large CD30$^+$ atypical cells. Citomorphologically the cells are large anaplastic cells, large pleomorphic cells or immunoblasts; small/medium and signet-ring cell morphology may be observed. The majority of neoplastic cells are CD30$^+$ and generally have a T-helper phenotype (CD3$^+$, CD4$^+$, CD8$^-$), though CD8$^+$ cytotoxic phenotypes are not uncommon. CD56$^+$ cases are not common but more frequent than in LP. Monoclonal rearrangement of the TCR genes is usually present whereas is absent the t(2;5).

Subcutaneous "panniculitis-like" T-cell lymphoma

The definition of subcutaneous "panniculitis-like" T-cell lymphoma includes exclusive involvement of the subcutaneous fat and α/β cytotoxic T-cell phenotype. Patients are adults and rarely children. Lesions are characterized by subcutaneous erythematous not ulcerated plaques and tumors arising preferentially on the extremities. Skin lesions may simulate erythema nodosum,

lupus panniculitis or other pannniculitic diseases. In a minority of patients there are accompanying symptoms such as fever, malaise, fatigue, and weight loss. A emophagocytic syndrome may be seen in advanced stages, and can be the cause of death in these patients. Histopathology reveals dense, nodular or diffuse infiltrates of small, medium and (rarely) large pleomorphic cells admixed with variable numbers of macrophages within the subcutaneous fat with the pattern of lobular panniculitis. Neoplastic cells are arranged in small clusters or as solitary units around the single adipocytes (rimming of the adipocytes). Necrosis is often a prominent feature. Immunohistology shows an α/β T-suppressor phenotype (βF1$^+$, CD3$^+$, CD4$^-$, CD8$^+$) of neoplastic cells. Cytotoxic markers (TIA-1, granzyme B and perforin) are always expressed but CD56 is negative. Monoclonal rearrangement of TCR is detected in the majority of the cases. The disease has usually protracted course with recurrent subcutaneous lesions. In many cases the treatment is based on systemic steroids. Systemic chemotherapy and radiotherapy have been used in many instances.

Aggressive cutaneous cytotoxic lymphomas

Extranodal NK/T-cell lymphoma, nasal type

It is commonly located in the upper respiratory tract, especially the nasal cavity, but involvement of other organs can be observed, particularly the skin. The disease may be primary cutaneous and staging investigations can be negative at presentation. Patients are adults, and children rarely affected. The skin lesions are erythematous or violaceus plaques and tumors, sometimes ulcerated. The nasal cavity and the upper respiratory tract should be checked carefully. Symptoms of nasal obstruction and/or epistaxis should be investigated for evidence of nasal lymphoma. The variant described in the past as lethal midline granuloma is associated with large ulcers of the nose and adjacent tissues. Histopathology reveals a diffuse proliferation of small-, medium- or large-sized pleomorphic cells involving the dermis and often the subcutaneous tissue. Usually present a prominent angiocentricity and angiodestruction. Cells are negative for T-cell markers (CD3$^-$, CD4$^-$, CD5$^-$, CD8$^-$) and positive, in practically all cases, for CD2, CD56 and cytotoxic proteins (TIA-1, granzyme B, perforin). EBV can be demonstrated by in situ hybridization in practically all cases. A germline configuration of the TCR genes is present in most of the cases.

Primary cutaneous peripheral T-cell lymphoma, NOS

Primary cutaneous aggressive epidermotropic CD8$^+$ cytotoxic T-cell lymphoma

It is a disease characterized by aggressive behaviour clinically and proliferation of epidermotropic CD8$^+$ T lymphocytes histopathologically. Distinction from CD8$^+$ MF is made on the basis of clinical presentation and behaviour. Patients are adults and present with generalized patches, plaques and tumors, usually ulcerated. The clinical features are indistinguishable from those of cutaneous γ/δ T-cell lymphoma and to those of generalized pagetoid reticulosis. Involvement of the mucosal regions is common and the tumor often spread to the central nervous system. Histology reveals a nodular or diffuse proliferation of small-, medium- or large-sized pleomorphic cells with marked epidermotropism which may be partially loss in advanced stages. Invasion and destruction of adnexal structures is common. Immunohistology shows the phenotypic profile of neoplastic lymphocytes (βF1$^+$, CD2$^{-/+}$, CD3$^+$, CD4$^-$, CD7$^+$, CD8$^+$, TIA-1$^+$, CD45Ra$^+$, CD45RO$^-$). CD56 is usually negative. Monoclonal rearrangement of the TCR genes is detected in the majority of the cases.

Primary cutaneous γ/δ T-cell lymphoma

Cutaneous γ/δ T-cell lymphoma is included as a provisional entity in the WHO-EORTC classification, and as a distinct entity in the WHO 2008 classification. A major problem for precise characterization of this rare lymphoma is the lack of a reliable marker of γ/δ T-lymphocytes on routinely fixed, paraffin-embedded sections of tissue. Patients are adults. They present with generalized patches, plaques and tumors, often ulcerated. The patches of the disease, unlike the patches of MF, reveal the clinical picture of severe interface dermatitis with a red-brown aspect and small superficial erosions. Involvement of the mucosal regions is common. Hemophagocytic syndrome is a frequent complication. For the diagnosis is crucial to exclude a history of MF. The histology shows nodular or diffuse infiltrates of small/medium- to large-sized pleomorphic cells usually with both epidermotropism and involvement of the subcutaneous tissue. The phenotypic profile of neoplastic cells is tipically $\beta F1^-$, $CD3^+$, $CD4^-$, $TIA-1^+$, $CD56^+$, $CD57^-$ and CD8 may be expressed in some cases. Immunohistology on frozen sections reveals positivity for δ1.
Molecular biology shows a monoclonal rearrangement of the TCR genes. γ/δ T-cell lymphomas are characterized by overexpression of genes of NK-cell-associated molecules such as killer cell immunoglobulin-like receptor (KIR) genes and killer cell lectin-like receptors (KLRC).

Cutaneous small-medium pleomorphic T-cell lymphoma

This lymphoma is listed as a provisional entity in the WHO-EORTC classification. Since MF and Sézary syndrome are characterized by the predominance of small-medium pleomorphic T lymphocytes, the diagnosis of small-medium pleomorphic T-cell lymphoma can only be accepted if MF and Sézary syndrome are ruled out by a complete clinical examination. Patients are adults or elderly. They present usually with solitary erythematous or purplish tumors, commonly located on the face and neck or upper trunk, but multiple tumors can be seen. Ulceration is uncommon. Histology reveals dense, nodular or diffuse infiltrates of predominantly small/medium-sized lymphocytes with pleomorphic nuclei within the entire dermis, often involving the superficial part of the subcutaneous fat. Large cells, when present, should not exceed 30% of the neoplastic infiltrate. Epidermotropism is usually absent. A granulomatous reaction can be observed. The neoplastic cells show a T-helper phenotype, sometimes with loss of pan-T-cell antigens. A reactive infiltrate of B lymphocytes is commonly seen. Monoclonal rearrangement of TCR genes is commonly present.

CUTANEOUS B CELL LYMPHOMAS

Cutaneous follicle center lymphoma (FCL)

It is defined as the neoplastic proliferation of germinal center cells confined to the skin.
Complete staging investigations must be performed in all patients, as the clinicopathologic features alone cannot distinguish with certainty between FCL and secondary involvement of extracutaneous lymphoma with a similar morphology. FCL is currently defined by the absence of extracutaneous manifestations after complete staging investigations have bee performed. Patients are adults. Onset in children is exceptional. FCL presents clinically with erythematous papules, plaques and tumors, usually non-ulcerated, located mostly on the head and neck and on the trunk. A distinct clinical presentation with plaques and tumors on the back surrounded by erythematous macules and papules expanding centrifugally around the central tumors has been described as Crosti's lymphoma. Association with infections such as Borrelia burgdoferi, HCV or HHV8 has been described in sporadic patients. Histology shows nodular or diffuse infiltrates characterized by predominance of centroblasts and centrocytes admixed with small reactive lymphocytes. The pattern of growth can

be purely follicular, purely diffuse, or mixed. The epidermis is spared as a rule. Cases with a purely diffuse pattern of growth should be carefully differentiated by diffuse large-B-cell lymphoma as prognostic features are completely different. Neoplastic cells are positive for B-cell markers such as CD20 and CD79. Most cases with a diffuse pattern of growth are negative for CD10 and do not show a network of CD21 follicular dendritic cells in the background. In contrast, cases of follicle center lymphoma with a follicular pattern of growth are positive for markers of germinal center cells such as CD10 and Bcl-6. Negativity for Bcl-2, MUM-1 and FOX-P3 is usually observed. MIB-1 staining shows reduced proliferation of neoplastic follicles. Monoclonal rearrangement of IgH gene is detected in the majority of cases. t(14;18) is absent in most cases.

Cutaneous marginal zone lymphoma (MZL)

Patients are typically younger adults. Onset in childhood has been observed. Association with Borrelia burgdoferi has been detected in some cases. They present with red to reddish brown papules, plaques and nodules localized particularly to the upper extremities or the trunk. Lesions are commonly solitary but may be multiple characterized either by clustered or scattered lesions. Cutaneous recurrences are frequent. Complete staging investigations should be performed, particularly to exclude cutaneous involvement from other types of extranodal marginal zone lymphoma or MALT. Histology shows patchy, nodular or diffuse infiltrates involving the dermis and sometimes the superficial part of the subcutaneous fat. The epidermis is not involved. A characteristic pattern can be observed: nodular infiltrates with follicles sometimes containing reactive germinal centers, are surrounded by a pale-staining peri- and interfollicular population of small- to medium-sized cells with indented nuclei, inconspicuous nucleoli and abundant pale cytoplasm (marginal zone cells, centrocyte-like cells). In addition, plasmacells, lymphoplasmacytoid cells, small lymphocytes and occasional large blasts are observed. In typical cases, the neoplastic population is composed of marginal zone cells, a few lymphoplasmacytoid lympocytes an several plasmacells. In some lesions neoplastic plasmacells predominate admixed with few marginal zone cells, resembling the picture of cutaneous palsmocytoma. These cases are classified as cutaneous MZL, plasmacytic variant. Cases with predominance of blasts are rare. The marginal zone cells reveal a $CD20^+$, $CD79a^+$, $Bcl-2^+$, $CD5^-$, $CD10^-$ and $Bcl-6^-$ phenotype. Monoclonal expression of the immunoglobulin light chain is often observed. A monoclonal rearrangement of the IgH gene can be observed in approximately 50-60% of cases.

Cutaneous diffuse largeB-cell lymphoma, leg type (DLBCL)

It is a lymphoma of intermediated behaviour, occurring mostly on the legs in elderly patients. It is important to distinguish DLBCL with FCL diffuse type because of their different clinical and prognostic features. The disease predominantly affects elderly patients. Patients present with solitary or clustered erythematous or reddish brown tumors, mostly located on the distal extremity of one leg. Ulceration is common. Lesions with similar histopathologic and phenotypical features can arise at cutaneous site other than the legs (DLBCL, leg type, occurs in approximately 80-85% of cases on the legs). Histology shows a dense, diffuse infiltrate within the entire dermis and subcutis. Involvement of the epidermis is possible. The neoplastic infiltrate consists predominantly of large cells with round nuclei (immunoblasts and centroblasts). Reactive small lymphocytes are usually only sparse. A rare histopathologic variant of DLBCL, leg type, shows a starry sky and/or mosaic stone-like pattern similar of that of Burkitt-like lymphoma. Neoplastic cells express B-cell markers (CD20, CD79a). Bcl-2, MUM-1, and FOX-P1 are positive in the great majority of cases. Bcl-6 and CD10 are often expressed demonstrating a derivation from germinal center cells. Stainings for immunoglobulin light chains usually show a monoclonal population. The tumors reveal a monoclonal rearrangement of the IgH gene in the majority of cases.

