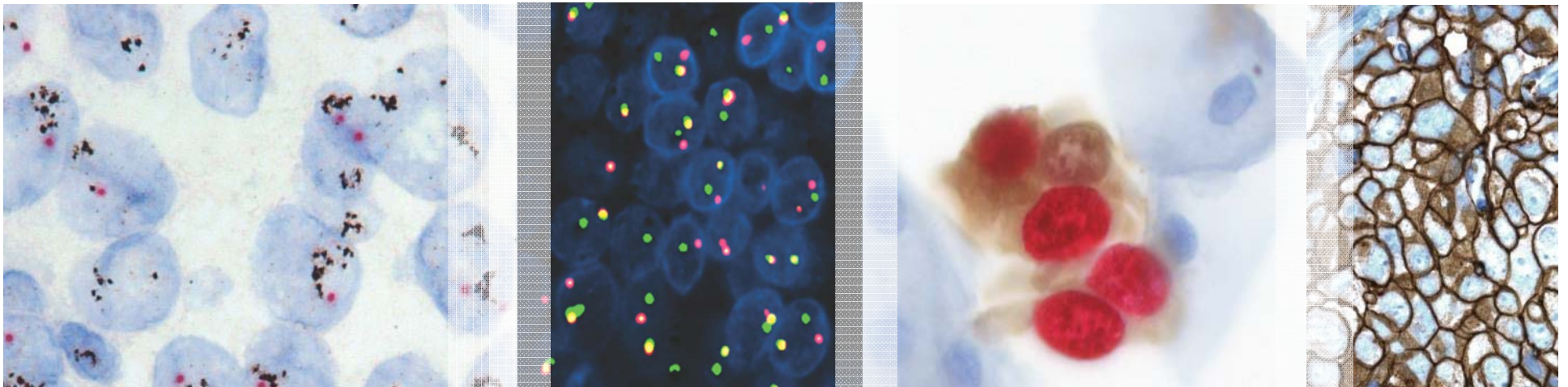
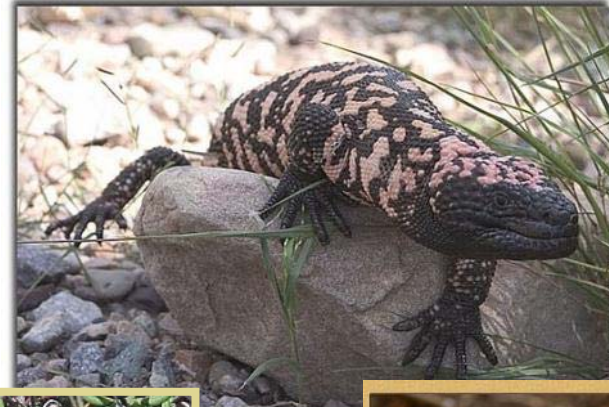
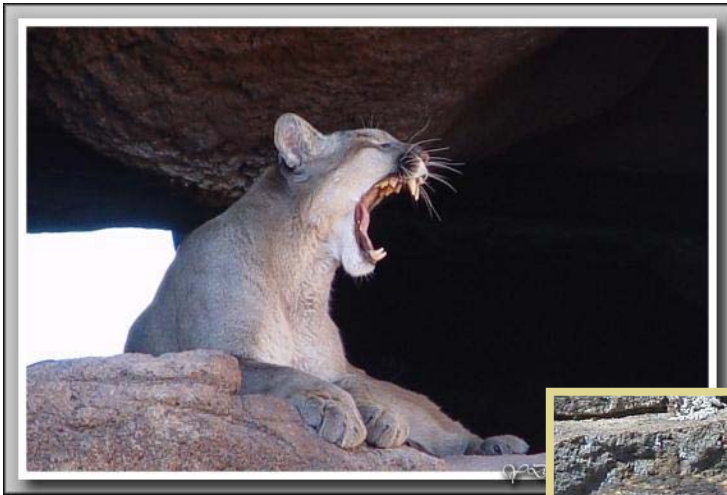


