Approach to microscopic examination of skin tumors

- Tumor vs. "pseudotumor" *(e.g., pseudoepitheliomatous hyperplasia in lichen simplex chronicus)*
- Differentiation *(e.g., epithelial, melanocytic, etc.)*
- Benign vs. malignant *(vs. "intermediate", hyperplasia, malformation, hamartoma)*
- Need (or not) of immunohistochemistry or other ancillary methods *(e.g., molecular studies for soft tissue tumors)*
- Diagnosis *(or differential diagnoses)*
- For malignant tumors: prognostic features, if applicable
- Assessment of surgical margins and other information relevant to further management of the patient

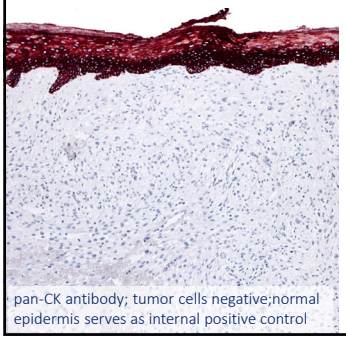
Microscopic diagnosis is the synthesis of many aspects

- Overall architecture (i.e., scanning magnification) including orientation and pattern of growth
- Presence of specific structures / features *(e.g., nests in melanocytic tumors, ghost cells in pilomatrical differentiation, etc.)*
- Cell morphology *(including mitoses, necrotic cells)*
- If needed: immunohistochemical features *(never rely on a single antibody, judge a phenotypic pattern)*
- Rarely: molecular analyses *(several methods depending on the question that should be answered)*
- **A single histopathological feature is never diagnostic *(e.g., a malignant tumor can be symmetrical and well circumscribed)***

Immunohistochemistry

- Thousands of monoclonal (and some polyclonal) antibodies
- In skin biopsies internal positive controls for most antibodies
- Only a (relatively) limited panel necessary for routine histopathological examination of skin specimens
- Choice of which antibodies should be used depends on morphological diagnosis and differential diagnoses
- Applied mostly for tumors, but several antibodies useful also for inflammatory disorders (e.g., *Treponema pallidum* for syphilis, CD123 for lupus erythematosus, MPO and MNDA for histiocytoid Sweet syndrome)

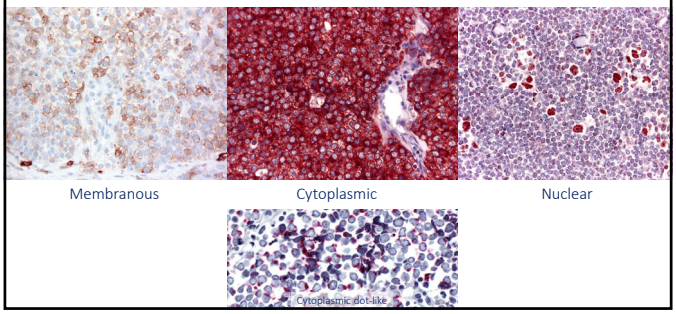
Immunohistochemistry – Controls

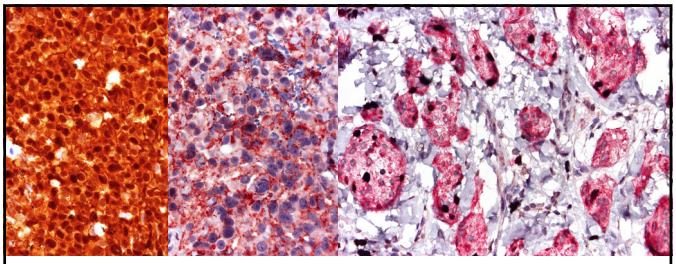


pan-CK antibody; tumor cells negative;normal epidermis serves as internal positive control

- Internal positive controls are available for the vast majority of antibodies used in routine immunohistochemistry
- Some antibodies need external controls to confirm negative results (e.g., BRAF)
- Repeat staining in negative cases if internal cells and/or structures serving as control are negative or not present on that particular section of tissue (e.g., normal sebaceous glands positive for PRAME)
- If several cases are stained at the same time, one positive case can serve as "external" control for negative ones

Immunohistochemistry – Pattern of positivity



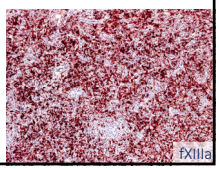


The staining pattern matters: for some antibodies only one type of staining is specific (in this example: ß-catenin, positive if nuclear; cytoplasmatic staining does not count)

"Double stainings" can be (rarely) helpful (here melan-A in red, cytoplasmatic to recognize melanocytes, and Ki-67 in brown, nuclear to highlight proliferating cells)

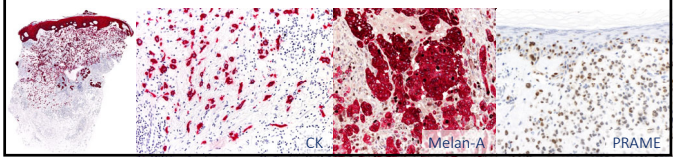
Immunohistochemistry in most common benign cutaneous tumors

- Seborrheic keratosis: *not needed*
- Cherry hemangioma: *not needed*
- Pyogenic granuloma: *not needed*
- Melanocytic nevi (some cases): *Melan-A, S100, SOX10, PRAME, Ki67, others*
- Achrocordon / Skin tag: *not needed*
- Dermatofibroma (some cases): *CD34, fXIIIa*
- Lipoma: *not needed*
- Viral warts & Condyloma: *not needed*
- Cysts (infundibular, tricholemmal): *not needed*



Immunohistochemistry in most common malignant cutaneous tumors

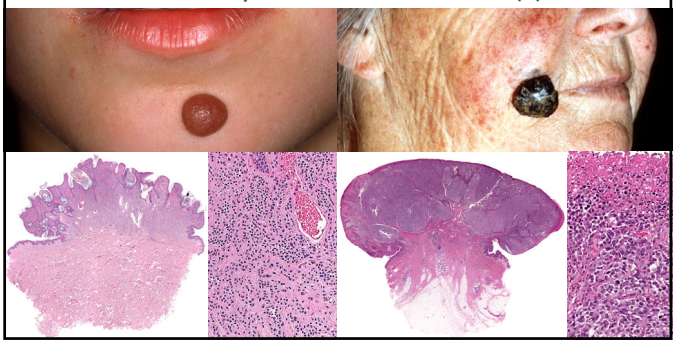
- Basal cell carcinoma: *in most cases not needed; rarely BerEP4, PHLDA1*
- Actinic keratosis: *not needed*
- Squamous cell carcinoma: *in most cases not needed; rarely CK (various)*
- Melanoma (some cases): *Melan-A, S100, SOX10, PRAME, Ki67, others*



Immunohistochemical panels (selected antibodies)