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM (CD4$^+$/CD56$^+$ HEMATODERMIC NEOPLASM)

In most cases, at presentation, blastic plasmocytoid dendritic cell neoplasm is confined to the skin or skin lesions are the first manifestation of the disease (90% of cases). Leukemic spread after variable periods of time is the rule. Patients are mostly elderly adults. Clinically they present with solitary (rarely), localized or generalized plaques and tumors with characteristic "bruise-like" violaceous aspect due to intratumoral hemorrhage. Ulceration is uncommon. Mucosal region may be involved. In a proportion of patients (30-40%) skin lesions are accompanied by systemic symptoms and extracutaneous manifestations in the blood, bone marrow and/or other organs. Lymph nodes are involved in approximately half of the cases at presentation. Trombocytopenia, anemia and neutropenia are commonly found. Histology shows a diffuse monomorphous infiltrate of medium-sized neoplastic cells with a blastoid morphology. The epidermis is not involved whereas involvement of the subcutis is common. In early lesions there are perivascular infiltrates of blastoid cells, admixed with reactive lymphocytes. The neoplastic cells are positive for CD4 and CD56. TdT is positive in the majority of the cases, whereas myeloid antigens, NK cell markers and cytotoxic proteins are negative. CD123 is usually positive. Other positive markers are Bcl-2, CD43, CD101 and TCL-1. Focal positivity for Bcl-6 and MUM-1 has been observed in some cases. CD3, CD5, CD20, and myeloperoxidase are usually negative.

WHO-EORTC classification of primary cutaneous lymphomas.

T-Cell Lymphomas (CTCL)	B-Cell Lymphomas
Mycosis fungoides • *Folliculotropic MF* • *Pagetoid reticulosis* • *Granulomatous slack skin* Sézary's syndrome Adult T-cell leukemia/ly. Primary cutaneous CD30+ lymphoproliferative disorders • *Lymphomatoid papulosis* • *Large cell CTCL, CD30+* Subcutaneous T-cell lymphoma Extranodal NK/T-cell ly., nasal-type Peripheral T-cell lymphoma, NOS • *Primary cutaneous CD8+ aggressive CTCL (provisional)* • *g/d CTCL (provisional)* • *Primary cutaneous CD4+ pleomorphic small-medium-sized CTCL (provisional)*	Primary cutaneous Marginal zone B-cell lymphoma Primary cutaneous Follicle center lymphoma Primary cutaneous Diffuse large B-cell lymphoma, leg-type Primary cutaneous Diffuse large B-cell lymphoma, other
Precursor hematologic neoplasm Blastic plasmocytoid dendritic cells neoplasm (CD4+/CD56+ hematodermic neoplasm)	

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1. Cerroni L, Gatter K, Kerl H. Skin Lymphoma. London: Wiley-Blackwell, 2009.

2. Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2008

3. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. Blood 2005 105: 3768-85.
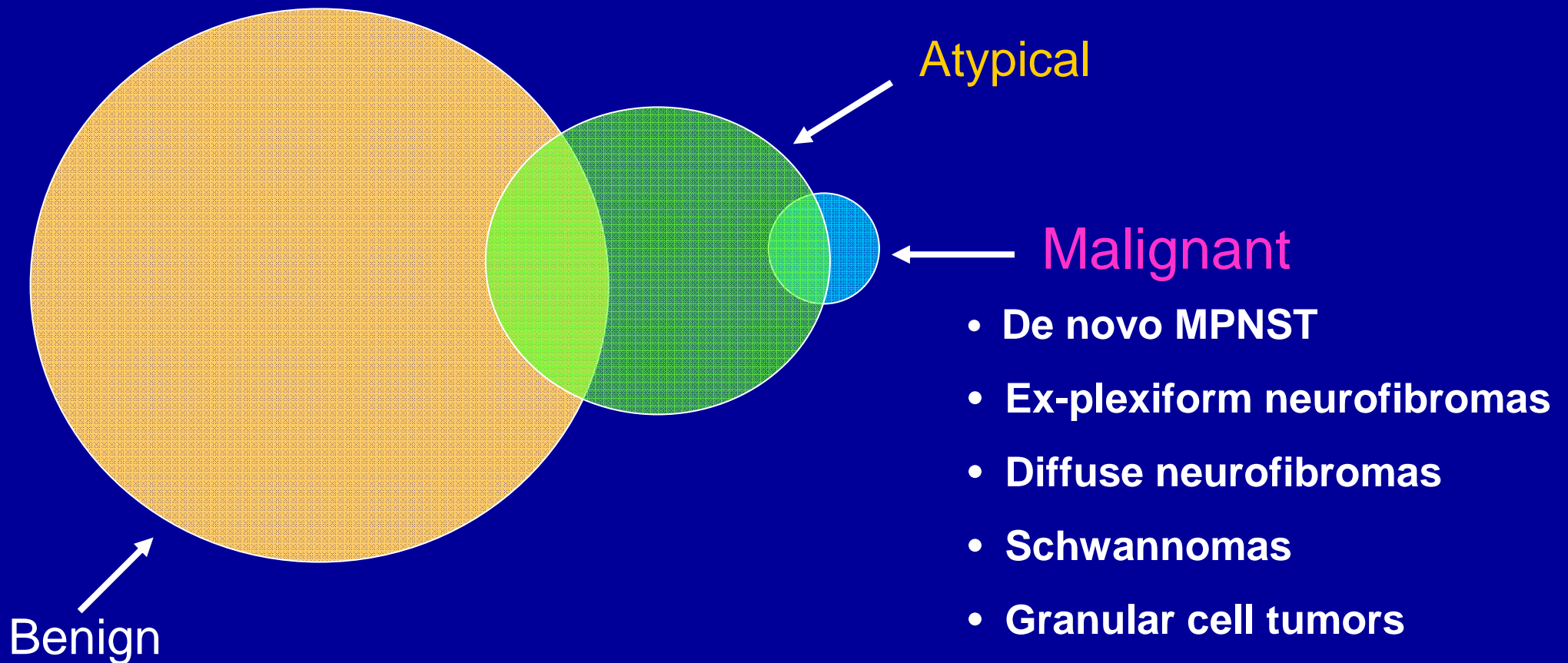
The Relevance of Cytologic Atypia in Cutaneous Neural Tumors

Recent Findings - New Developments –

New Problems

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Cardinal Features of Malignant Peripheral Nerve Sheath Tumors

- Size
- Necrosis
- Mitotic rate
- Cellularity
- Atypia

Growing Number of Cases
With Atypia Alone

What is the relevance of cytologic atypia in these tumors?

borderline tumors?

reactive changes?

low grade malignancy?
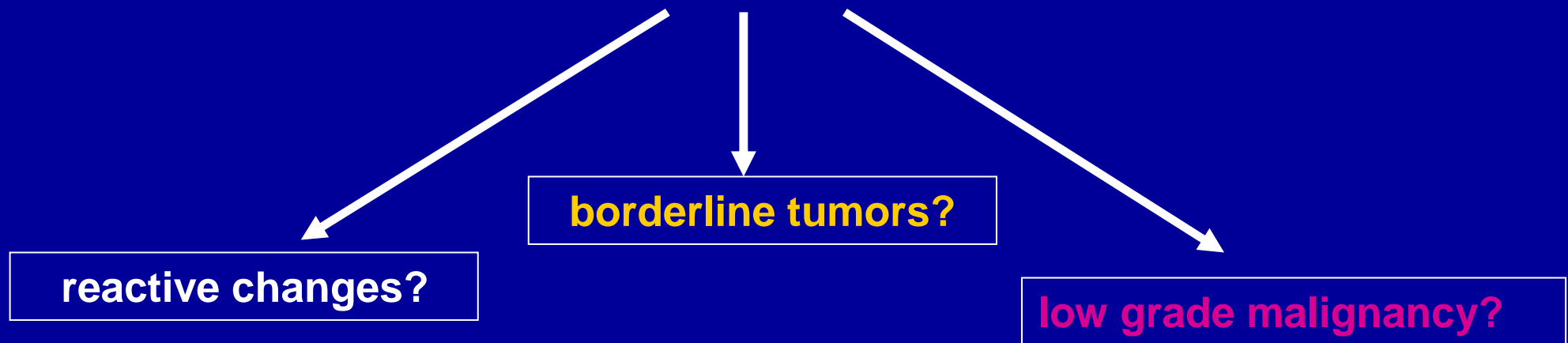
Very limited studies to determine outcomes

Growing Spectrum of Atypical Cutaneous Neural Tumors

"What constitutes atypia" in these tumors?

1. Cytologic deviation from normal cell types
   - epitheliod
   - pleomorphic
   - multinucleated cells
2. Nuclear atypia
3. Mitotic atypia
4. Cellularity
5. Growth pattern