Challenges in Creating a Scoring Algorithm for IHC Companion Diagnostics

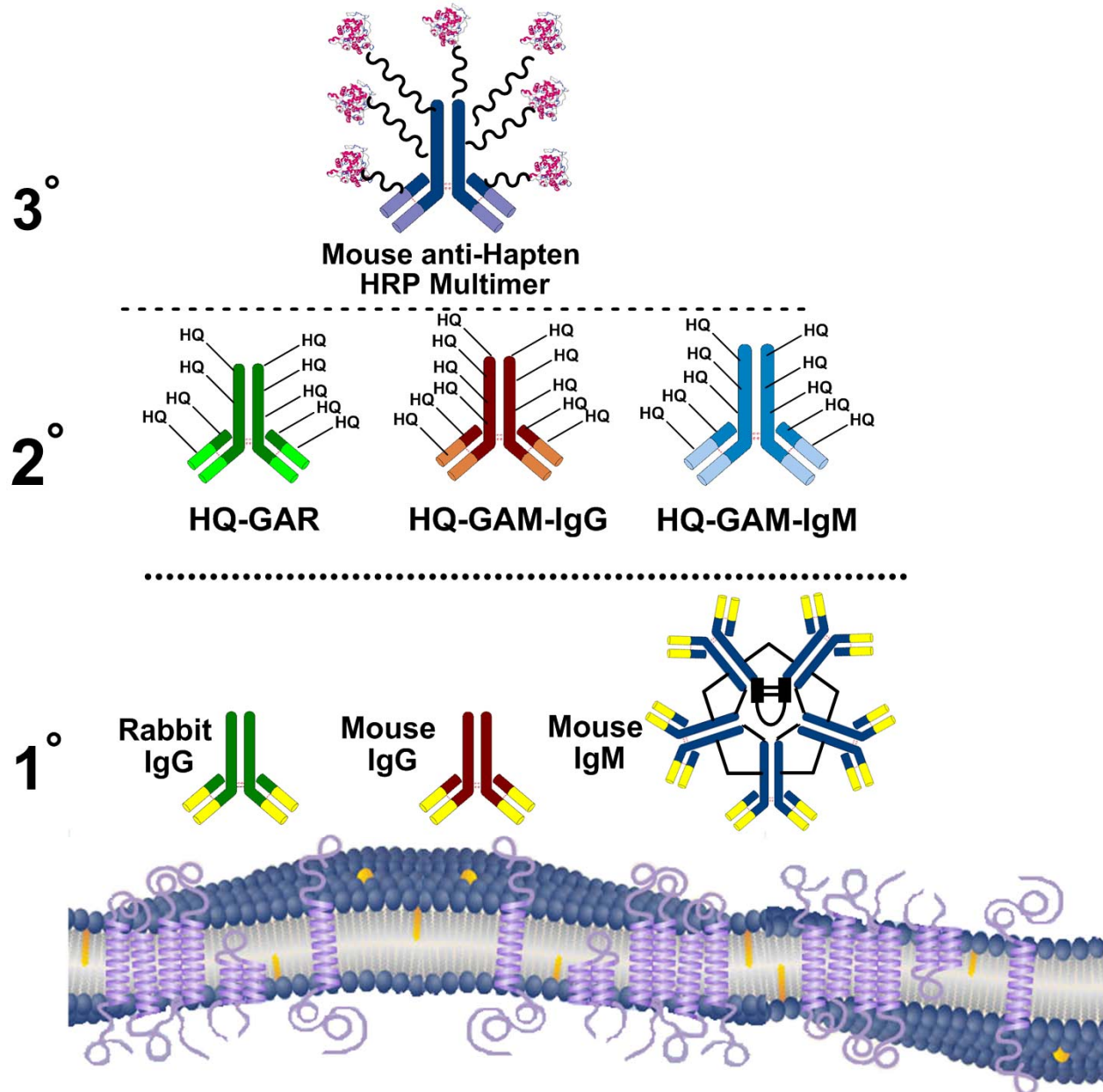
Patrick C. Roche, Ph.D.