- **Epithelial tumors:** CK (various types), EMA, CEA, Ber-EP4, PHLDA1, p53, p63, ß-catenin
- **Melanocytic tumors:** Melan-A, S100, SOX10, HMB45, tyrosinase, PRAME, Ki-67, others (e.g., ALK, BAP1, BRAF, p16, ß-catenin, etc.)
- **Fibro-histiocytic tumors:** CD34, f.XIIIa
- **Vascular tumors:** ERG, CD31, CD34, D2-40, Ki-67, c-myc, HHV8
- **Neural tumors:** S100, SOX10, EMA, NF
- **Muscular tumors:** SMA, desmin, caldesmon
- **Lipomatous tumors:** S100, CD34, MDM2, CDK4
- **Histiocytic tumors:** CD1a, CD14, CD68, CD123, CD163, CD207, S100, MNDA
- **Mast cell tumors:** CD117, NASDCl, CD4, CD25, CD30
- **Lymphoproliferative lesions:** T-cell (CD3, CD4, CD5, CD8, TCR-ß, TCR-δ) and B-cell markers (CD20, CD79a, PAX5), others (e.g., CD21, CD30, Bcl-6, Bcl-2, MUM1, kappa, lambda, EBER-1)
- **Other tumors:** CK20, MCPyV, chromogranin-A, synaptophysin, CD10, NKIC3, others
- **Cutaneous metastases:** CK7, CK20, TTF1, CDX2, PSA, others

Melanocytic nevi & Melanoma(s)



Main variants of melanocytic nevi & melanomas

- "Common" melanocytic nevus (junctional, compound, dermal)
- "Dysplastic" (Atypical / Clark) nevus
- Congenital melanocytic nevus
- Blue nevus & variants
- Spitz nevus & spitzoid tumors
- Acral melanocytic nevus
- Mucosal melanocytic nevus
- "Combined" melanocytic nevus
- Halo nevus
- Recurrent (persistent) nevus
- Lentigo maligna melanoma
- Superficial spreading melanoma
- Nodular melanoma
- Acral melanoma
- Mucosal melanoma
- Desmoplastic melanoma
- Melanoma arising in a nevus

The field of melanocytic tumors represents possibly the most problematic area in dermatopathology, comprising the largest number of variants, subvariants and problematic lesions; diagnosis, yet, is straightforward in >90% of cases.

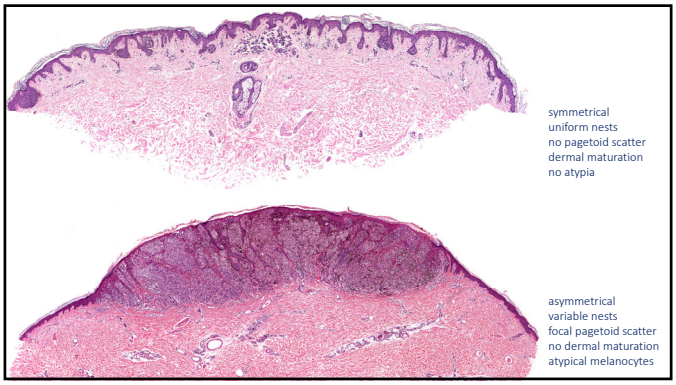
TABLE 4. Criteria for Histopathologic Diagnosis of Malignant Melanoma

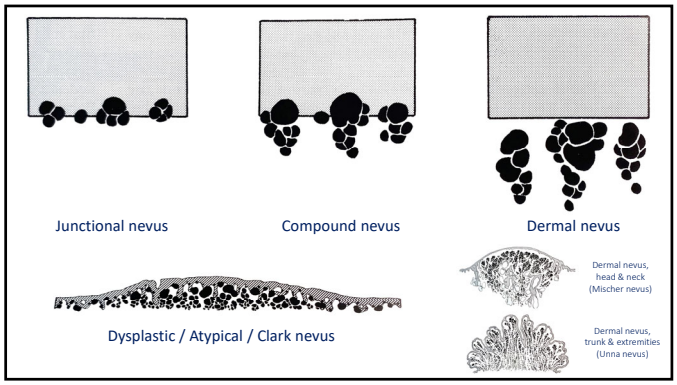
Architectural pattern

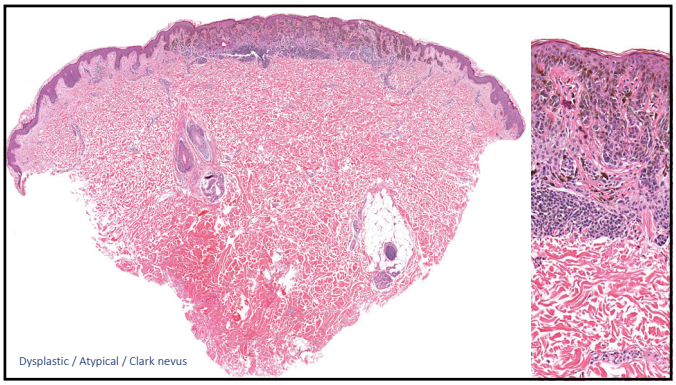
1. Asymmetric
2. Poorly circumscribed
3. No maturation of melanocytes with progressive descent into the dermis
4. Nests of melanocytes within the epidermis are not equidistant from one another
5. Nests of melanocytes vary markedly in sizes and shapes
6. Some nests of melanocytes become confluent
7. Melanocytes in some "nests" are not cohesive
8. Scatter of melanocytes above the dermo-epidermal junction
9. Melanocytes arranged as solitary units predominate over nests of melanocytes in some high-power fields
10. Melanocytes arranged as solitary units are not equidistant from one another
11. Melanocytes extend far down epithelial structures of adnexa in the same pattern as they are arrayed within the epidermis
12. Melanin is not distributed symmetrically within the epidermis, adnexa, and dermis

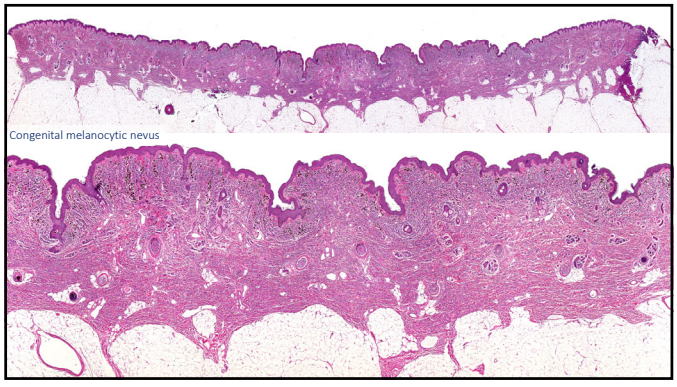
Cytologic features

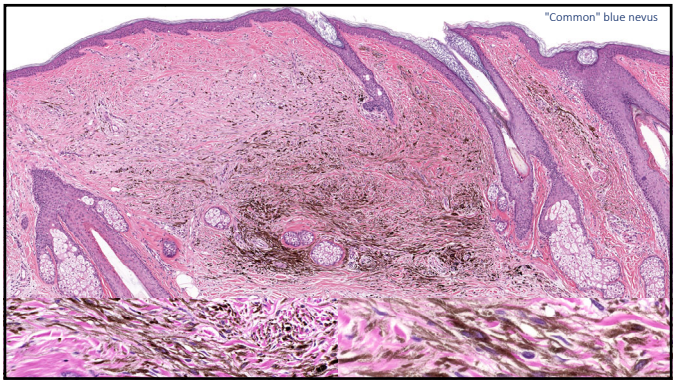
1. Melanocytes may be atypical
2. Melanocytes in mitosis
3. Melanocytes may be necrotic