The Most Common Cutaneous Neural Tumors With Atypia

1. Neurofibromas
2. Schwannomas
3. Neurothekeomas

Atypia in Neurofibromas
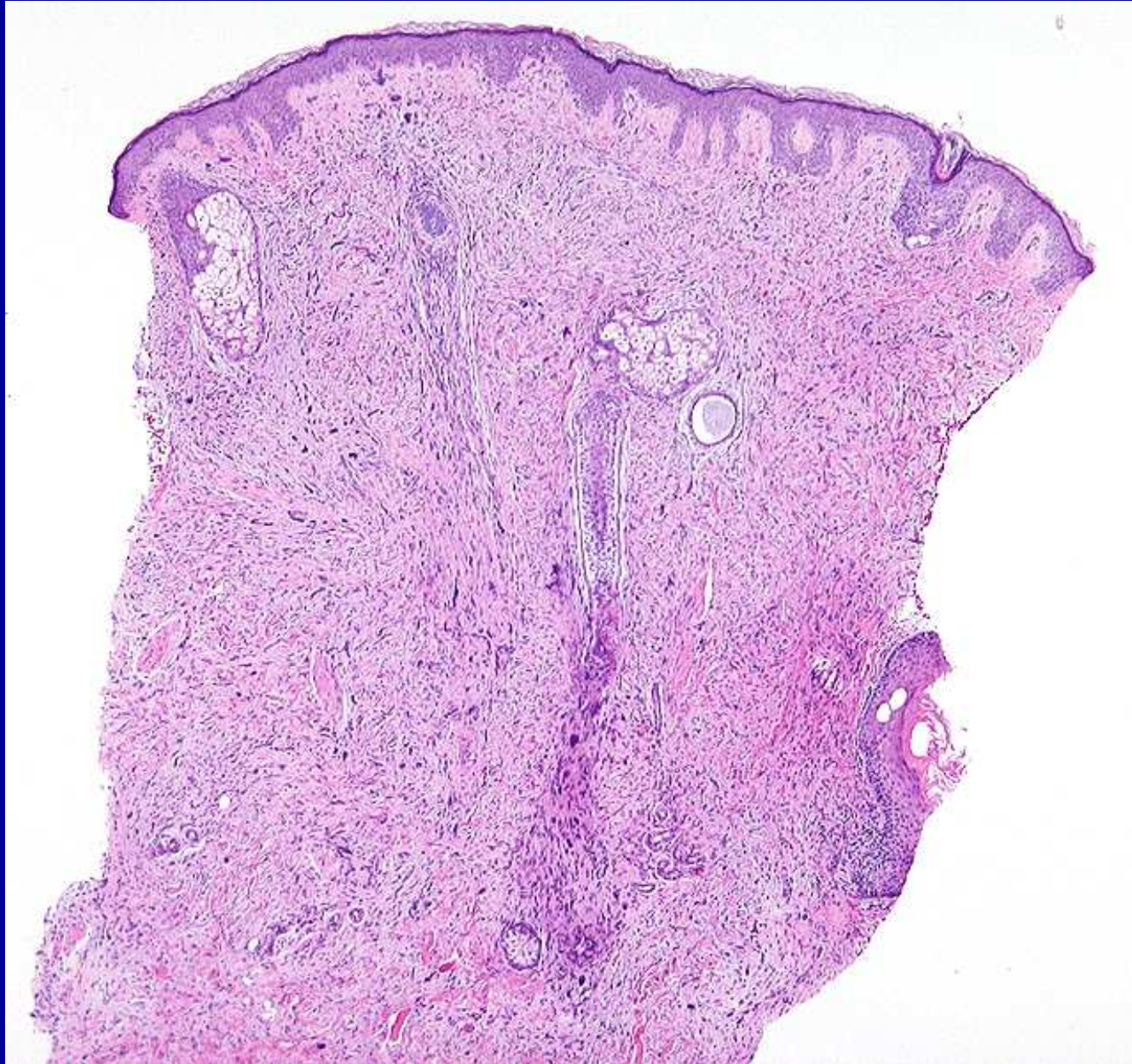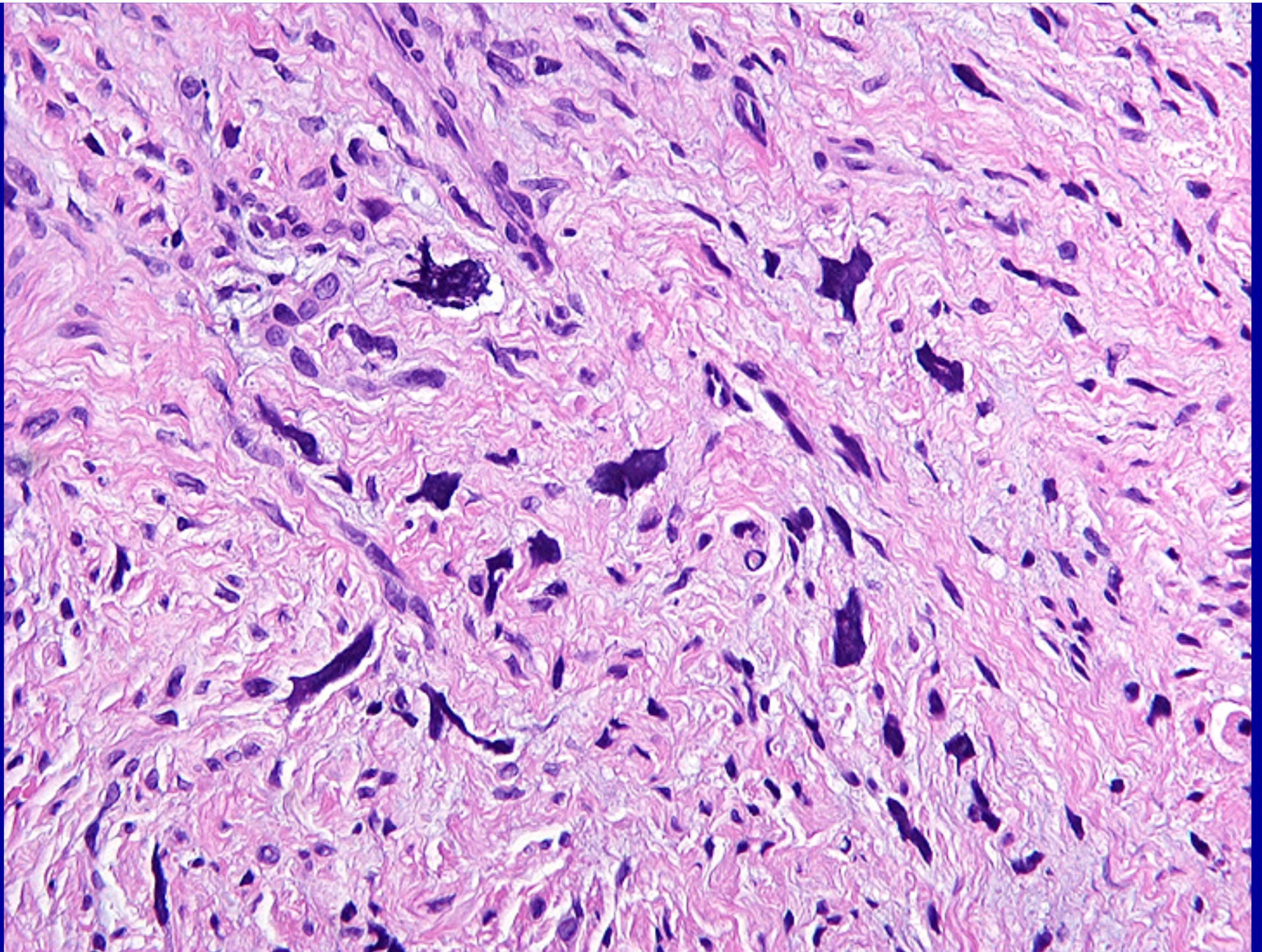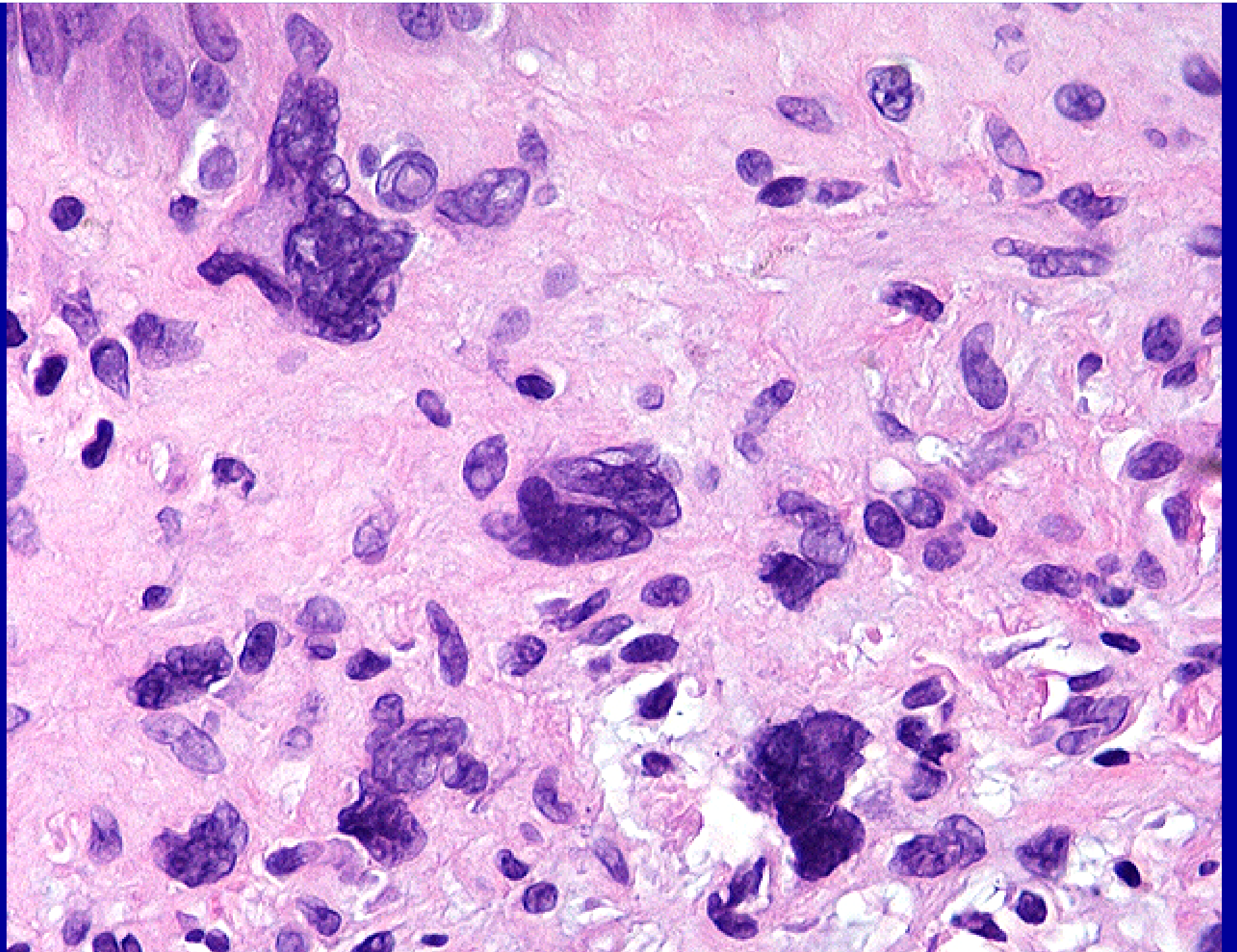
Atypical Neurofibroma
Differential Diagnosis

- Malignant peripheral nerve sheath tumor
- Pleomorphic liposarcoma
- Pleomorphic lipoma
- Myxoid MFH
- Cellular Schwannoma
- Cellular dermatofibroma

Relevance of Atypia Cutaneous Neurofibromatosis

- Important distinctions:
  - Plexiform type ⟶ high association with NF-1
  - Solitary, sporadic type; benign subtype or malignant?
- Prior studies:
  - Lin BT-Y, Weiss L, Medeiros LJ. Neurofibroma and cellular neurofibroma with atypia: a report of 14 tumors. Am J Surg Pathol 1997; 21: 1443
  - predominantly superficial soft tissues

Neurofibroma and Cellular Neurofibroma With Atypia

- Clinicopathologic study by Lin BT et al. Am J Surg Pathol 21(12):1443-1449, 1997.
- 14 cases of 6 patients, mean age 40, F:M = 2:1
- Head (5), trunk (5) and extremities (4)
- 3 patients with type I neurofibromatosis
- Benign behavior (limited F/U)

Neurofibroma and Cellular Neurofibroma with Atypia
Histopathologic features

- Usual growth pattern of neurofibromas
- Mild to severe cytologic atypia, nuclear enlargement, hyperchromasia, bizarre giant cells
- Mitotic activity 1 < 10 HPF (3 cases) or absent
- Degenerative changes
- Focal hypercellularity (3 cases)
- Lack of necrosis
- Low p53, Ki-67, and S-phase values as compared to MPNST

Jokinen CH., Argenyi ZB: Atypical neurofibroma of the skin and subutaneous tissue: clinicopathologic analysis of 11 cases. J Cutan Pathol 2009; 36: *in press*.

Atypical Neurofibroma of the Skin

Clinical Data

- 11 cases, 80% females, 20% males
  - Age range 8-70, mean
- Predominantly trunk
- 1 patient with NF
- No history of PMNST

