

IHC IN DIAGNOSTICS

THE SELECTION OF WHICH ANTIBODIES TO USE IN DIAGNOSTIC HISTOPATHOLOGY DEPENDS ON SEVERAL VARIABLE::

1) Ab AVAILABILITY FOR SPECIFIC ANTIGENS :

- **SPECIFIC FOR A TISSUE/ ORGAN AND OF THE IPATHOLOGICAL LESIONS ORIGINATING FROM IT - *LINEAGE-SPECIFIC*** (eg Thyroglobulin for the thyroid)
- **SPECIFIC FOR A GROUP OF TISSUES - *LINEAGE-RESTRICTED*** (eg cytokeratins in epithelial tissues)

2) POSSIBILITY OF ANTIBODIES TO REACT WITH ANTIGEN SPECIFICALLY IN *FORMALIN FIXED AND PARAFFIN EMBEDDED TISSUES*

3) POSSIBILITY OF SEARCHING IN POSITIVE OR NEGATIVE DIFFERENTANTIGENS TO CONFIRM THE DIAGNOSIS - *ANTIBODY PANEL*

4) HISTOLOGICAL RECOGNITION OF A SUSPECTED LESION FOR CONFIRMATION OR *DIFFERENTIAL DIAGNOSIS* BETWEEN A LIMITED NUMBER OF LESIONS (eg differential diagnosis between different types of spindle-cell sarcoma)