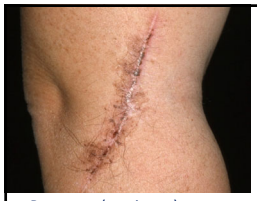


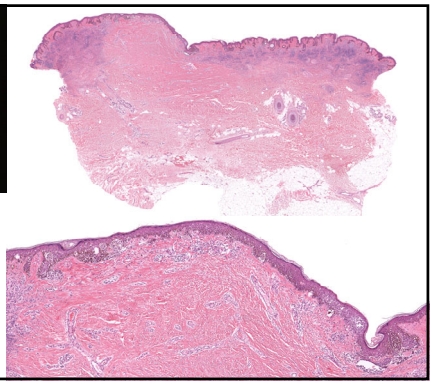






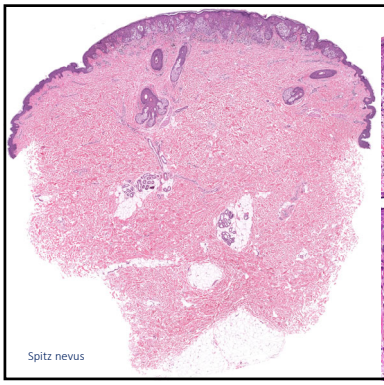




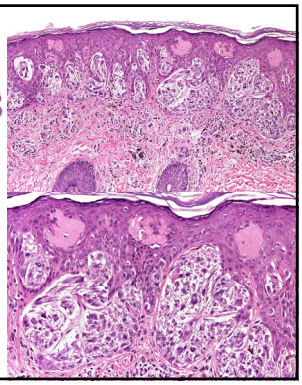


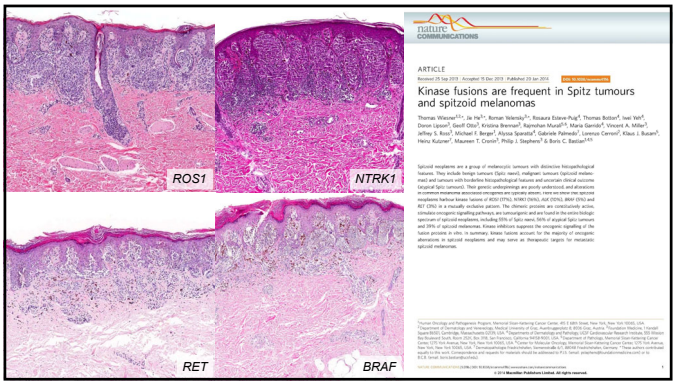
Recurrent (persistent) nevus

- Atypical distribution of melanocytes within the epidermis (melanoma-like) over the scar of a melanocytic nevus that has been incompletely excised
- The atypical features are restricted to the portion of the epidermis over the scar
- Onset usually <12 months from the primary excision (a lesion recurring after more than 12 months is suspicious for persistent melanoma rather than nevus)



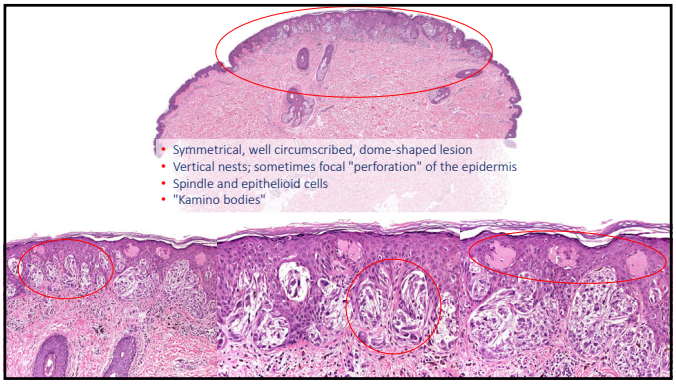
Spitz nevus



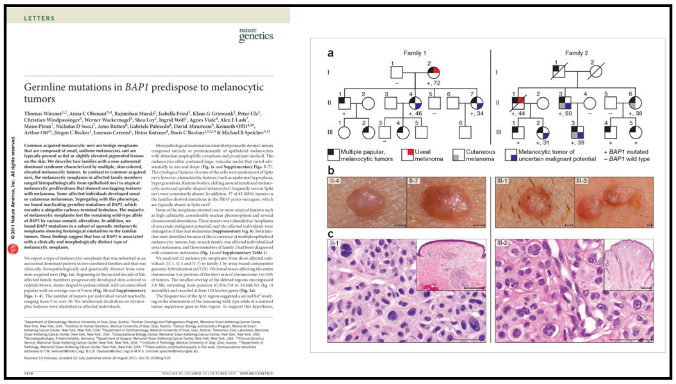


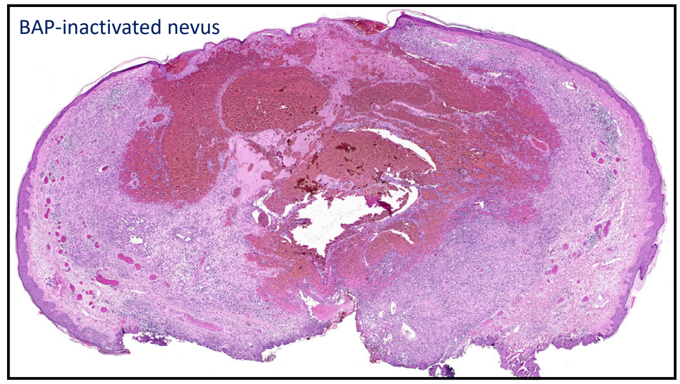
Does a "Spitz nevus" exist?

- Sophie Spitz did not describe a nevus
- "Spitzoid" melanocytes *(large epithelioid cells with abundant eosinophilic or amphophilic cytoplasm and large vesicular nuclei that contain prominent nucleoli)* are not restricted to "Spitz nevi"
- Molecular studies revealed many different genetic alterations related to benign and malignant "spitzoid tumors"
- Diagnosis of "Spitz nevus" should be restricted to (rare) prototypic examples of that melanocytic tumor



- Symmetrical, well circumscribed, dome-shaped lesion
- Vertical nests; sometimes focal "perforation" of the epidermis
- Spindle and epithelioid cells
- "Kamino bodies"