Atypical Neurofibromas of the Skin
Pathology

- Dermis/superficial subcutis
- Growth type: fibrillary and lamellar
- Cellularity: mild to moderate
- Cytologic atypia (5-50% of cellularity)
- Pleomorphic cells
- Mitotic figures:
  - Absent 9/11
  - Present 2/11
- No necrosis
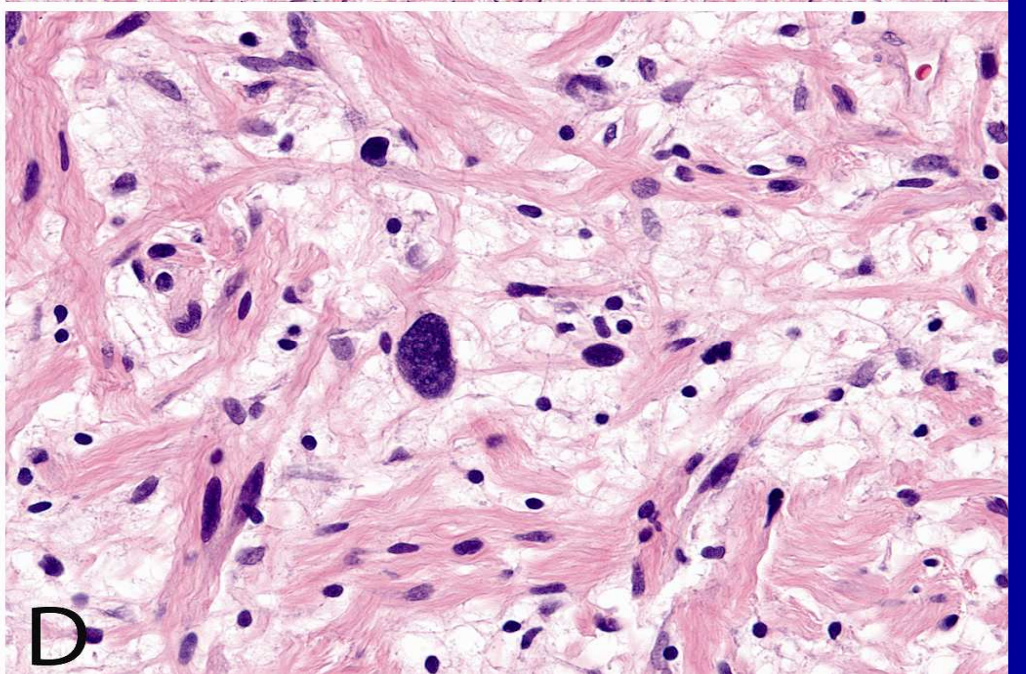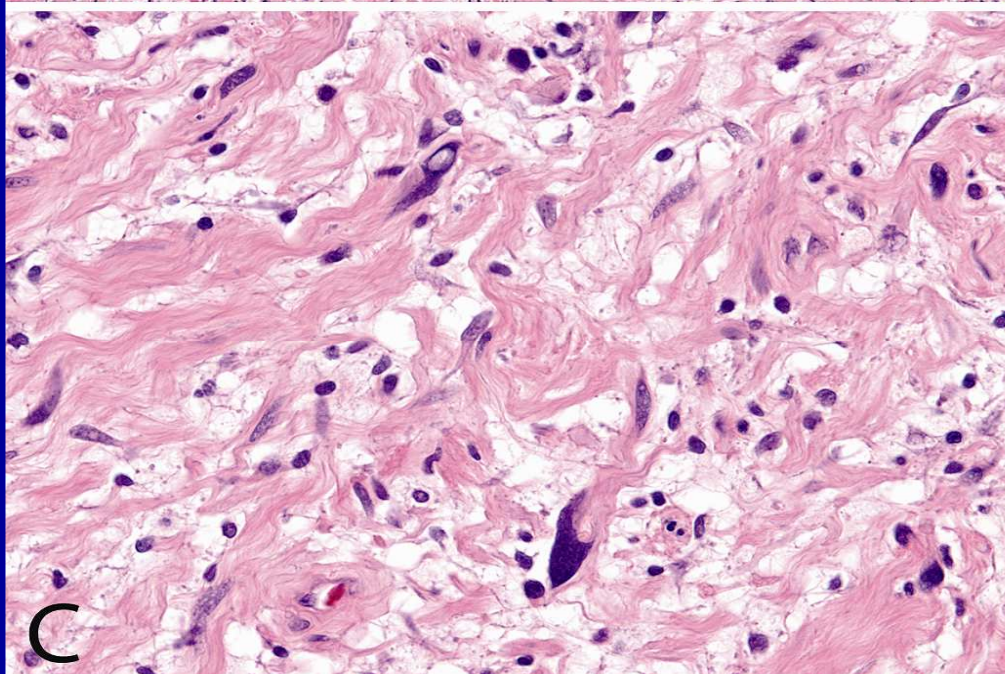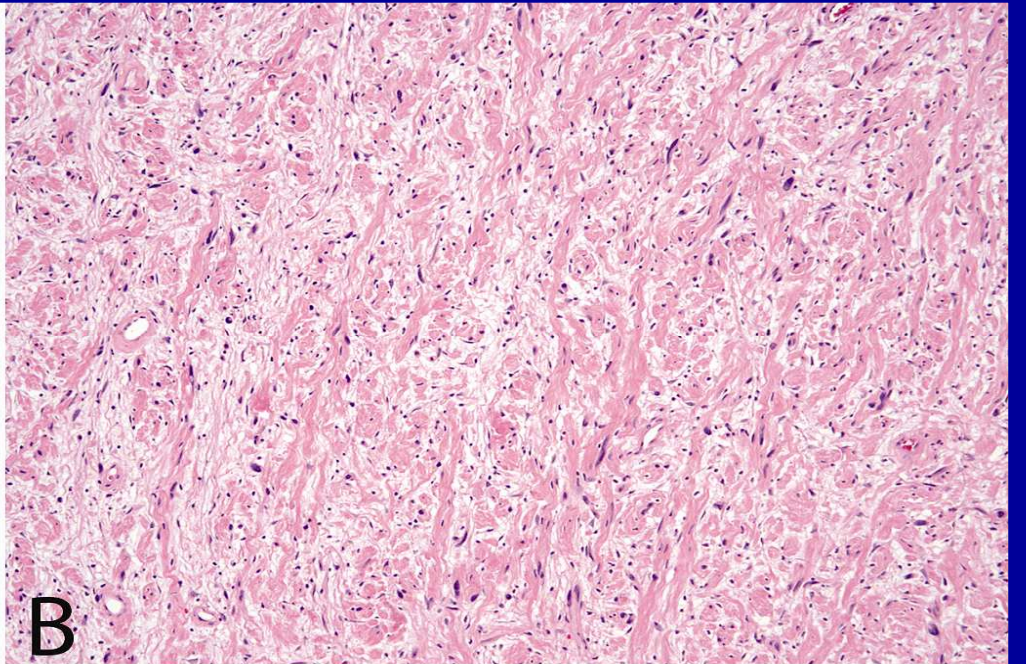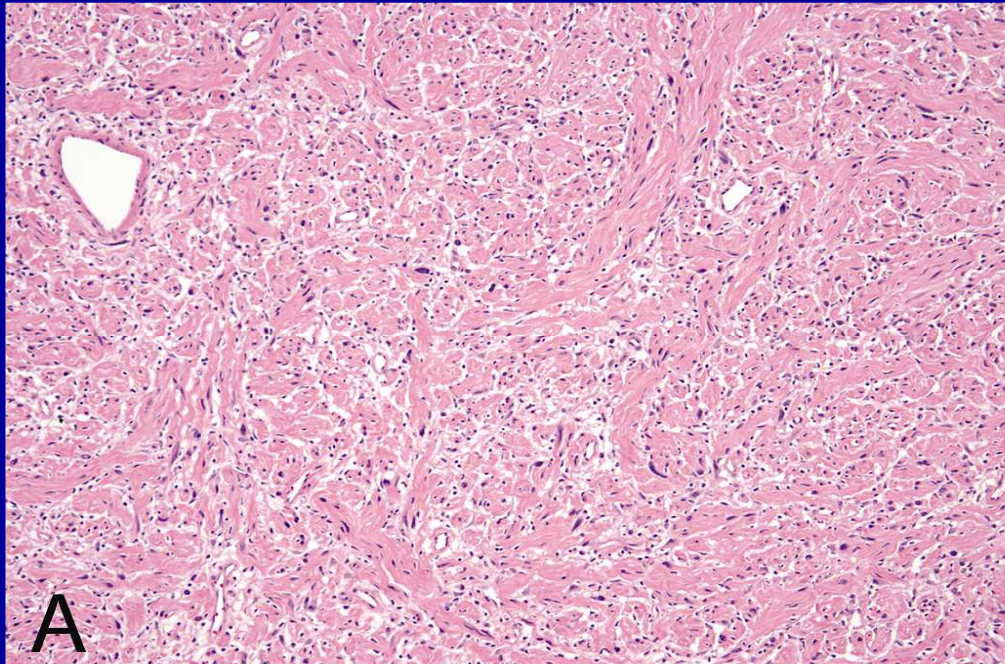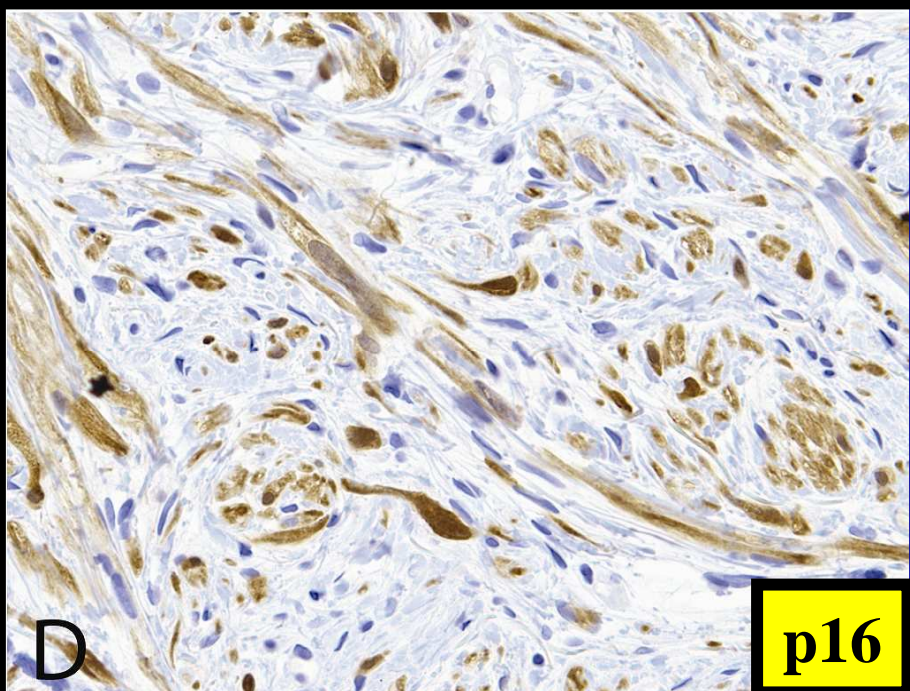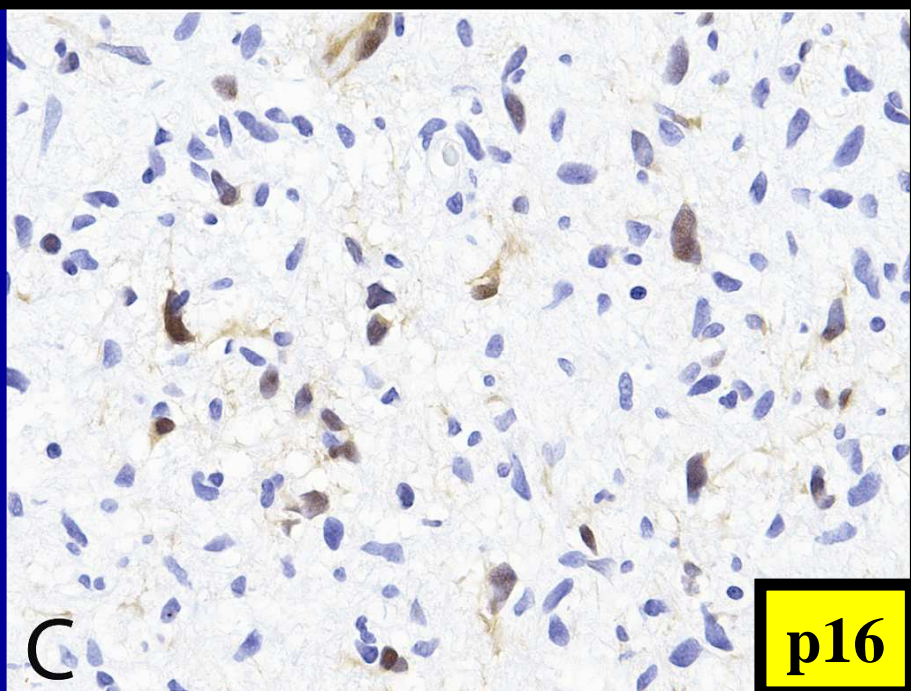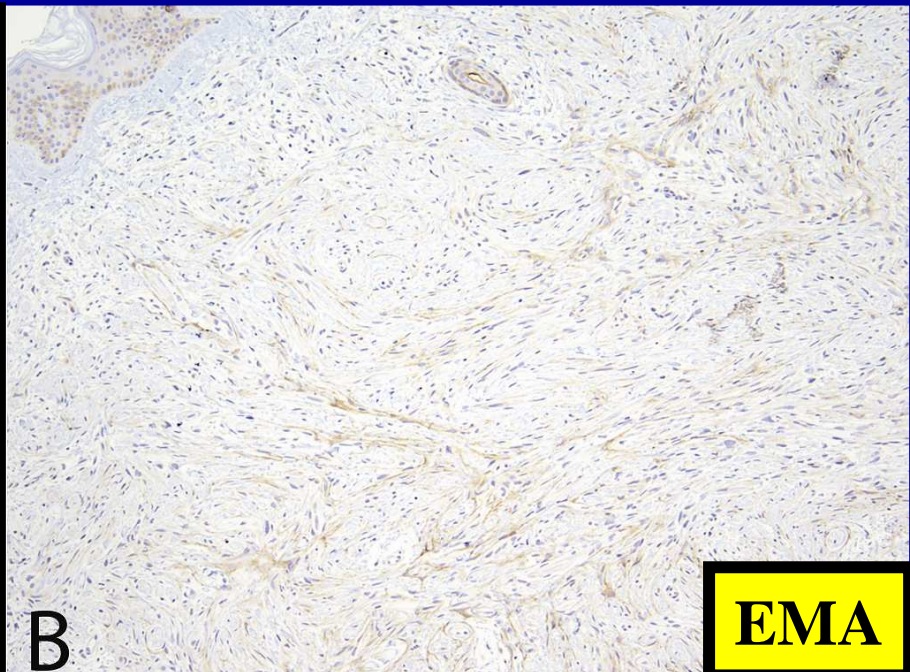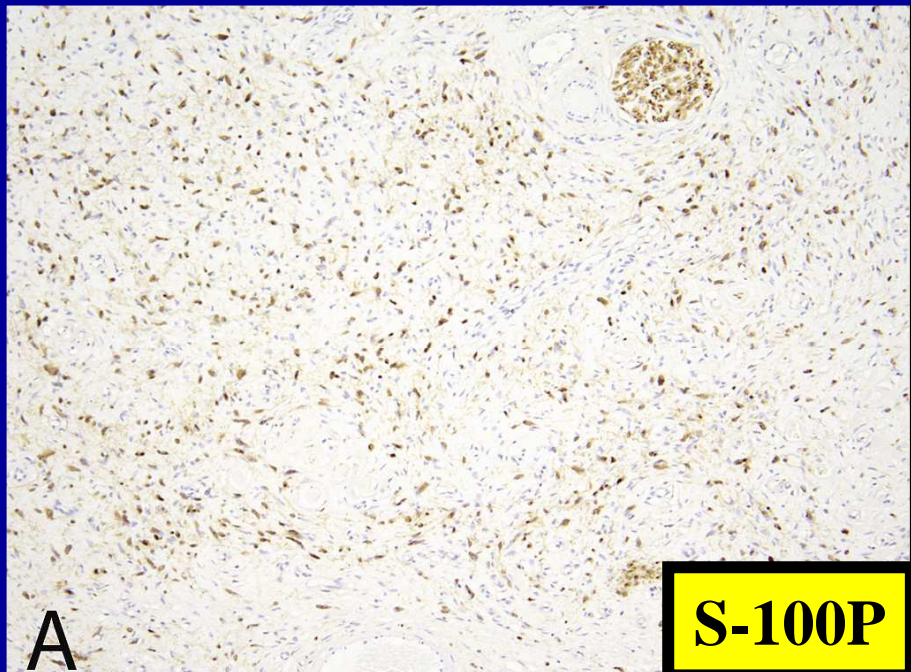
Atypical Neurofibromas of the Skin
Immunohistochemistry