IHC IN DIAGNOSTICS

1. It requires time, human and financial resources
2. Many antibodies with different sensitivity and specificity
3. Result depends on preanalytical factors
4. Semi-quantitative subjective interpretation. The intensity of the staining and the percentage of positive cells are evaluated.

$$\text{H score (histo)} = [1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)]$$

The score can vary in the range 0 - 300. Cases can be classified as positive or negative according to specific cut-off.

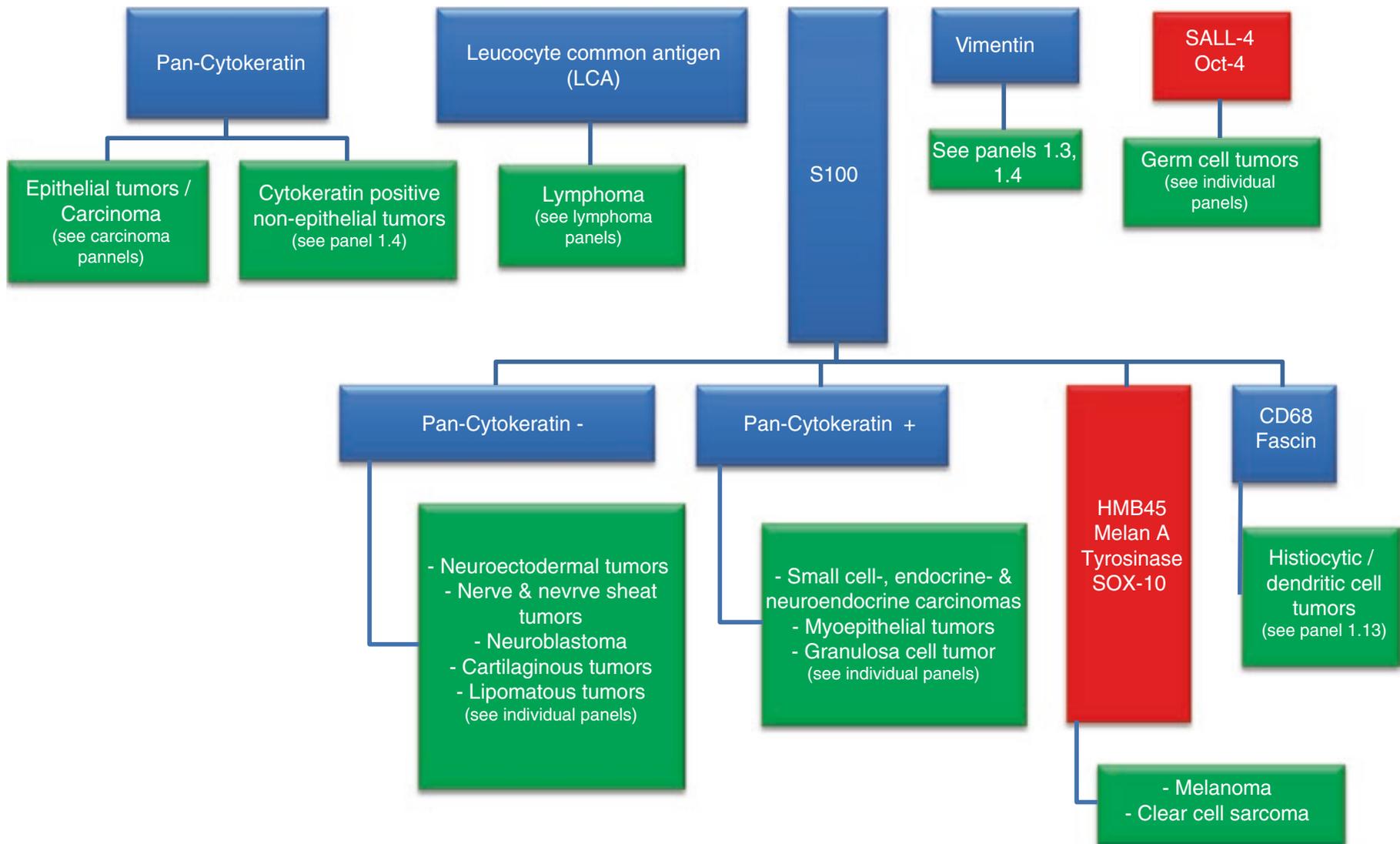
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Knowledge of the nature of targeted antigens is an important factor in the interpretation of the results. The following details are always to consider:

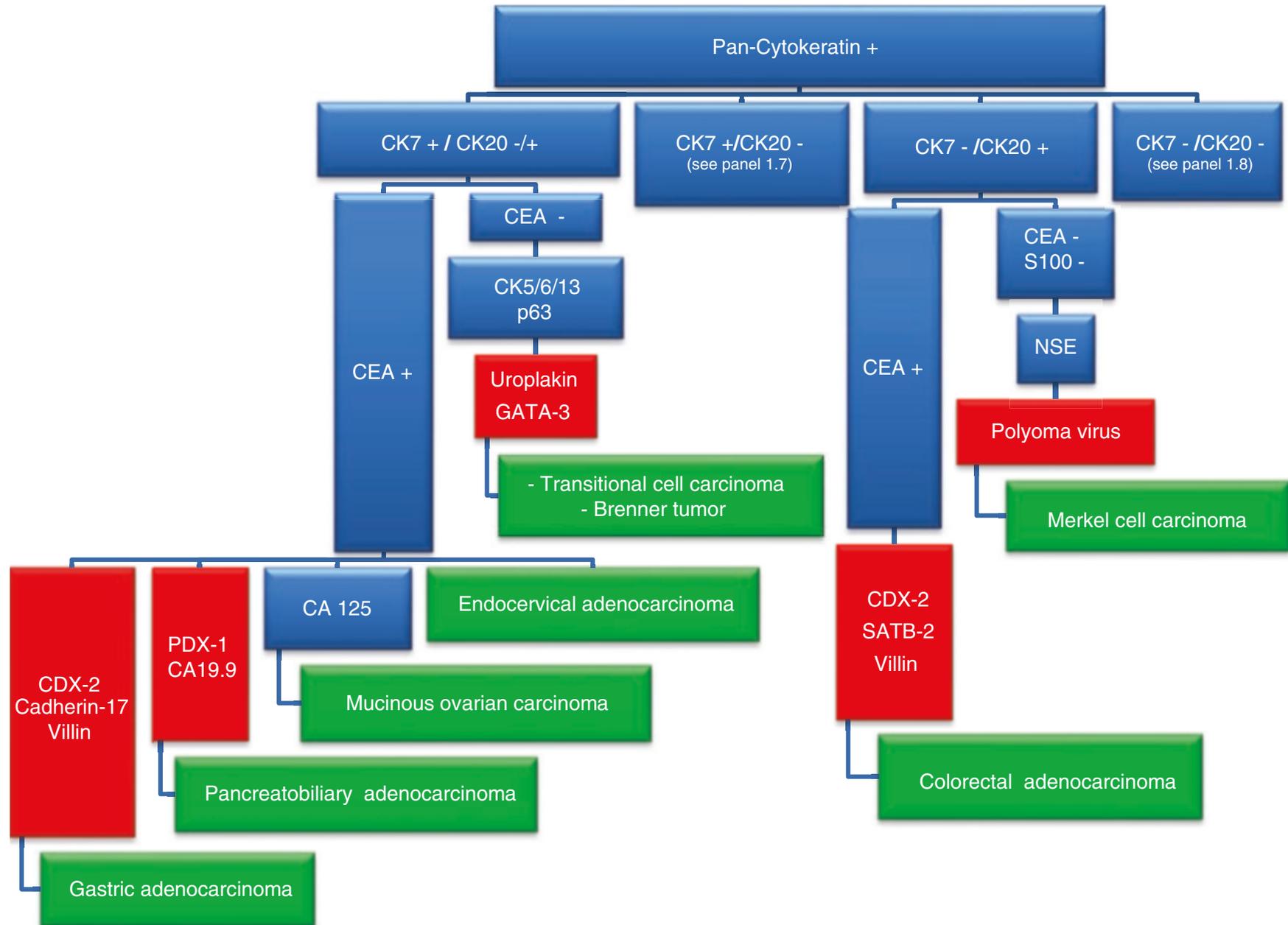
- ✓ The expression pattern of the antigens (nuclear, cytoplasmic, membranous, or extracellular).
- ✓ Stability of antigens during tissue processing. Optimal and standardized tissue fixation and processing and as a rule, bad H&E sections mean bad immunohistochemistry results.
- ✓ Histopathologists deal with neoplasia with heterogeneous cell populations with high potential of genotypic and phenotypic variations. The reason for the atypical antigen expression can be in the biology of the tumor or the nature of the antibodies used.
- ✓ Standardized reporting is very helpful in organizing the information to reach an accurate diagnosis.
- ✓ Despite the high sensitivity of IHC and the large number of available antibodies, immunohistochemistry—as any method—has its own limits.