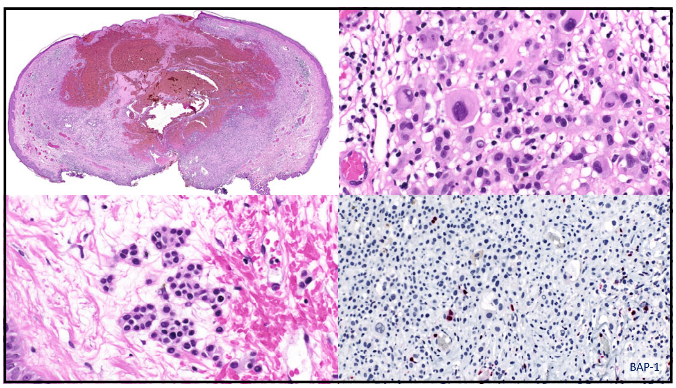
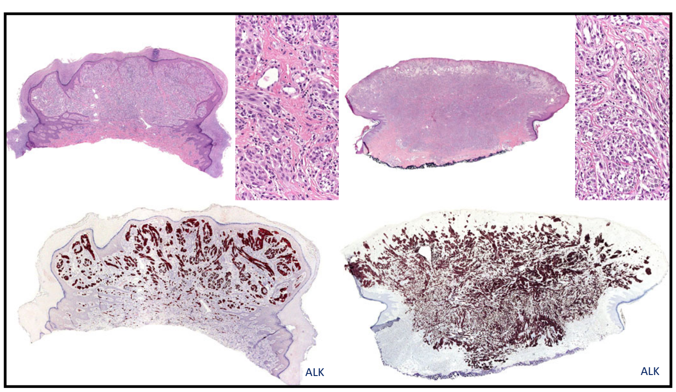
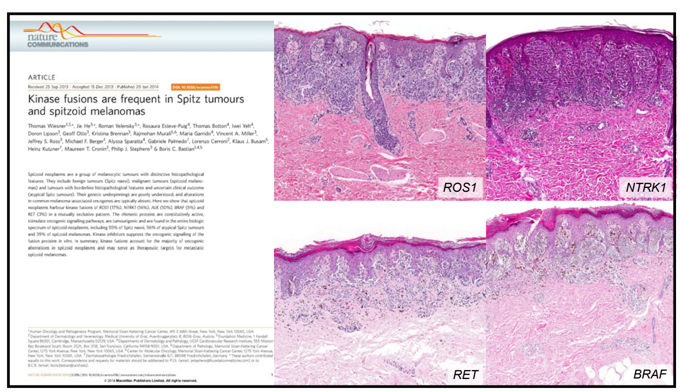
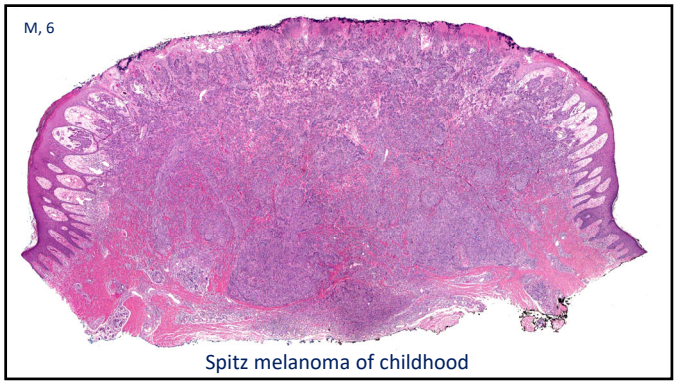


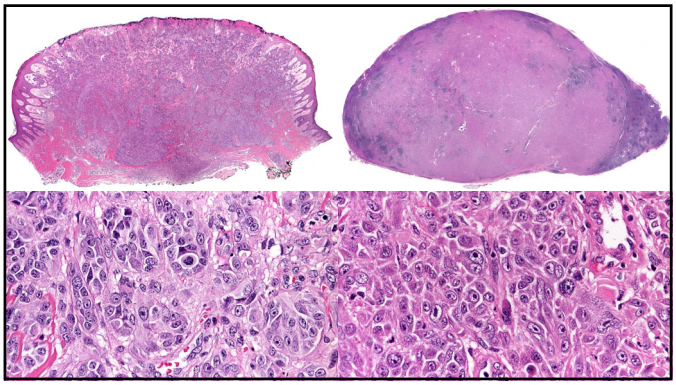
Table 1 | Frequency of kinase fusions in spitzoid neoplasms.

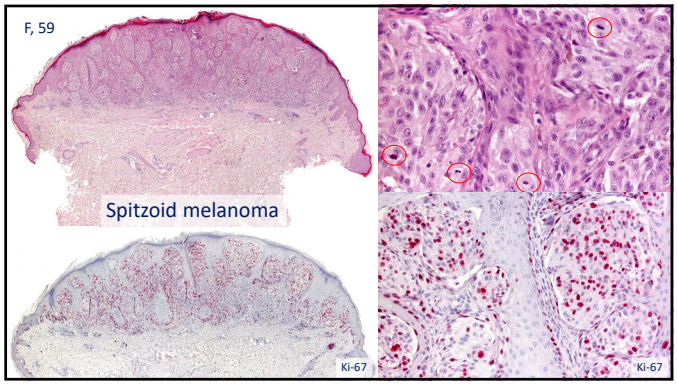
Fusion	Spitz naevus ($n=75$) % (number of cases)	Atypical Spitz tumour ($n=32$) % (number of cases)	Spitzoid melanoma ($n=33$) % (number of cases)	Total ($n=140$) % (number of cases)
ROS1	25.3 (19)	6.3 (2)	9.1 (3)	17.1 (24)
ALK	10.7 (8)	15.6 (5)	3 (1)	10 (14)
NTRK1	10.7 (8)	25 (8)	21.2 (7)	16.4 (23)
BRAF	5.3 (4)	6.3 (2)	3 (1)	5 (7)
RET	2.7 (2)	3.1 (1)	3 (1)	2.9 (4)
Total	54.7 (41)	56.3 (18)	39.4 (13)	51.4 (72)