IMMUNOHISTOCHEMISTRY						
Case number	S-100 protein	p16	p53	MIB-1	ER/PR	EMA
1	+++	++	-	+	-	+(P)
2	+++	++	-	-	ND	+(P)
3	+++	++	-	-	ND	+(P)
4	+++	++	-	+	ND	+(P)
5	+++	+++	-	-	ND	++(P)
6	+++	+++	-	-	ND	+++(D)
7	+++	+++	-	-	ND	+(D)
8	+++	+++	-	+	-	++(P)
9	+++	++	-	-	-	++(D)
10	+++	++	-	-	-	-
11	+++	ND	ND	ND	ND	ND

Cutaneous Atypical Neurofibroma
Clinical Outcome

- No tumor recurrence

- No new malignancy

- Follow-up range 6 – 63 months

    (mean: 33 months)

Atypical Neurofibroma
Conclusions

- Distinct subset of neurofibromas.
- The designation should be better accepted.
- Atypia could be analogous to lesions in:
  - Ancient Schwannomas with degenerative changes
  - Cellular Schwannomas with limited mitotic activity
- Cytologic atypia alone in NF does not appear to be associated with Neurofibromatosis type-1
- There is no apparent short term risk for recurrence or malignancy.
- Awareness of atypia helps better patient management
- These lesions can be treated conservatively.

Problematic Aspects of Atypical, "Cellular Schwannomas"

1. The diagnostic criteria are still disputed → difficult to apply for cutaneous lesions.

2. Confusing terminology for cutaneous lesions → unclear biologic potential.

3. Expanded differential diagnosis in the skin → malignant melanoma.

Cellular Schwannoma

The term was coined by Woodruff in 1981

Synonyms: atypical Schwannoma (Reed)

cellular Schwannoma (Woodruff)

transformed Schwannoma (Reed)

low-grade malignant Schwannoma (Ducatman)

Cellular Schwannoma
General features

1. Affect mainly females.
2. Tumors of the deep soft tissue (mediastinum, pelvis).
3. Association with NF-1 less than 5%.
4. Evolving histologic criteria.
5. Considered benign.
6. Cutaneous involvement is <u>rare</u>.

Cutaneous Cellular Schwannomas

1. Cheek, 59 year old male, Woodruff et al. 1981.

2. Hand, 90 year old male, Davidson et al. 1989.

3. Back, 34 year old male, Megahed et al. 1994.

4. Hand, 66 year old male, De Nuntiis et al. 1996.

5. Oral mucosa, 34 year old male, Koizumi et al. 2002.
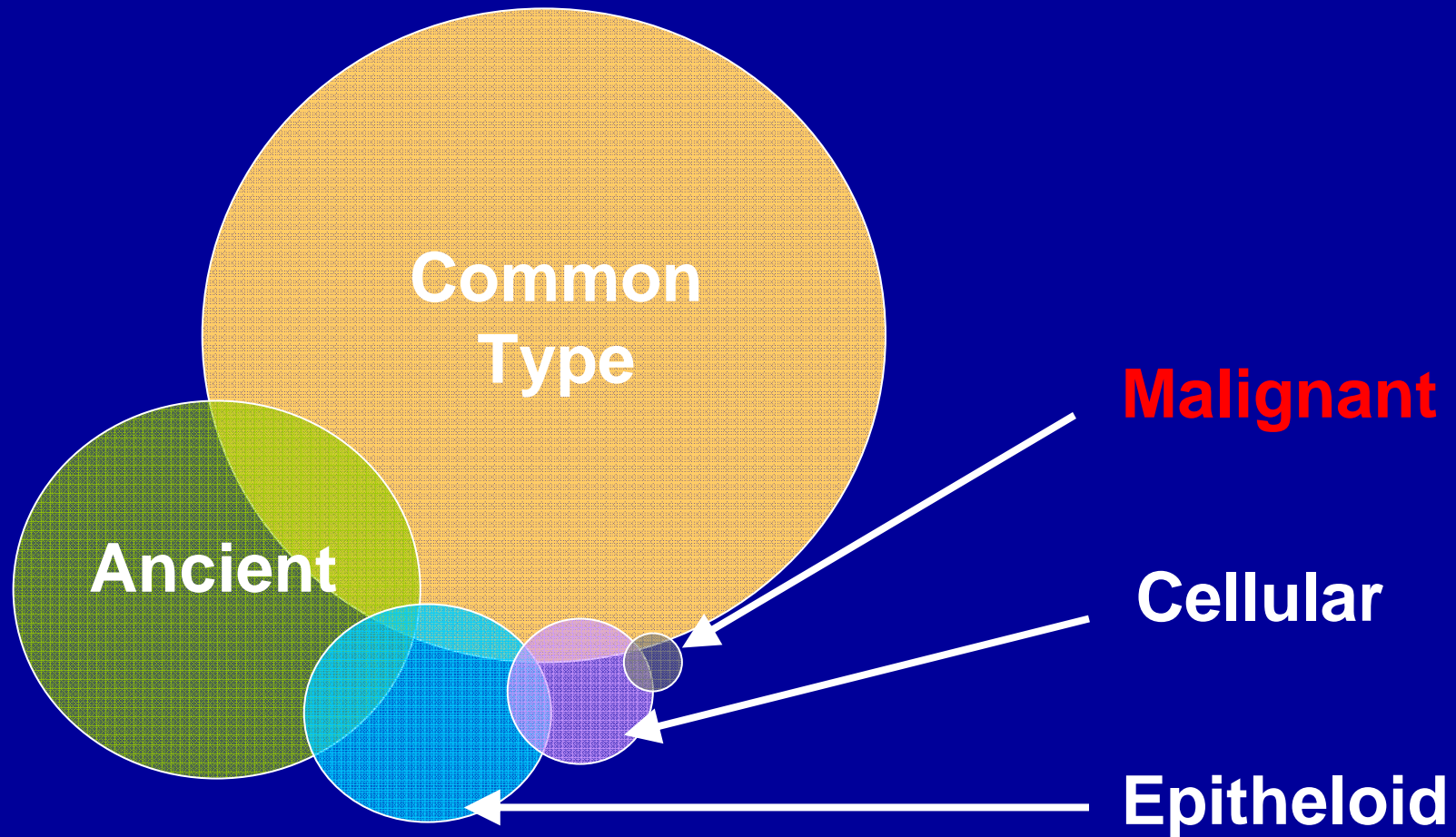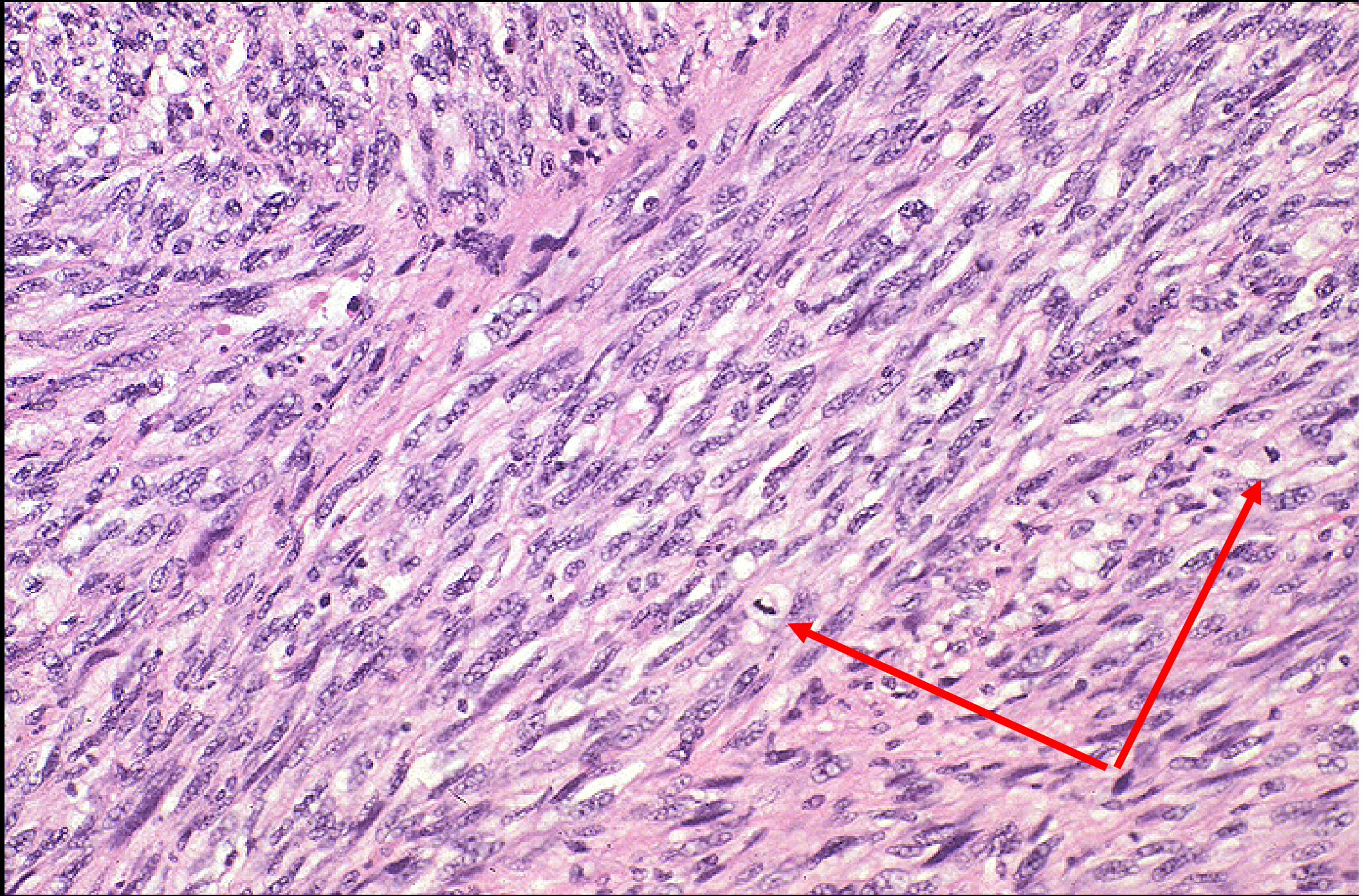
Cellular Schwannoma (deep soft tissue type)
Key Histopathologic Features