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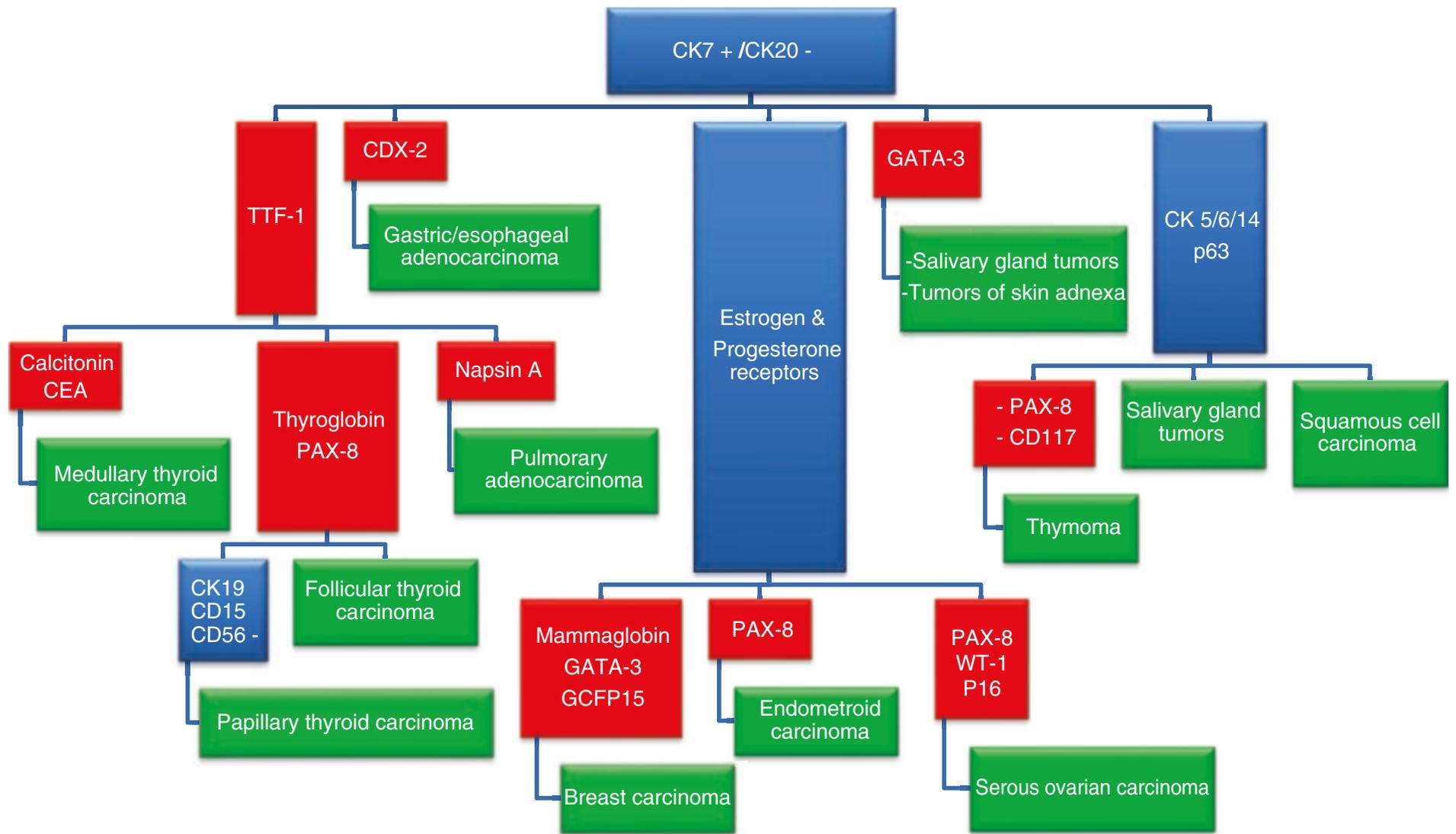
- 1. EPITHELIAL Ab EPITELIALI***
- 2. MESENCHIMAL Ab***
- 3. HEMOLYMPHOPOIETIC Ab***
- 4. HORMONES, HORMONAL RECEPTORS AND RELATED PROTEINS***
- 5. ONCOPROTEIN, GROWTH FACTORS AND THEIR RECEPTORS***
- 6. CELL CYCLE ASSOCIATED MARKERS***
- 7. MARKERS OF EXTRACELLULAR MATRIX (es Laminins)***