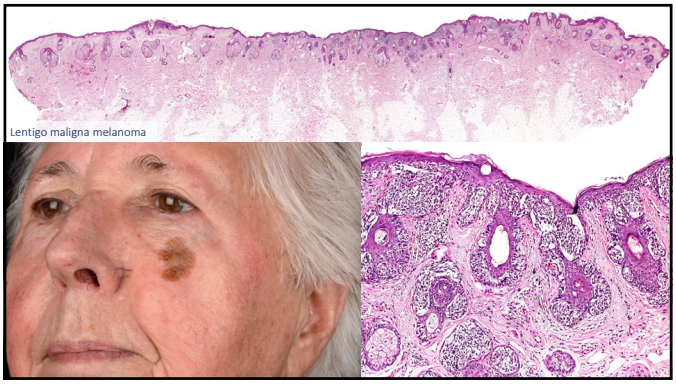


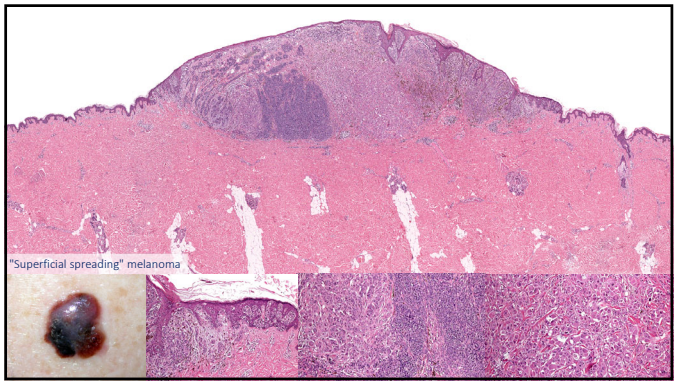


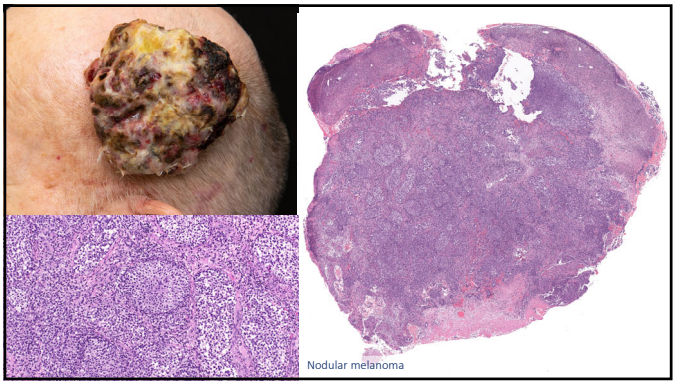


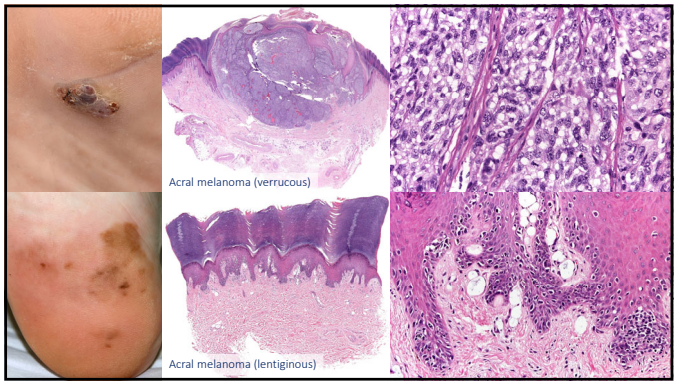
Working classification of "spitzoid" lesions

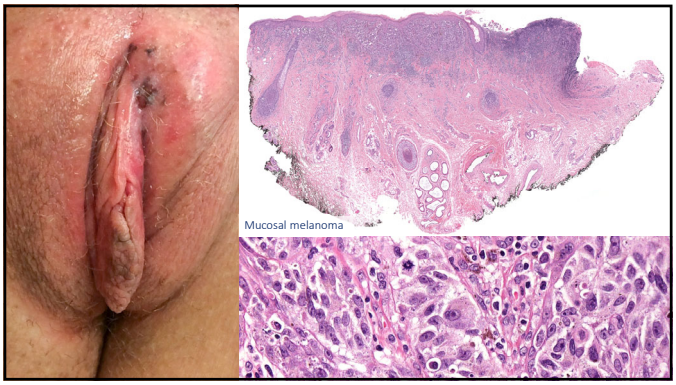
- **"Conventional" Spitz nevus:** *HRAS* mutations
- **Spitz "lineages":** *MAP2K1* fusion, *MAP3K8* fusion, *BRAF* fusion, *ALK* fusion, *ROS1* fusion, *NTRK1* fusion, *NTRK3* fusions, *RET* fusion, and probably other molecular aberrations; within each lineage, "Spitz nevus", atypical Spitz tumor, Spitz melanoma
- **Spitz melanoma (of childhood):** Spitz lineage initiating event; additional progression events (homozygous loss of *CDKN2A*, mutations in *TERT*, *CDK4*, *p53*, etc.); spread beyond local lymph nodes, but prognosis seems better than classic melanoma
- **Spitzoid melanoma:** "conventional" melanoma that histopathologically resembles a Spitz nevus; initiating mutations in *BRAF*, *NRAS*; other mutations related to tumor progression

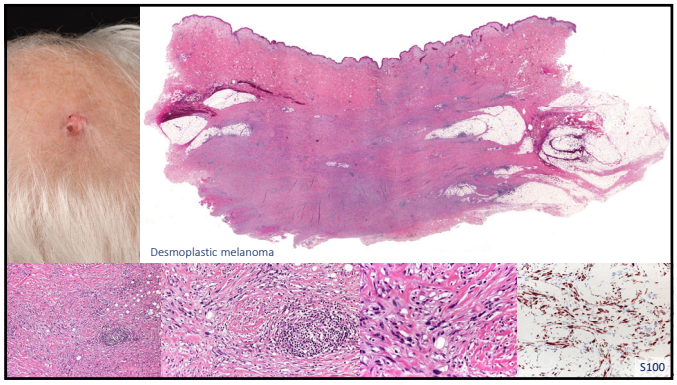






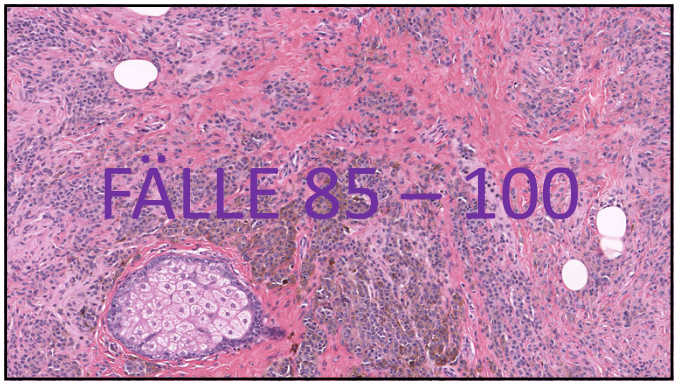






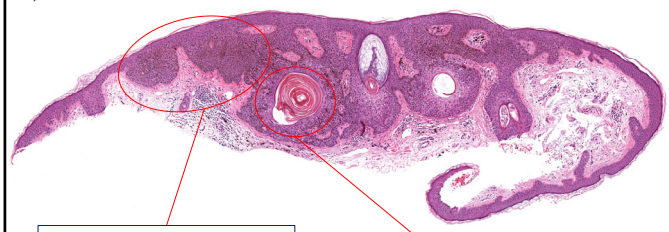
Immunohistology for melanocytic tumors

- **Differential diagnosis (undifferentiated malignant tumors):** *confirm melanocytic lineage by immunohistochemical stainings*
- **Differential diagnosis (melanocytic nevus vs. melanoma):** *the architecture of the lesion (symmetry, circumscription) may be better appreciated with immunostainings; PRAME, Ki-67 may provide useful diagnostic information; some Spitz tumors show specific staining according to the molecular pathway (yet no differentiation benign / malignant); some other stainings helpful in specific contexts*
- **Minimal invasion ("microinvasion"):** *a staining for melanocytes should be performed before rendering a diagnosis of MM "in situ"*
- **Depth of invasion:** *in some cases immunohistochemistry may be helpful in determining the depth of invasion (e.g., desmoplastic MM)*
- **Margins of excision:** *evaluation of the surgical margins may be easier with immunohistochemical stainings*



CASE 85
M, 75. Face.