Arizona – Everything Bites

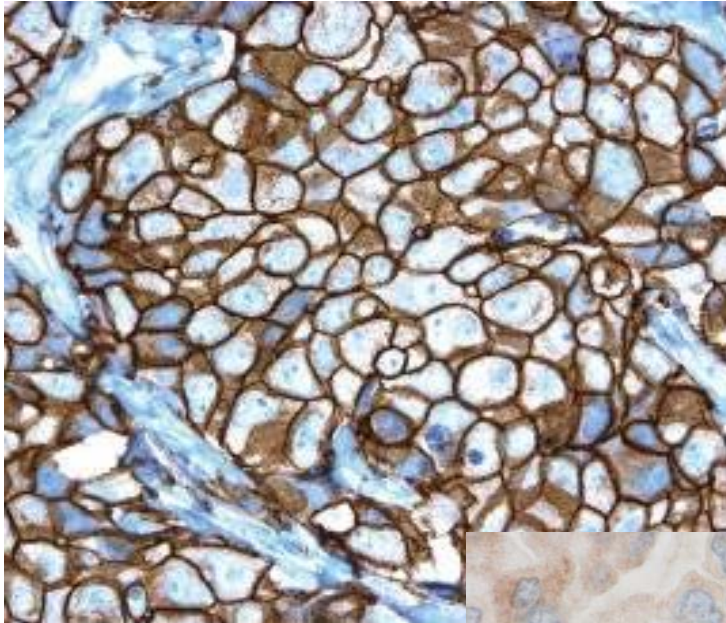


Immunohistochemistry

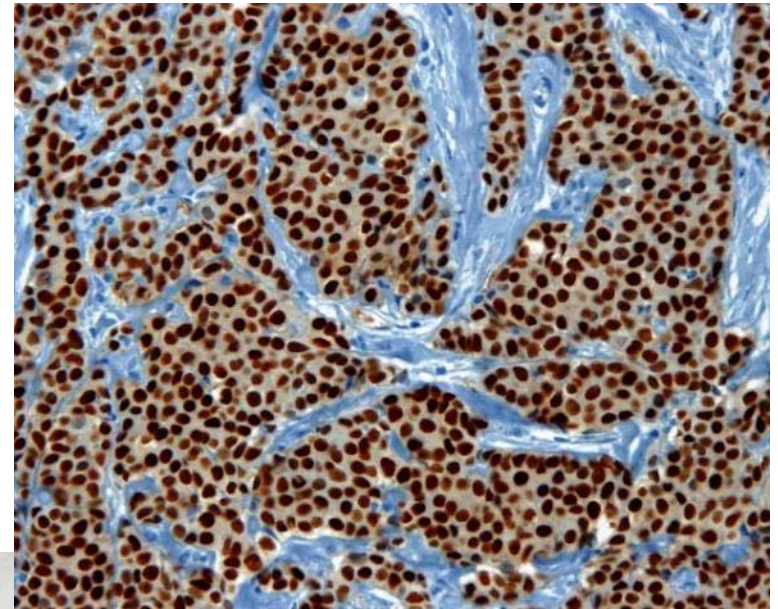


Different Staining Patterns

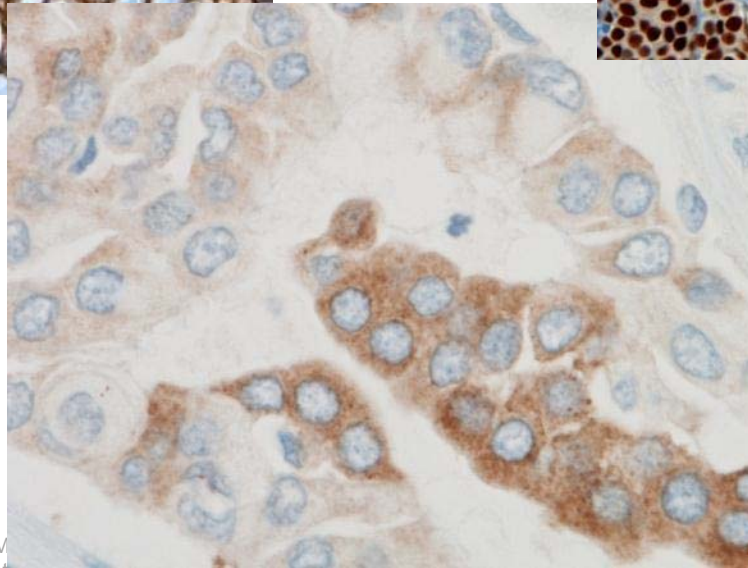
Membrane



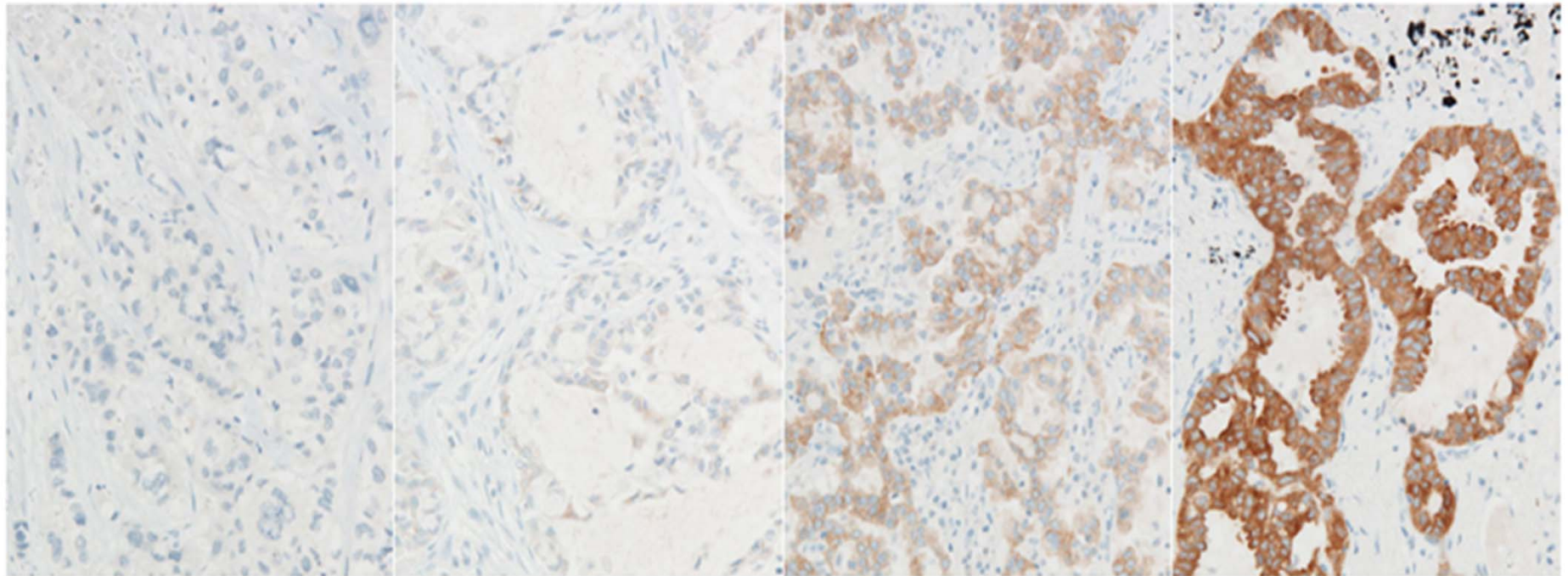
Nuclear