Algorithm 1.1 Primary Screening Antibody Panel



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Algorithm 1.6 CK7/CK20 Expression Pattern in Carcinomas



Algorithm 1.7 Cytokeratin CK7+/CK20– Carcinoma

EPITHELIAL TISSUES MARKERS: CYTOKERATINS

↳ Cytokeratins are the most important markers used for the diagnosis of epithelial neoplasms. Cytokeratins are intermediate filament proteins building an intracytoplasmic network between the nucleus and cell membrane of epithelial cells. Cytokeratins are a complex family composed of more than 20 isotypes and divided into 2 types: **type I (acidic group)** including cytokeratins 9–20; **type II (basic group)** including cytokeratins 1–8

↳ **Moll's catalog**- it groups the cytokeratins according to their MW and isoelectric point. Therefore they are subdivided into 3 groups: high MW, mean MW and low MW.

↳ **Simple epithelium: 7-18, 8-18**

EPITHELIAL TISSUES MARKERS: CYTOKERATINS

	BASIC		ACIDIC	
	Number	kDa	Number	kDa
CK-HMW	1-2	65-67	9	64
	3	64	10-11	56
			12	55
CK-MMW	4	59	13	51
	5	58	14-15	50
	6	56	16	48
			17	46
CK-LMW	7	54	18	45
	8	52	19	40
			20	46

- Simple epithelium and glandular type

Different cytokeratins are expressed in different epithelial tissues and at different stages of differentiation; consequently, different epithelial types of tumors have different specific cytokeratin expression profiles, which usually remain constant after neoplastic transformation

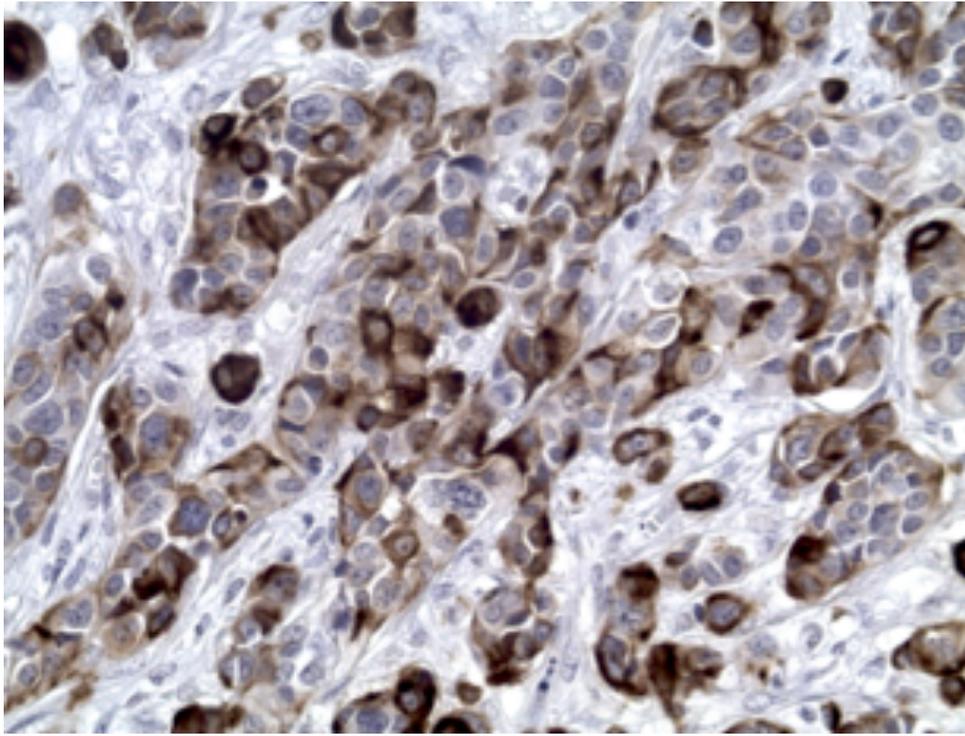
EPITHELIAL TISSUES MARKERS: CYTOKERATINS

-To define the epithelial origin of a neoplasm and characterize it.