Seborrheic keratosis

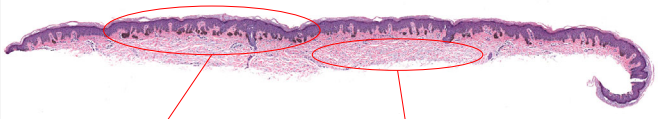


Irregular acanthosis of the epidermis with partly pigmented basaloid cells without atypia

"Pseudo" horn cyst

CASE 86
M, 57. Hand.

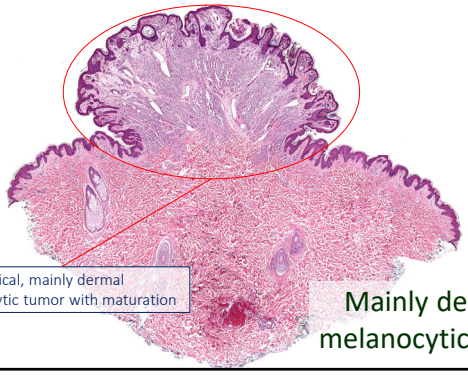
Lentigo actinica



Elongated, pigmented rete ridges without increase in the number of melanocytes

Actinic elastosis

CASE 87
M, 32. Back.

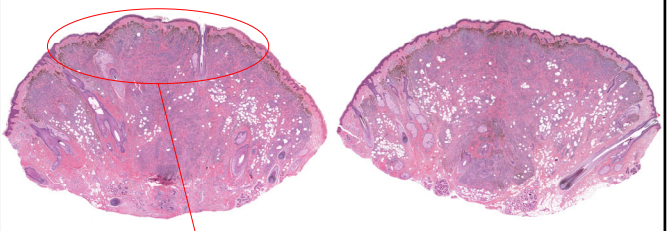


Symmetrical, mainly dermal melanocytic tumor with maturation

Mainly dermal melanocytic nevus

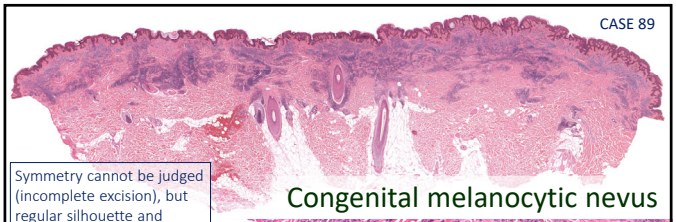
CASE 88
F, 38. Chin.

Dermal melanocytic nevus



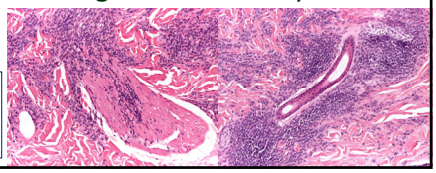
Symmetrical, dermal melanocytic tumor with pigmentation located mostly within the upper portion of it

CASE 89



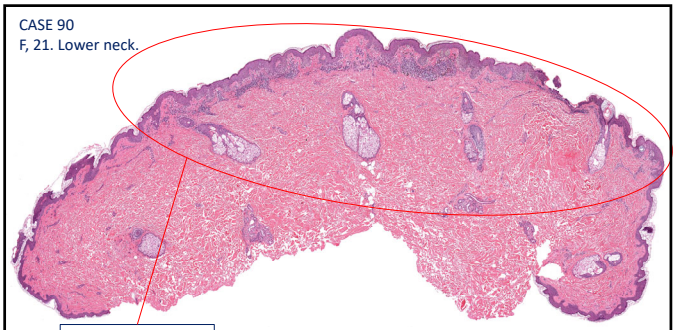
Symmetry cannot be judged (incomplete excision), but regular silhouette and regular distribution of pigment

Congenital melanocytic nevus



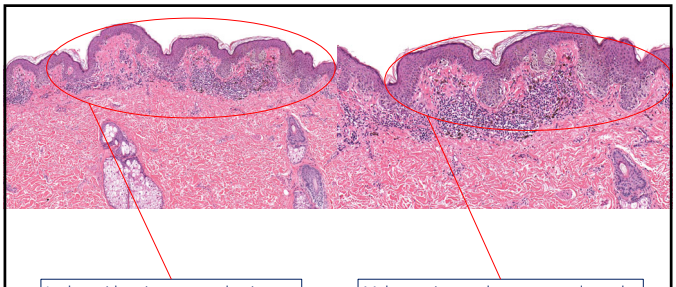
Nevoid melanocytes without atypia growing along and focally within the adnexal structures

CASE 90
F, 21. Lower neck.



Slightly asymmetrical melanocytic tumor

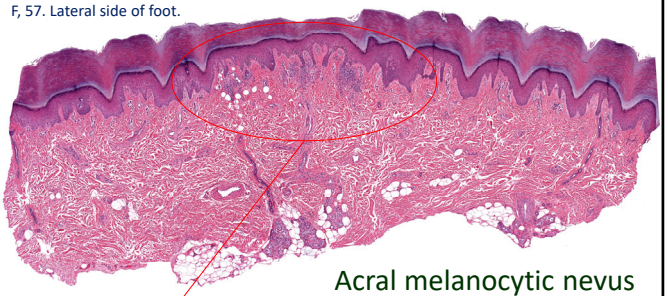
"Dysplastic" melanocytic nevus



In the epidermis nests predominate over solitary melanocytes

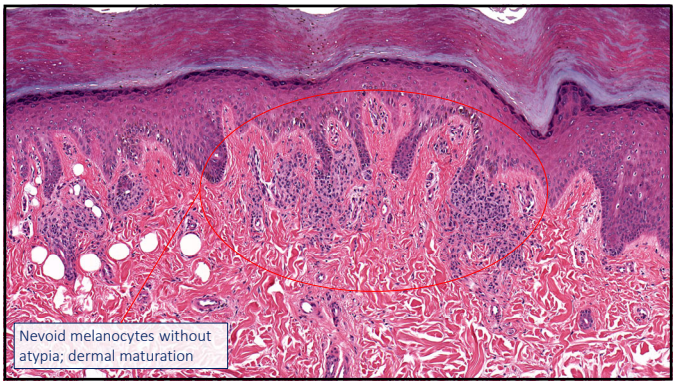
Melanocytic complexes arranged mostly at the dermo-epidermal junction

CASE 91
F, 57. Lateral side of foot.