Cytoplasmic



Different Levels of Target Expression



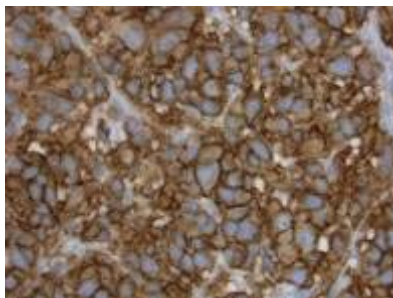
Negative

Weak

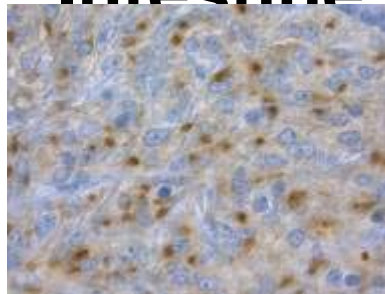
Moderate

Strong

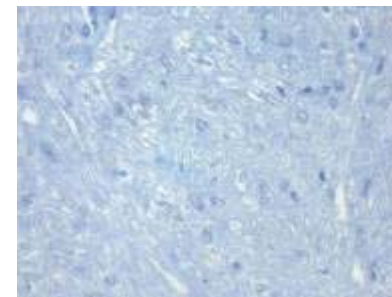
c-KIT Staining in GIST and Normal Intestine