- Well circumscribed
- Nodular
- Hypercellular (Antoni A-Type)
- Fascicles and whorls
- Hyperchromasia
- Spindled atypical nuclei
- Mitotic rate < 4/10 HPF (depending on authors)
- Thick-walled vessels
- Lymphoid aggregates in the wall of the capsule
- Diffuse, strong S-100 protein expression
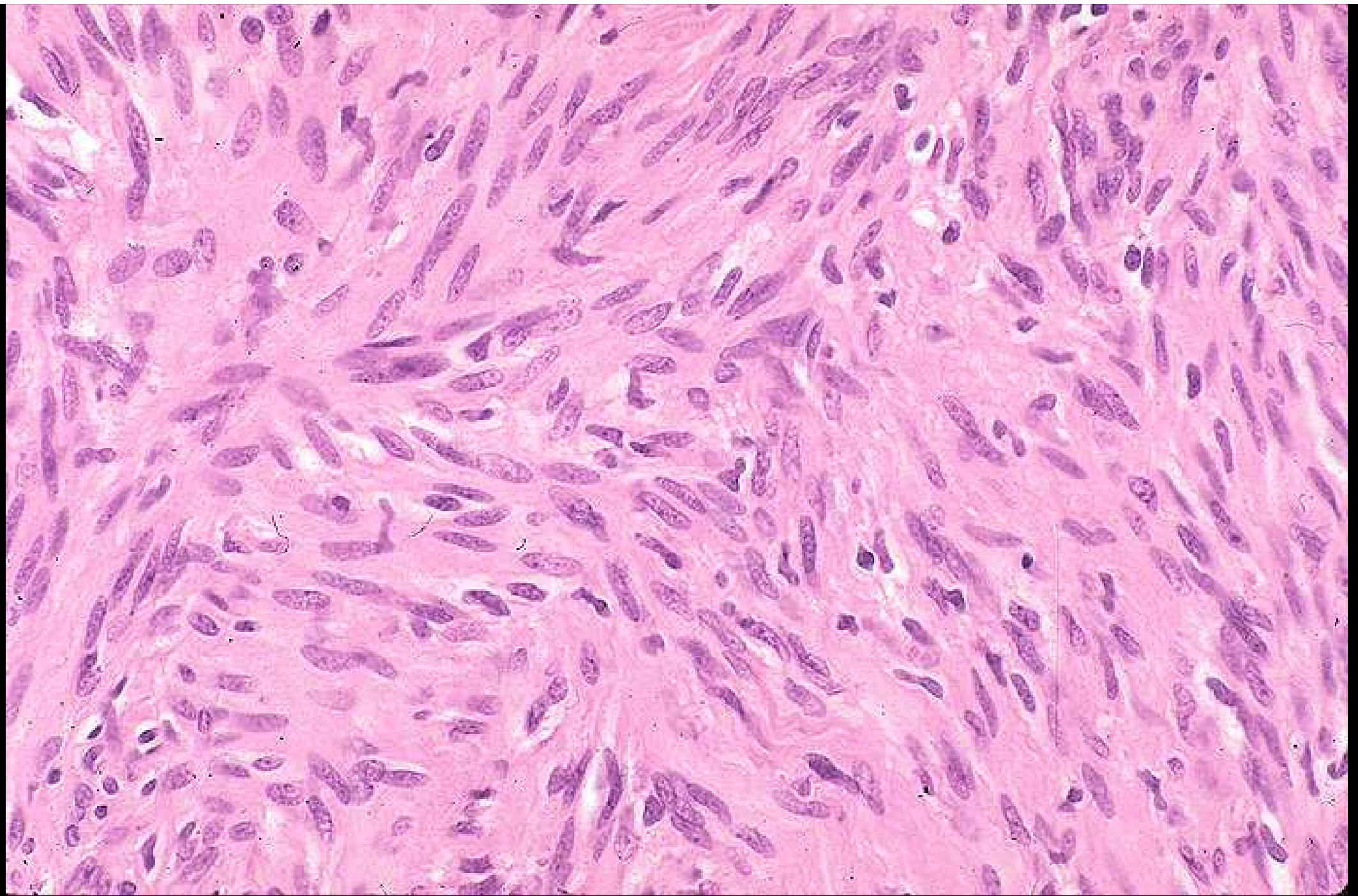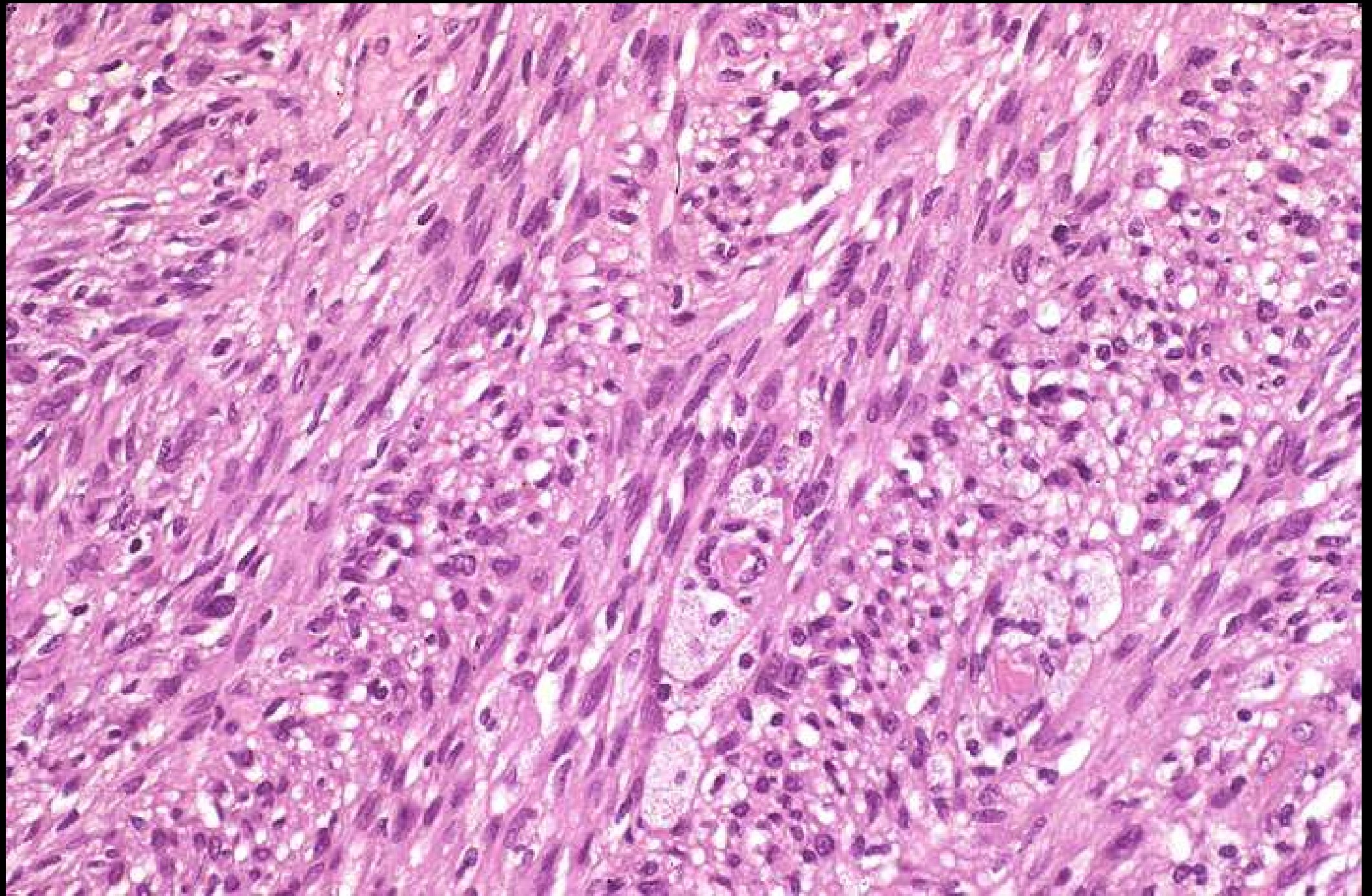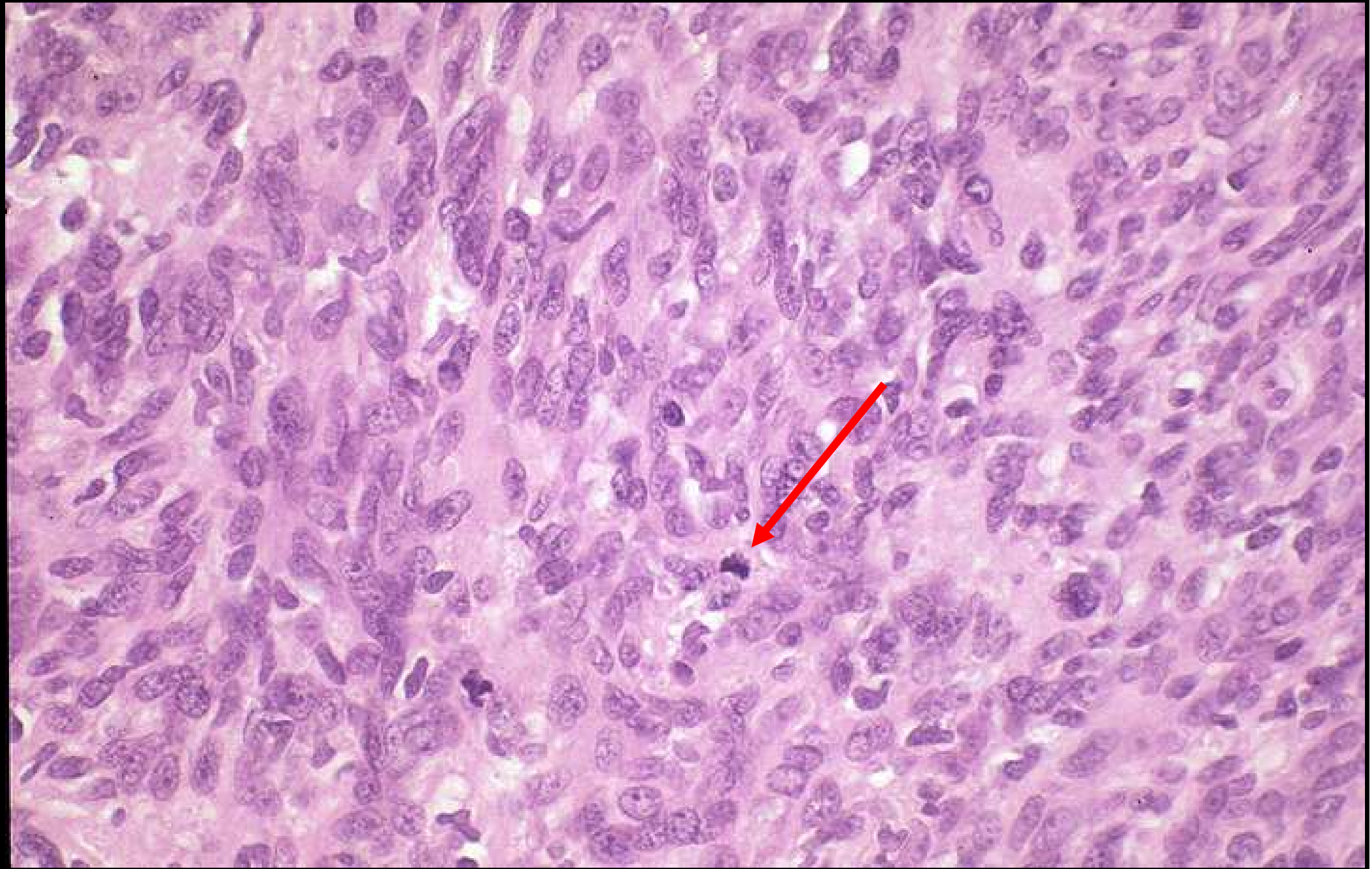
Histopathologic Spectrum of "Cellular Schwannomas" in the cutaneous literature

|	"Atypical"	"Cellular"	"Transformed"
- Hypercellularity	++	+++	+++
- Interlacing fascicles	++	+++	+++
- Storiform pattern	+/-	++	++
- Cytologic atypia	+	++	++
- Hyperchromatism	+/-	++	+++
- Mitotic rate	<2/10 HPF	2-10/10 HPF	>10/10 HPF
- Stellate necrosis	-	+/-	+
- Nuclear palisading	+	+/-	+/-
- Verocay bodies	+	-	-
- Lymphoid follicles	-	+	+/-
- Epithelioid cells	-	+	++
- Lipid laden histiocytes	+/-	+	+