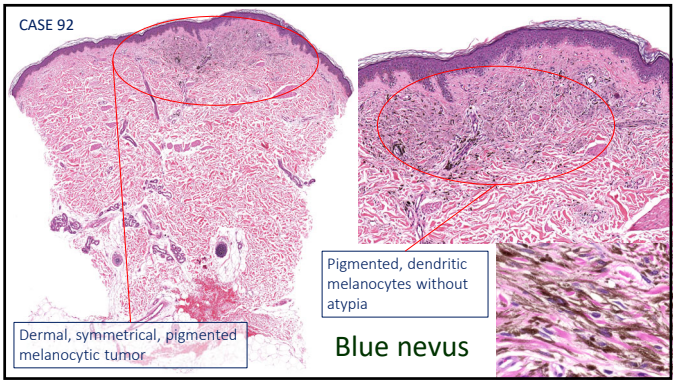
Symmetrical melanocytic tumor on acral skin

Acral melanocytic nevus



Nevoid melanocytes without atypia; dermal maturation

CASE 92

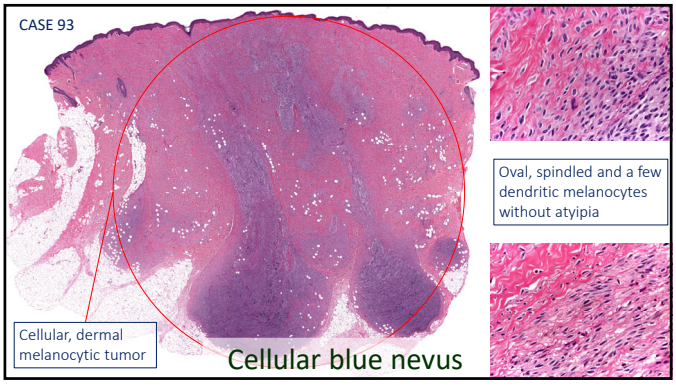


Dermal, symmetrical, pigmented melanocytic tumor

Pigmented, dendritic melanocytes without atypia

Blue nevus

CASE 93

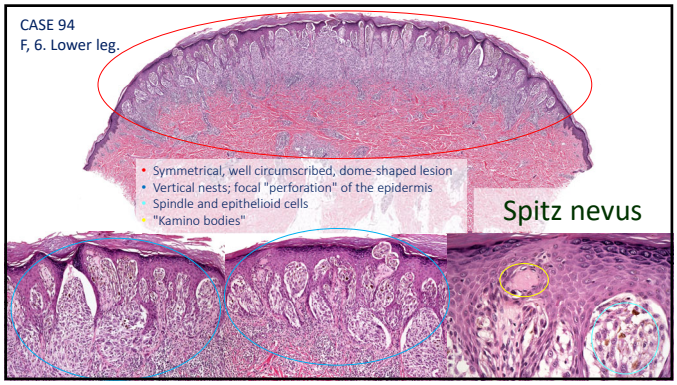


Cellular, dermal melanocytic tumor

Oval, spindled and a few dendritic melanocytes without atyipia

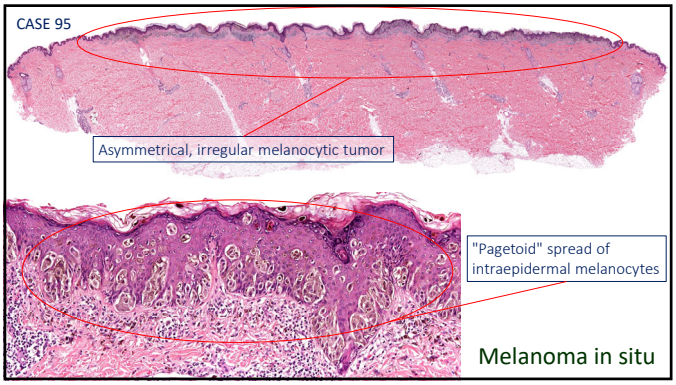
Cellular blue nevus

CASE 94
F, 6. Lower leg.



Spitz nevus

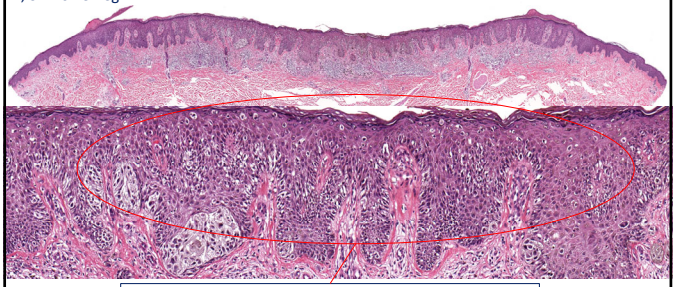
CASE 95



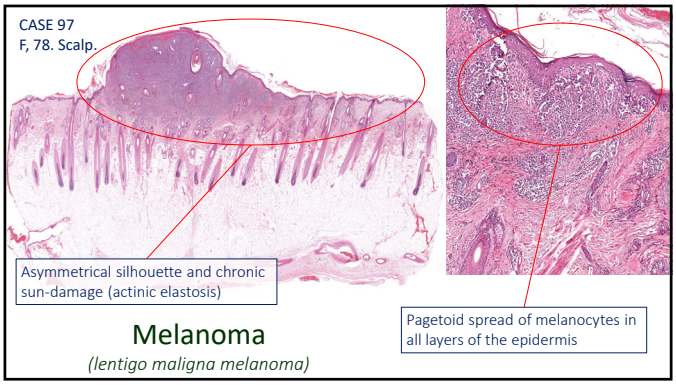
Melanoma in situ

CASE 96
F, 31. Lower leg.

Melanoma *(superficial spreading)*

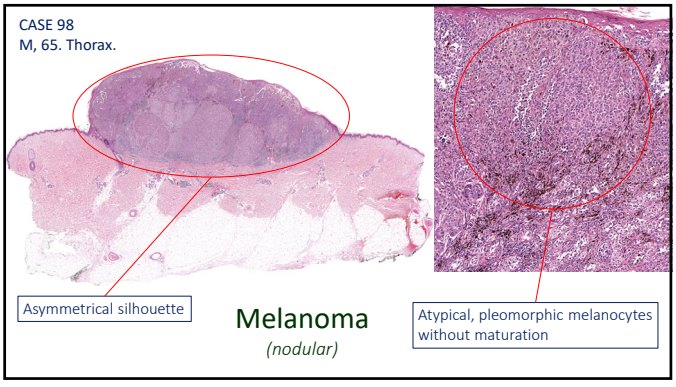


CASE 97
F, 78. Scalp.



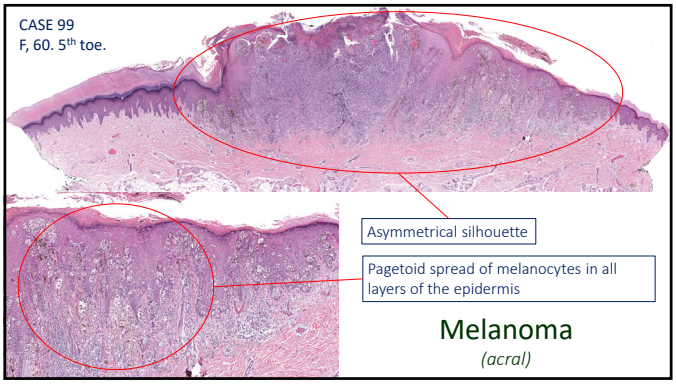
Melanoma
(lentigo maligna melanoma)

CASE 98
M, 65. Thorax.