Membranous - GIST



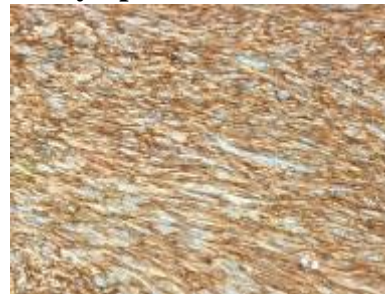
Dot-like paranuclear cytoplasmic - GIST



Negative - GIST



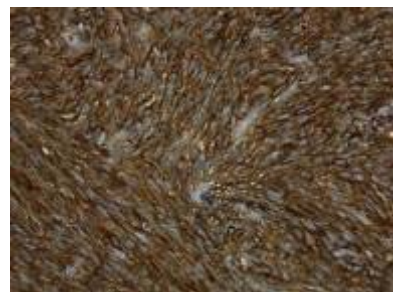
Positive (weak) - GIST



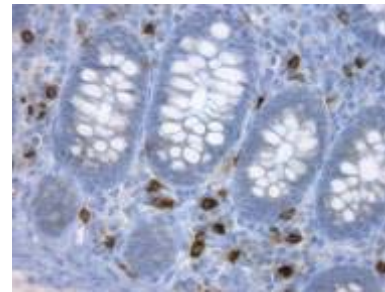
Positive (moderate) - GIST



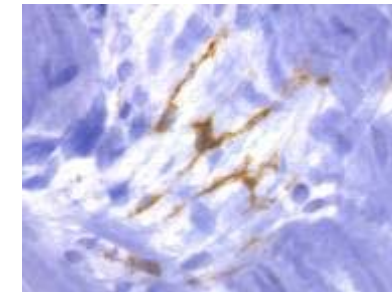