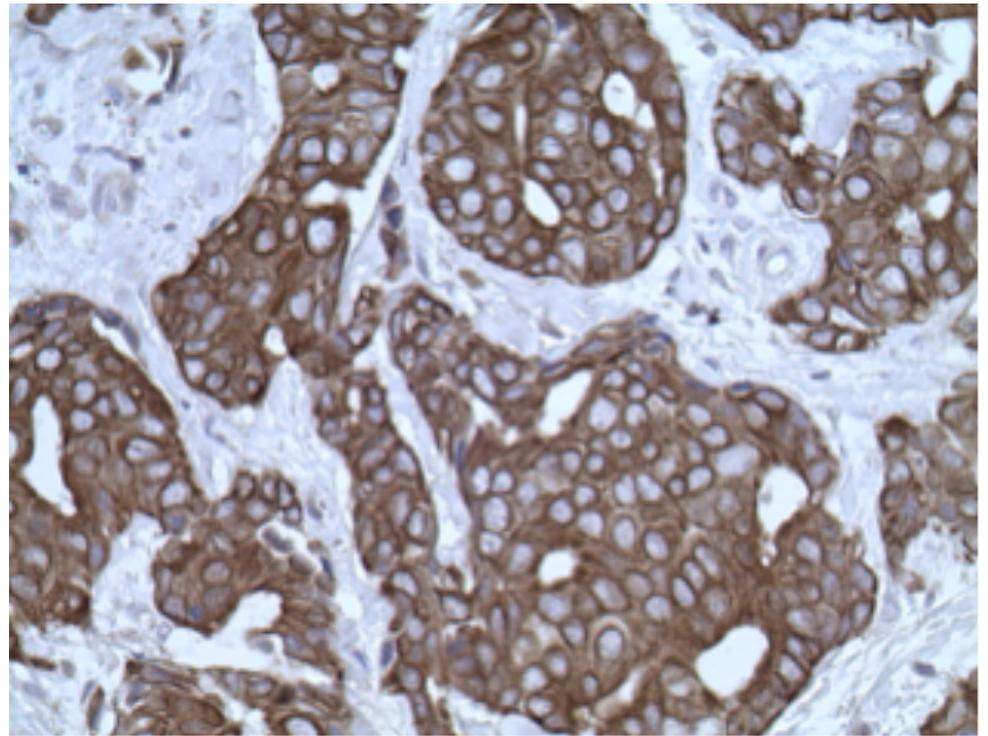
In IHC sections, cytokeratins reveal typically a diffuse cytoplasmic expression pattern; nevertheless, abnormal staining patterns such as perinuclear and dot-like expression patterns are characteristic for different neuroendocrine tumors.

Ab used :

- **- Broad spectrum cytokeratins (AE1/AE3 e CAM5.2):** Ab cocktail recognizing most epithelial neoplasms..
 - **HMW Cytokeratins (34 β E12 o 309):** it reacts with K1,5,10 and 14 of Moll's catalog. They are expressed in complex epithelium such as glandular, transitional, squamous epithelium and also in and in prostatic gland.
 - **LMW Cytokeratins (35 β H11):** it reacts withn K8 which is expressed in **simple epithelial cells** (eg. liver, mammary gland...), it is used in panel.
 - **-K20:** Gastric and colorectal epithelium, umbrella cells of transitional epithelium. As the expression of cytokeratin 20 is restricted to a limited number of carcinomas, it is a helpful marker to differentiate between different carcinoma types. **(useful to discriminate between lung and breast tumors)**
 - **-EPITHELIAL MEMBRANE ANTIGEN (EMA)- membranous-cytoplasmatic** Epithelial membrane antigen (EMA) also known as MUC-1 is a trans- membrane glycoprotein composed of cytoplasmic and extracellular domains. EMA is also one of the major components of the mucosal layer protecting gastric mucosa. EMA is highly expressed in different types of epithelial cells mainly **glandular epithelium and neoplasms originating from these epithelial types**, whereas very low expression level is found in squamous and transitional cell carcinomas.
- other epithelial markers - CA-125, CEA**



CK5,6, breast cancer



CK8, breast cancer

MARKERS FOR MUSCLE CELLS

-DESMIN: *Desmin stains cardiac, skeletal, and smooth muscle cells and tumors derived from these cells. The intensity of desmin expression correlates with the differentiation grade of muscle or muscle tumor. The main diagnostic use is for tumor with skeletal muscle differentiation/rhabdomyosarcoma. It is expressed in myofibroblasts, but not in myoepithelial cells.*

-Muscular Actin (HHF35): *it reacts with both skeletal and smooth muscle actins and accordingly stains both smooth muscle and skeletal muscle tumors. It is expressed by myofibroblasts as well as myoepithelial cells.*

-MYOGENIN (MyoD1): *it is a myogenic transcriptional regulatory factor. It takes part in the early stages of skeletal muscle differentiation. Main diagnostic use: Rhabdomyosarcoma.*