# Cutaneous Cellular Schwannoma
Differential Diagnosis

- Malignant peripheral nerve sheath tumor (MPNST)
- Malignant melanoma (primary or metastatic) (broad panel of immunohistochemistry, electron microscopy, clinico-pathologic correlation)
- Leiomyosarcoma (growth pattern + immunohistochemistry)
- "Benign imitators" ; ancient, epitheloid schwannomas, angiomyomas, neuromas

Cutaneous Cellular Schwannoma
"Benign Imitators"

- Ancient Schwannoma
- Epitheloid Schwannoma
- Palisaded Encapsulated Neuroma
- Epitheloid Angiomyoma
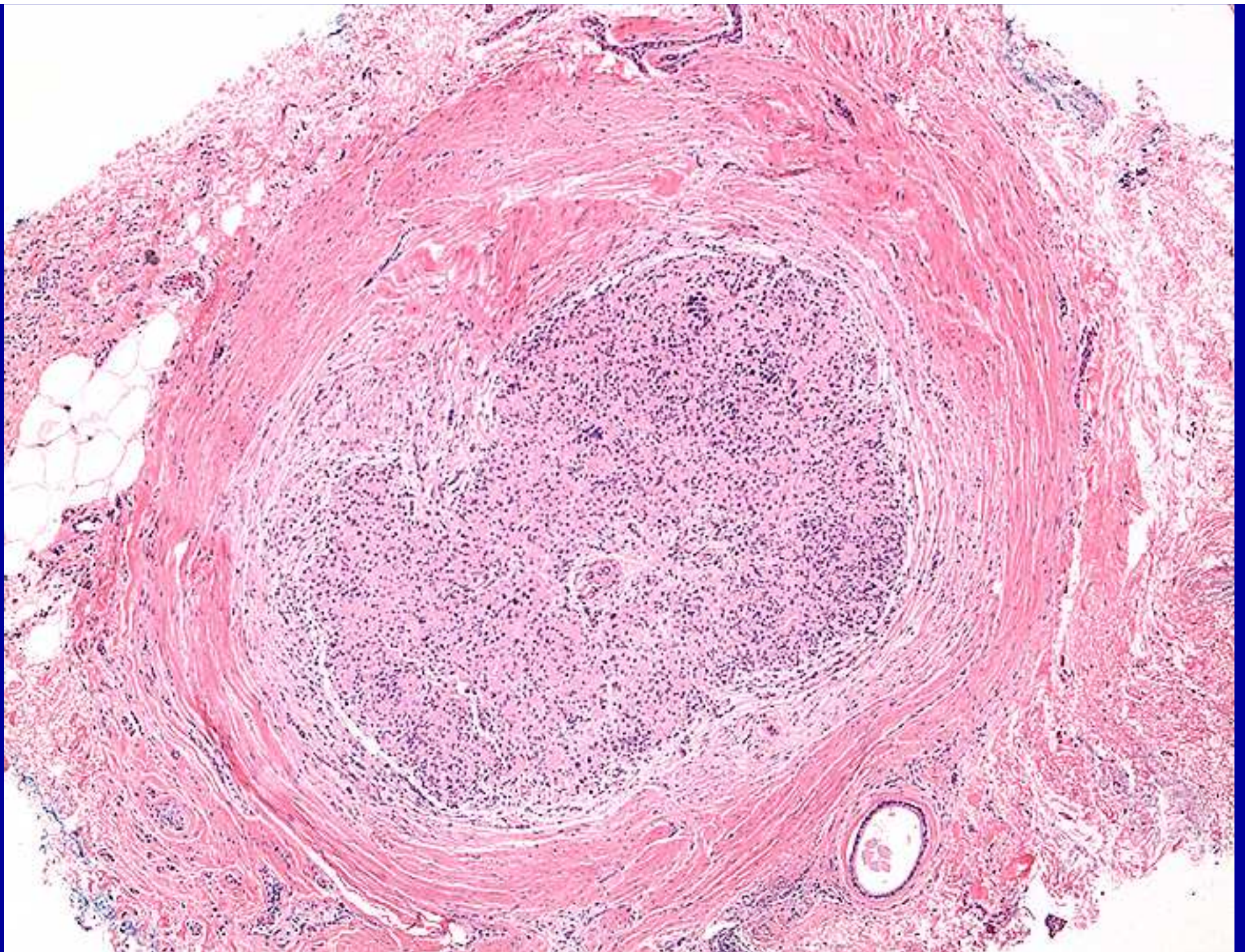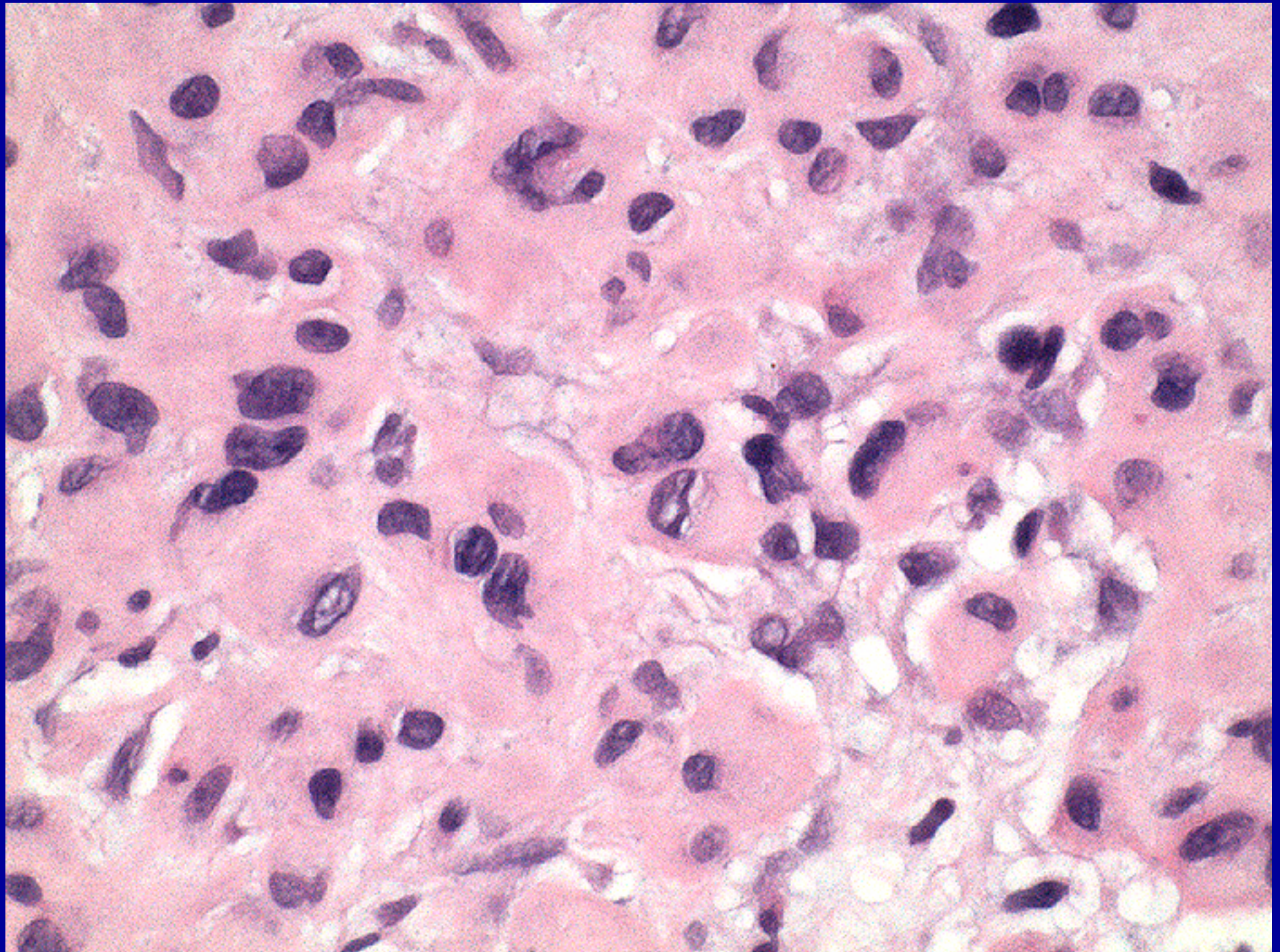
Differential Diagnosis of Schwannomas with Atypia

	Epitheliod	Cellular	MPNST
Growth Pattern	nodular, oblong	nodular, oblong	variable
Encapsulation	usually present	well-preserved	partial➜infiltrative
Cellularity	++	+++	+++
Architecture	trabecular, nodular, syncytial, myxoid	fascicular	fascicular "herring bone"
Cell Type	**epitheloid**/with or without spindled cells	**spindled** cells	spindled or pleomorphic
Atypia	mild	moderate	moderate to severe
Mitotic Rate	< 1/10 HPF	< **4/10** HPF	> 4/10 HPF
Necrosis	none	none or very focal	geographic
Vascularity	focal mild	focal, prominent	not characteristic
Lymphoid Infiltrate	variable	**patchy aggregates**	not characteristic

Cutaneous Cellular Schwannoma
Conclusions

1. No comprehensive studies on cutaneous cellular schwannoma and MPNST

2. Application of diagnostic criteria is often "arbitrary"

3. Important differential diagnostic challenge (melanoma vs. MPNST)

Cellular Neurothekeoma

- Entity described in 1986 by Rosati et al.
- Well-established clinico-pathologic features.
- Controversial histogenesis; neural vs. other
- Synonyms: - cellular nerve sheath myxoma
  - immature nerve sheath myxoma
  - "epithelioid" nerve sheath myxoma
  - and a plethora of others….
- Recently recognized atypical variants