Positive (strong) - GIST



Positive (very strong) - GIST

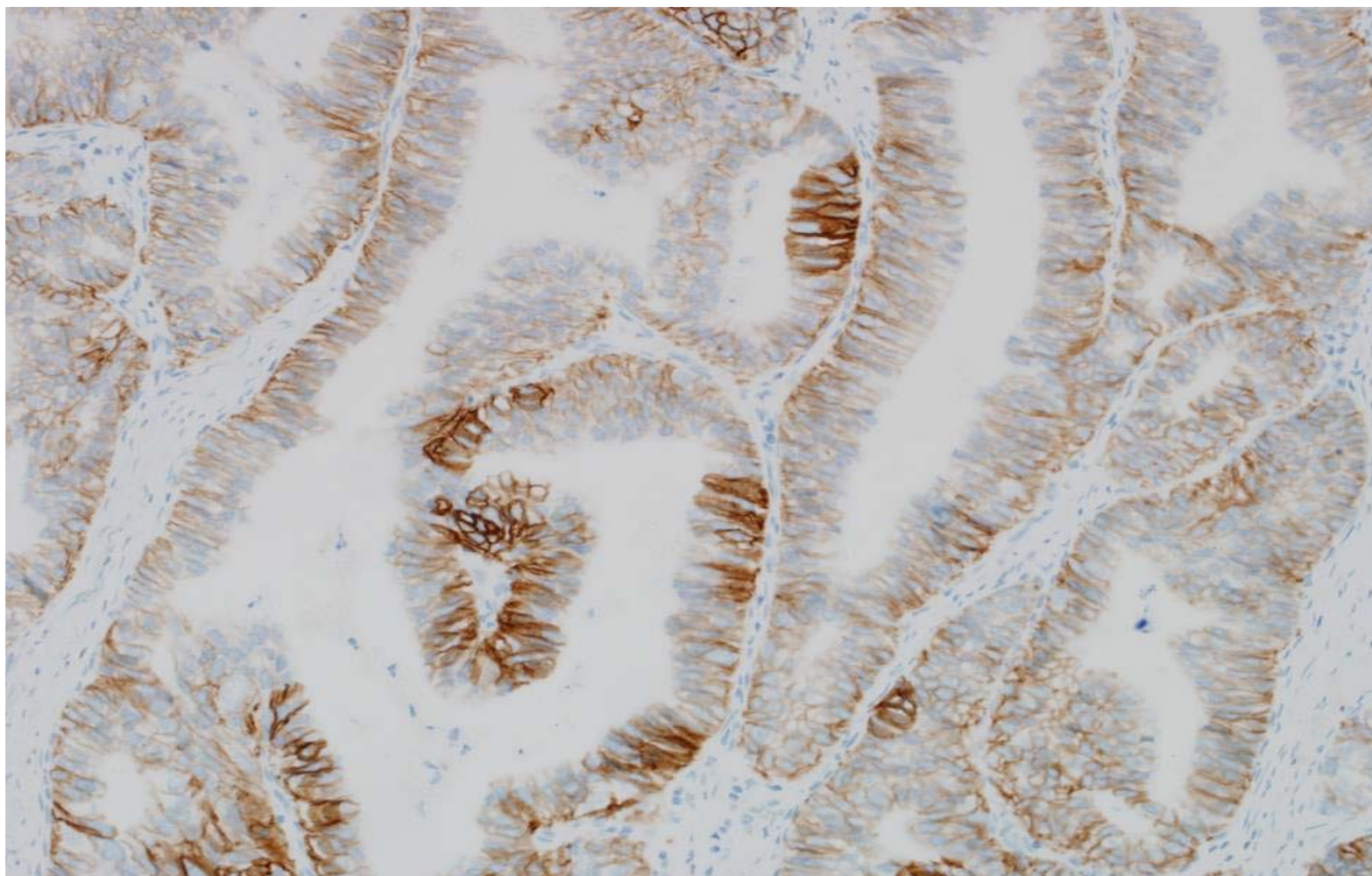


Positive mast cells - intestine

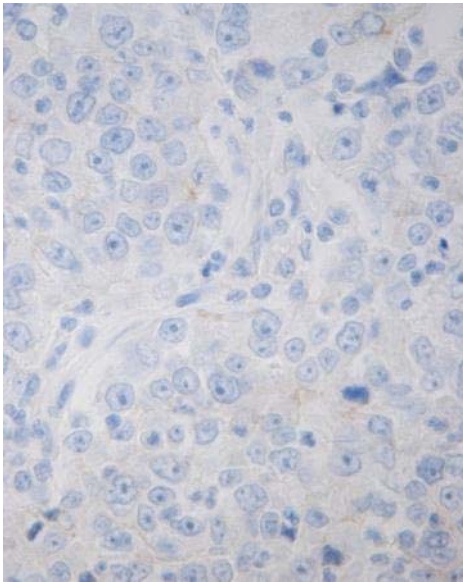


Positive ICC - intestine

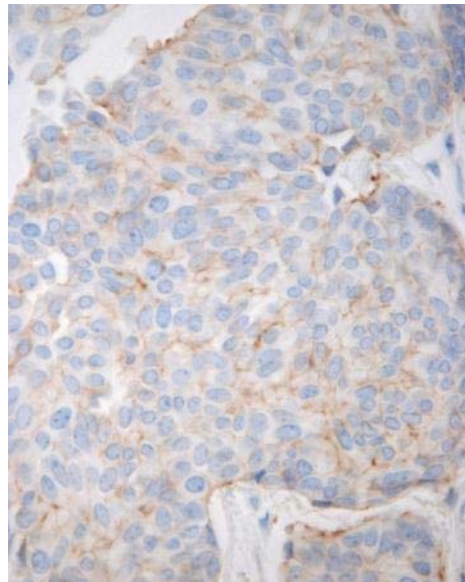
Heterogeneous HER2 Staining in Gastric Cancer