-MIOGLOBIN AND α -SARCOMERIC ACTIN: *Rhabdomyosarcoma*

-ACTINA PER MUSCOLO LISCIO: *per leiomiosarcomi.*

STEROID HORMONES RECEPTORS

Estrogen receptors (ER) are a member of the steroid family of ligand- dependent transcription factors and include two types encoded by two different genes on different chromosomes, the alpha type (ER- α) and beta type (ER- β). The ER- α type is mainly expressed in both epi- thelial and stromal cells of the breast, uterus, pla- centa, liver, CNS, endothelium, and bone. Progesterone is a steroid hormone involved in the differentiation of breast parenchyma and endometrium in addition to milk protein synthesis. Progesterone receptors (PgR) are good marker for breast carcinomas and have more speci city than estrogen receptors as they are expressed only in a limited number of tumors such as endometrial carcinoma .

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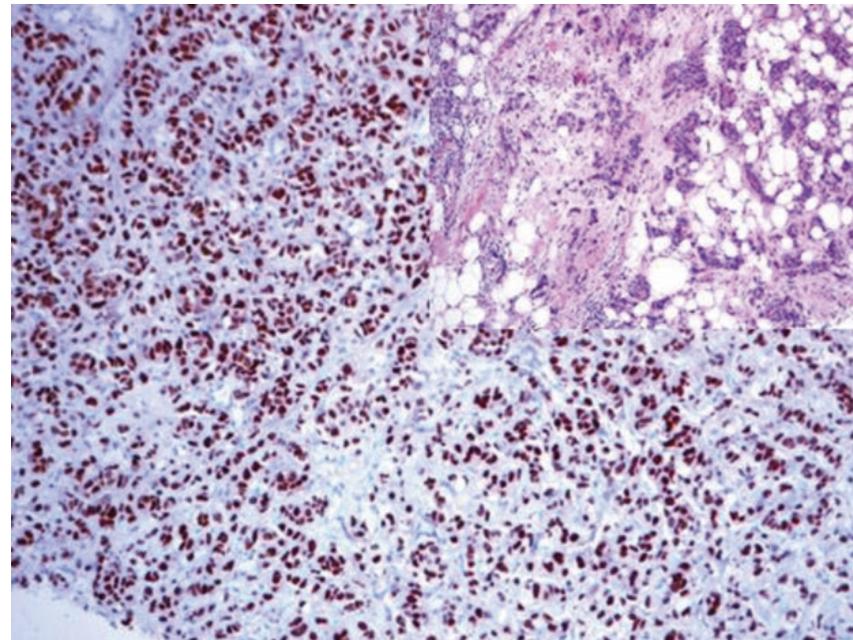