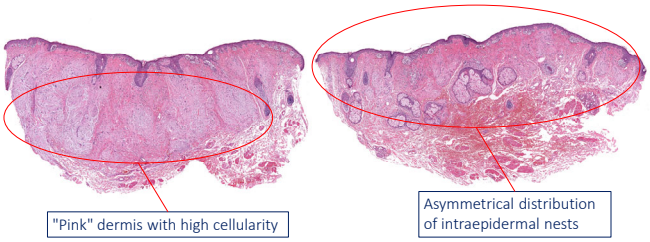
Melanoma
(nodular)

CASE 99
F, 60. 5th toe.

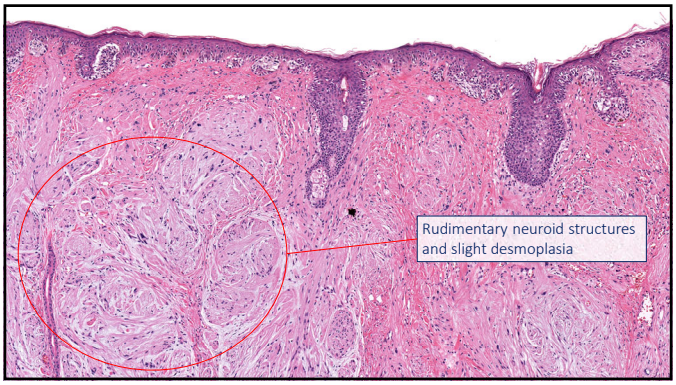


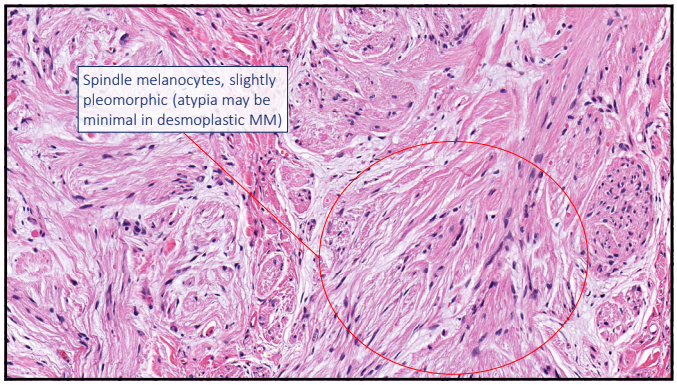
Melanoma
(acral)

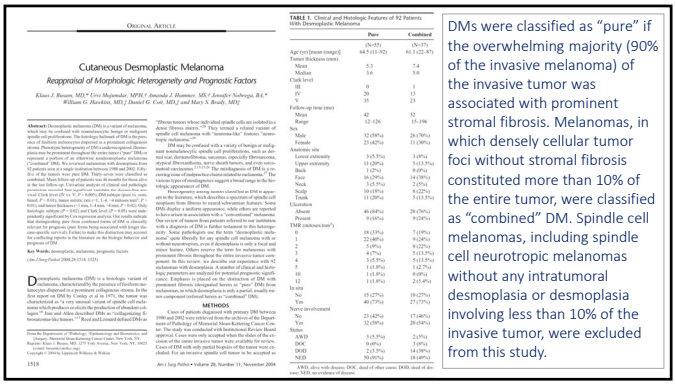
CASE 100
F, 56. Corner of the eye.



Desmoplastic ("neuroid") melanoma







ORIGINAL ARTICLE

Cutaneous Desmoplastic Melanoma

Reappraisal of Morphologic Heterogeneity and Prognostic Factors

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TABLE 1. Clinical and Histologic Features of 92 Patients With Desmoplastic Melanoma

	Pure	Combined
	(N=55)	(N=37)
Age (yr) [mean (range)]	64.5 (11–92)	61.1 (22–87)
Tumor thickness (mm)		
Mean	5.3	7.4
Median	3.6	5.0
Clark level		
III	0	1
IV	20	13
V	35	23
Follow-up time (mo)		
Mean	42	52
Range	12–126	15–196
Sex		
Male	32 (58%)	26 (70%)
Female	23 (42%)	11 (30%)
Anatomic site		
Lower extremity	3 (5.5%)	3 (8%)
Upper extremity	11 (20%)	5 (13.5%)
Back	1 (2%)	0 (0%)
Face	16 (29%)	14 (38%)
Neck	3 (5.5%)	2 (5%)
Scalp	10 (18%)	8 (22%)
Trunk	11 (20%)	5 (13.5%)
Ulceration		
Absent	46 (84%)	28 (76%)
Present	9 (16%)	9 (24%)
TMR (mitoses/mm²)		
0	18 (33%)	7 (19%)
1	22 (40%)	9 (24%)
2	5 (9%)	8 (22%)
3	4 (7%)	5 (13.5%)
4	3 (5.5%)	5 (13.5%)
5	1 (1.8%)	1 (2.7%)
10	1 (1.8%)	0 (0%)
12	1 (1.8%)	2 (5.4%)
In situ		
No	15 (27%)	10 (27%)
Yes	40 (73%)	27 (73%)
Nerve involvement		
No	23 (42%)	17 (46%)
Yes	32 (58%)	20 (54%)
Status		
AWD	3 (5.5%)	2 (5%)
DOC	0 (0%)	3 (8%)
DOD	2 (3.5%)	14 (38%)
NED	50 (90%)	18 (49%)

AWD, alive with disease; DOC, dead of other cause; DOD, dead of disease; NED, no evidence of disease.

DMs were classified as "pure" if the overwhelming majority (90% of the invasive melanoma) of the invasive tumor was associated with prominent stromal fibrosis. Melanomas, in which densely cellular tumor foci without stromal fibrosis constituted more than 10% of the entire tumor, were classified as "combined" DM. Spindle cell melanomas, including spindle cell neurotropic melanomas without any intratumoral desmoplasia or desmoplasia involving less than 10% of the invasive tumor, were excluded from this study.

Desmoplastic melanoma & variants

- "Pure" or "mixed" variants depending on the amount of desmoplastic stroma; pure variants have a better prognosis (mixed variants should probably be referred to as "melanoma with desmoplasia")
- Overlapping features with spindle cell melanoma, neuroid melanoma, neurotropic melanoma, myxoid melanoma (variants of the same type?)
- Common on face in elderly persons, but may be encountered on any area of the body and also in children
- In-situ component helpful for diagnosis if present, but may be missing (particularly in partial biopsies)
- Patchy lymphoid infiltrates within a desmoplastic spindle cell neoplasm represent an important clue
- Melan-A, HMB-45, MiTF oft negative; S-100, SOX-10, p75 NGFR positive
- Margins may be difficult to assess, particularly in re-excision specimens (beware of S-100 positivity in scars)

Melanomas and melanocytic nevi – not only H&E

- Several molecular pathways have been identified in melanomas arising at different cutaneous sites (e.g., chronic sun-damaged skin, intermittently sun-exposed skin, oral mucosa, genital mucosa, palmo-plantar sites)
- Several molecular pathways identified also for melanocytic nevi at different sites; numerous fusions and mutations described for Spitz nevi and tumors (and melanomas related to these lesions)
- Some "combined" nevi show presence of more molecular alterations in the different parts of the tumor, thus representing "evolving" (and more concerning) lesions
- Integration of all data helpful in controversial cases