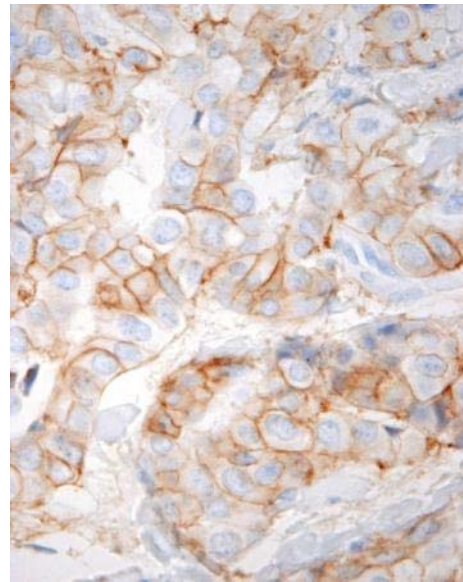
FDA Approved Scoring Algorithm for HER2 Protein Expression by Immunohistochemistry



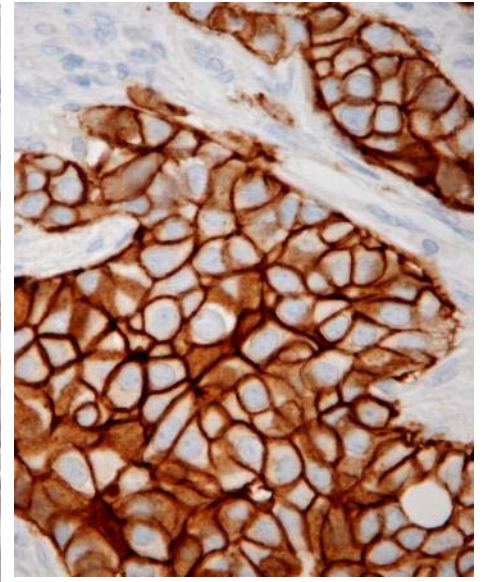
IHC 0



IHC 1+



IHC 2+



IHC 3+

- IHC is scored on a 0-3+ scale based on staining intensity and completeness of membrane staining



FDA Approved HER2 Descriptive Guidelines for Her2 Protein Expression

Staining Pattern	Score (report to treating physician)	HER2 Protein Overexpression Assessment (report to treating physician)
No staining is observed or membrane staining is observed in less than 10% of tumor cells	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of tumor cells. The cells are stained in only part of their membrane	1+	Negative
A weak to moderate complete membrane staining is observed in more than 10% of tumor cells	2+	Weakly positive
A strong complete membrane staining is observed in more than 10% of tumor cells	3+	Strongly positive

Scoring Systems

The scoring system which is employed is decided upon by mutual agreement between Diagnostics and Pharma

1. Positive / Negative
2. Percent positivity
3. Categorical staining
4. Percent positivity and a single categorical value
(combination of (2) and (3) above)
5. H Score*
6. Optical density scores / Positive pixel counts
(Image analysis)

H Score System

Staining intensity is measured on a semi-quantitative scale of 0 to 3+. The percentage of cells staining positively at each intensity for each cellular compartment (nucleus, cytoplasm, and membrane) level is recorded.

$$\begin{aligned} & (1 \times [\text{percentage of cells staining at an intensity of 1}]) \\ & + (2 \times [\text{percentage of cells staining at an intensity of 2}]) \\ & + (3 \times [\text{percentage of cells staining at an intensity of 3}]) \end{aligned}$$