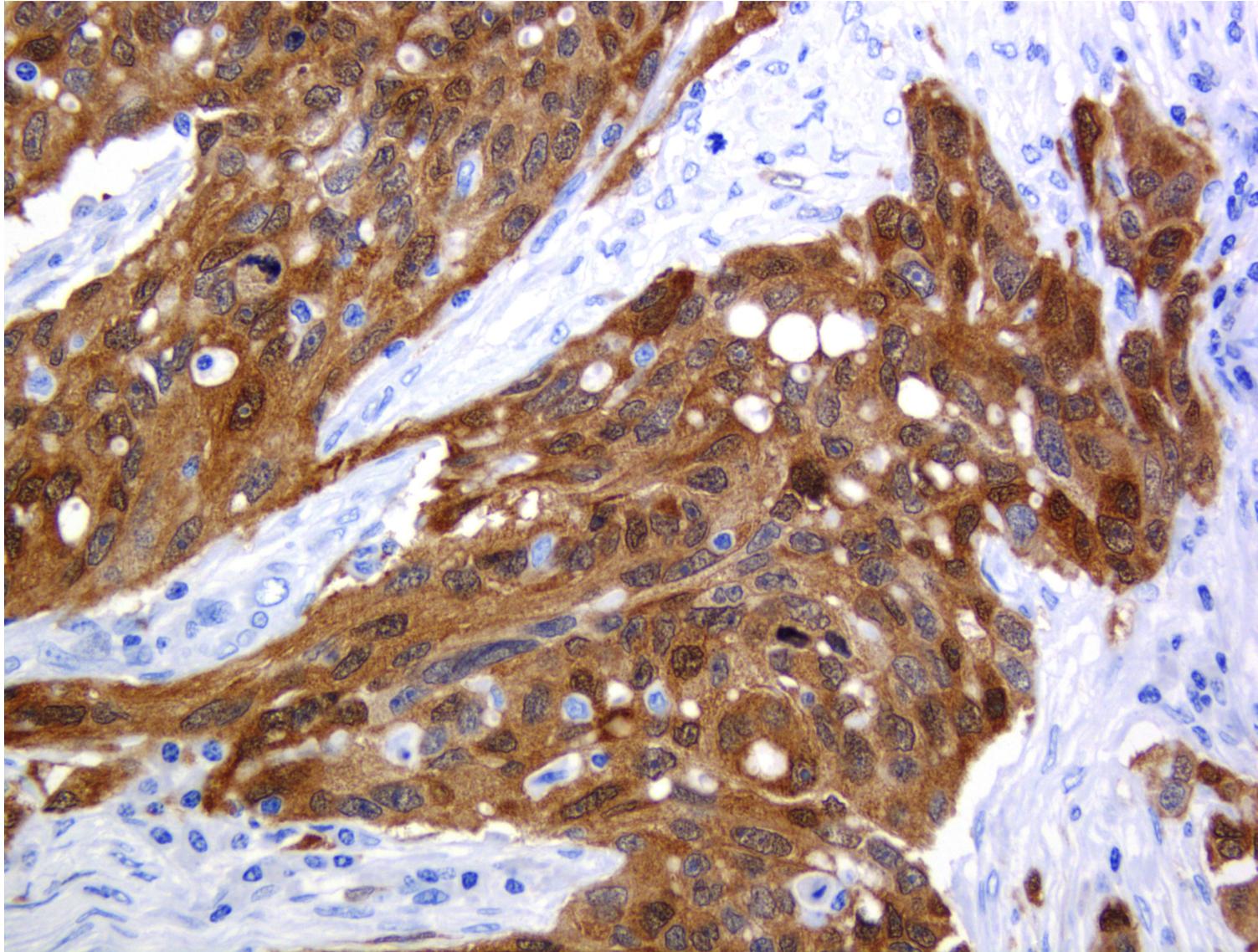
Fig. 10.1 Strong nuclear expression of estrogen receptors in breast carcinoma

CELL PROLIFERATION MARKERS

- PCNA (Proliferating Cells Nuclear Antigen)**: it is a DNA polymerase associated protein. It is highly expressed in the late G1 phase and during S phase of the cell cycle.
- Ki-67 (MIB1)**: proliferation index – it is expressed in all cell cycle phases, excluded G0.

MARKERS OF TUMOR AGGRESSIVENESS

- EGFr**: Epidermal growth factor receptor-1 (EGFR, Erb1) is a member of type I receptor tyrosine kinase family, a transmembrane glycoprotein normally expressed on the membrane of various types of normal epithelial and non-epithelial cells. It binds EGF or TGF α . Higher EGFR expression is associated to higher tumor aggressiveness.
- C-erbB2 (HER2 / neu)**: Human epidermal growth factor receptor-2 (HER-2). During carcinogenesis, the amplification of the HER-2 occurs, causing the overexpression of the HER-2 receptor, which is characteristic for few types of human carcinomas, mainly breast and gastric adenocarcinomas.
- P53**: p53 mutation is a common event in cancer. Mutations within the TP53 cause the overexpression and accumulation of mutated p53 protein not able to bind DNA to stimulate the p21 synthesis acting as a stop signal in the cell cycle, consequently causing an uncontrolled proliferation of involved cells. The overexpression of p53 is associated with different neoplastic and preneoplastic lesions.



P53 in HGSO carcinoma