Cellular Neurothekeoma
Histologic Features

1. Dermal/subcutaneous tumor
2. Multi-lobular or fascicular growth
3. Not encapsulated
4. Scant mucin
5. Epithelioid or spindle cells
6. Characteristic nuclei
7. Rare mitotic figures
8. Heterogeneity of cell components can occur
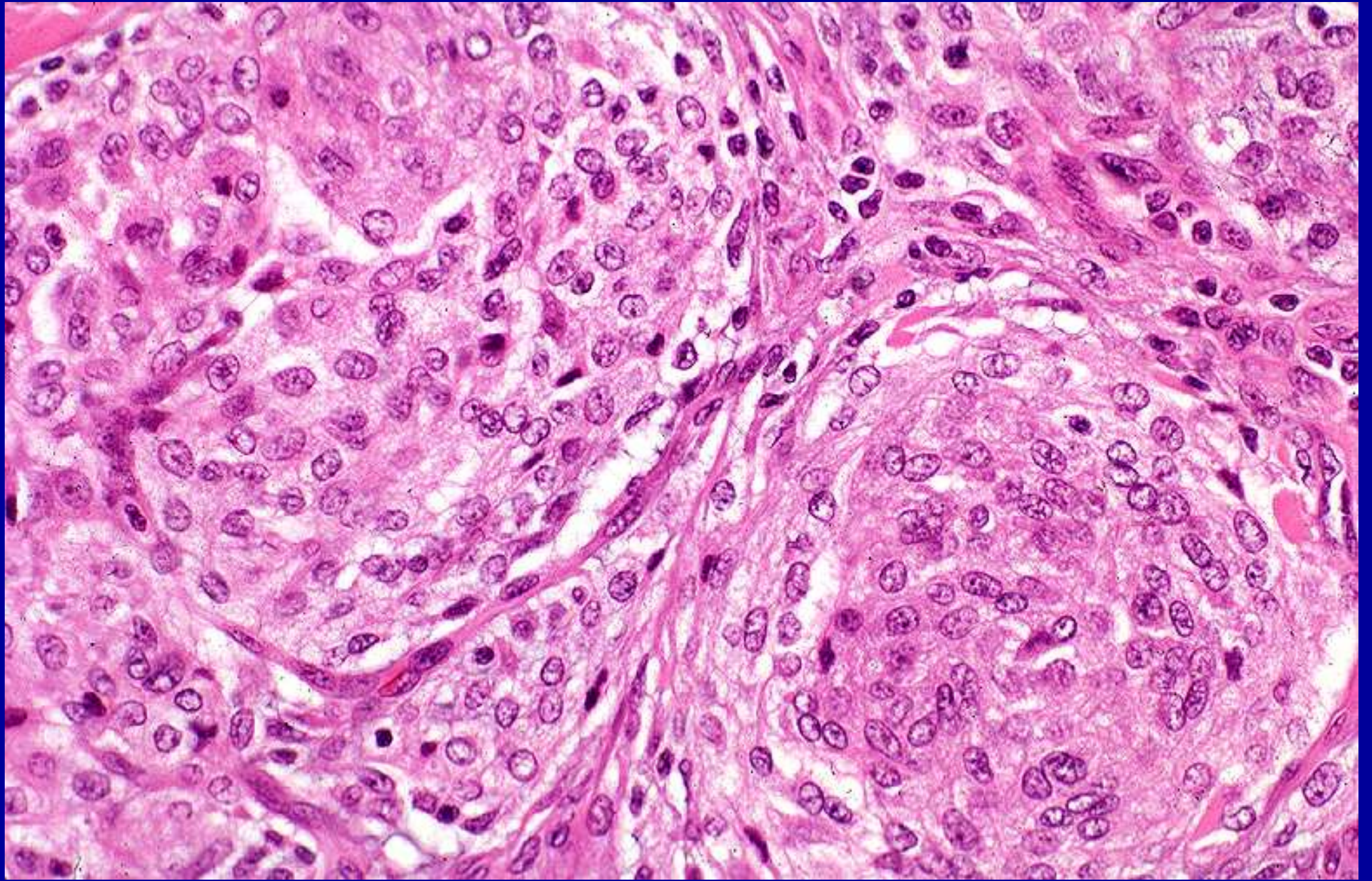
Cellular Neurothekeoma
Atypical (? malignant variants)

- **Atypical variants** (Busam et al. 1998)

  Clinical   - 10 patients
                    - Median age 20.5 years
                    - head and neck

  Pathological: - Large size
                    - Deep penetration
                    - Diffusely infiltrative borders
                    - Vascular invasion
                    - "Marked" cytologic atypia
                    - Mitotic rate > 5/10 HPF

  Follow up: - (1-5 years) - no recurrence

- **Additional Cases:** - Bhatia et al. 2003
                       - Benbenisty et al. 2006 (advocating Mohs surgery)
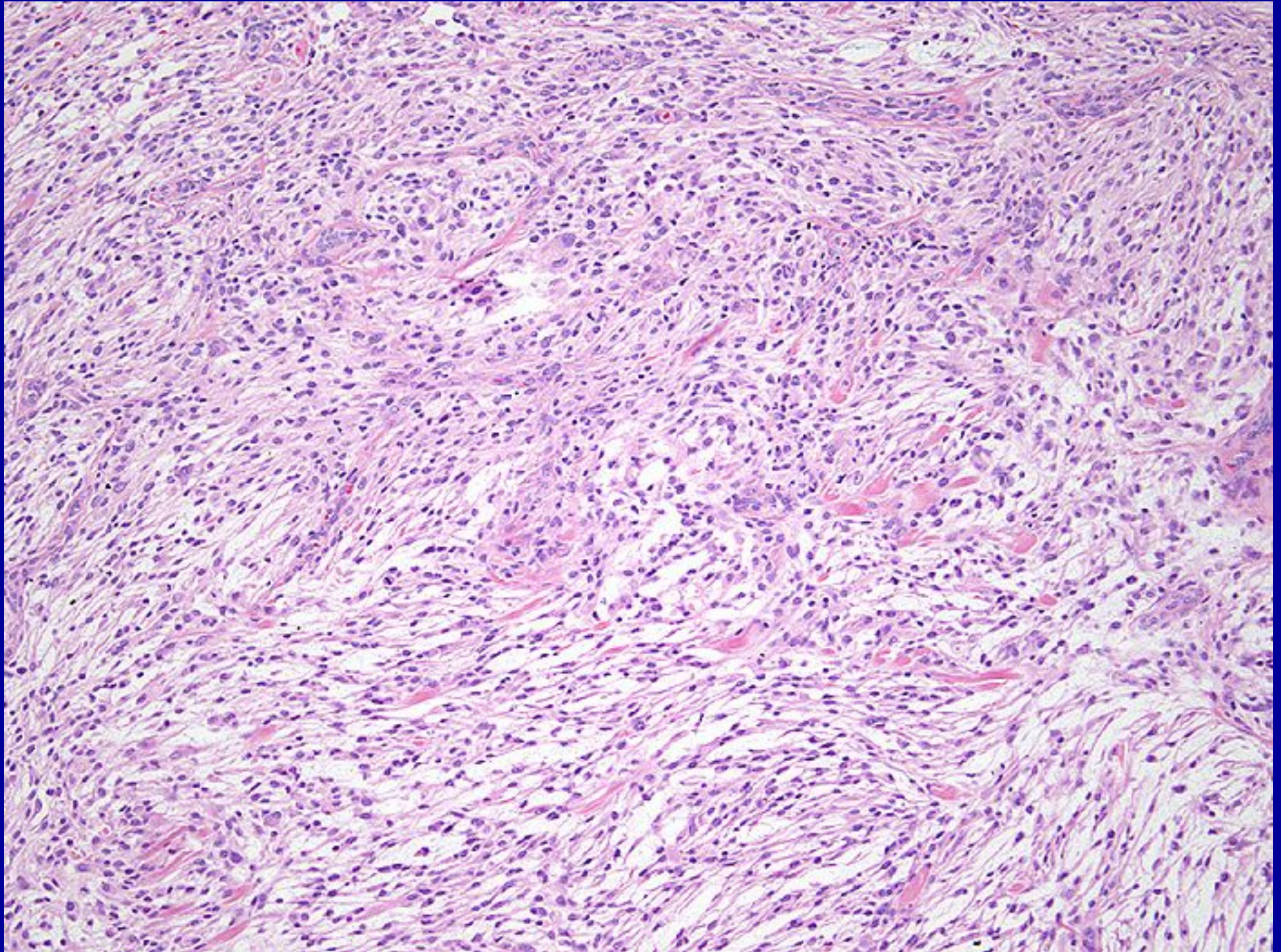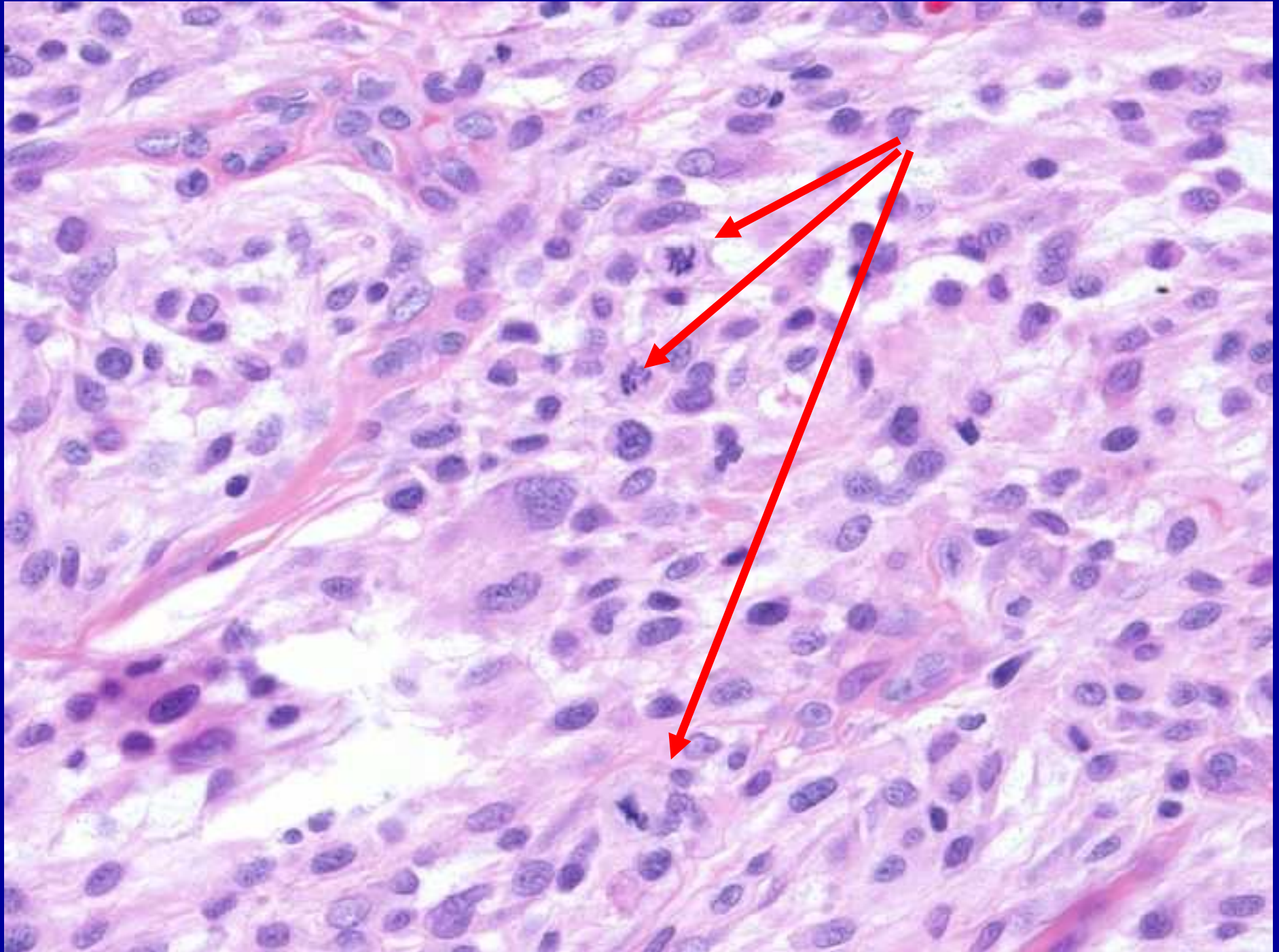
Atypical Neurothekeoma
Additional observations; Hornick and Fletcher, 2007

- Clinical follow-up on 69 cases with the mean F/U of 44 mo. showed recurrence of 10 cases.

- Atypical morphologic features of 133 cases;
  - size>2cm (10%), mitosis >5/10HPF(21%), pleomorphism (25%), infiltration of fat (25%) were too common to represent increased risk for local recurrence.

  Conclusions:

  Only head and neck location and incomplete surgical excision correlated with recurrence.

Cellular Neurothekeoma
Practical Conclusions

- CNT has distinct enough clinical and pathological features to accept as a distinct entity, with the notion that the histogenesis is still uncertain.
- Atypical variants are reported more often than original studies indicated.
- Currently there are somewhat contradicting data regarding the significance of atypia in Cellular Neurothekeomas.
- Considering the uncertain biologic potential a cautious clinical approach with adequate excision seems prudent.