H Score

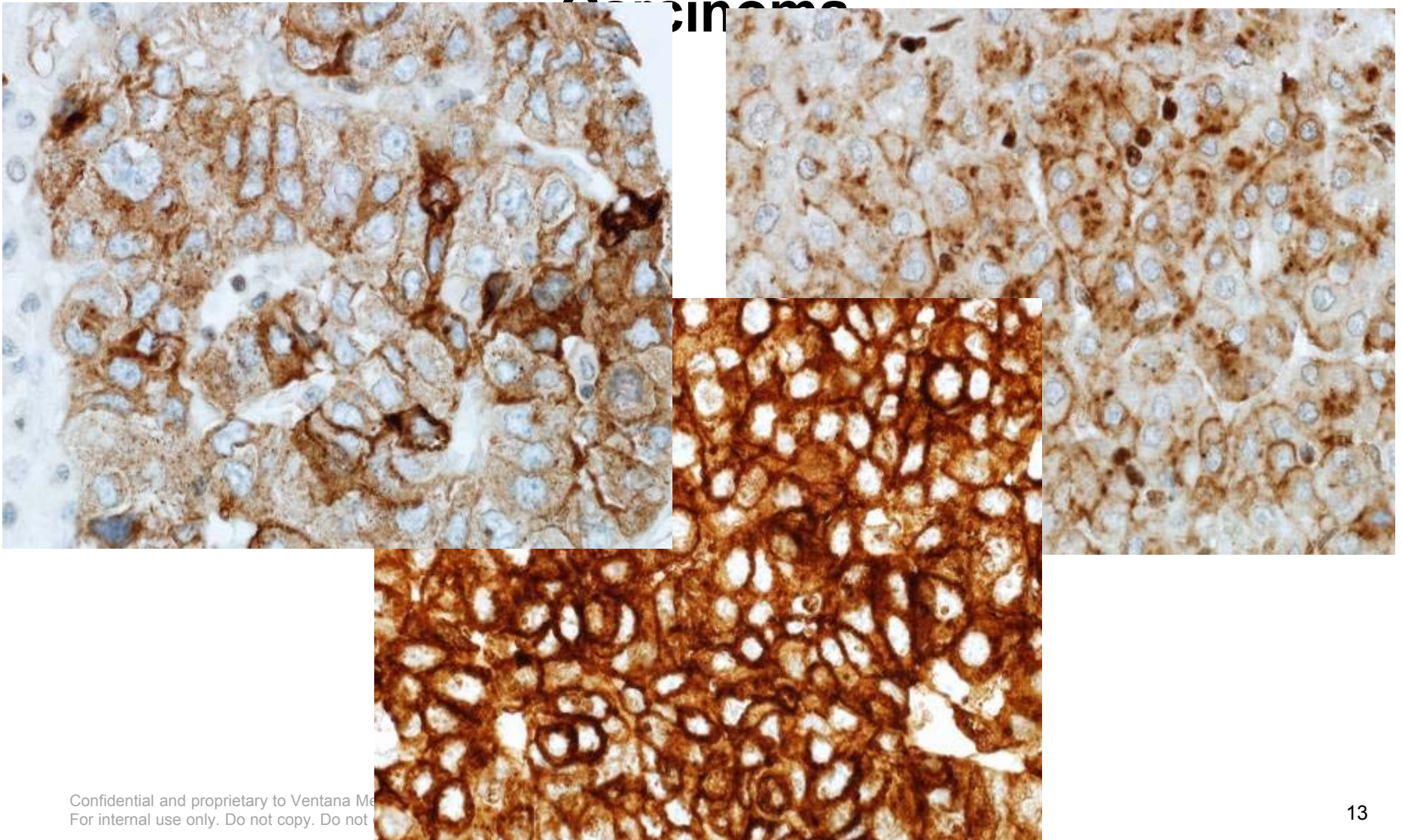
[McCarty KS Jr, et al.](#) *Cancer Res.* 1986 46(8 Suppl):4244s-4248s.

The Value of the H Score

Rather than having a single data set to compare to outcome, the data can be analyzed a number of ways leading to an increased probability of finding a correlation. The following data are readily accessible:

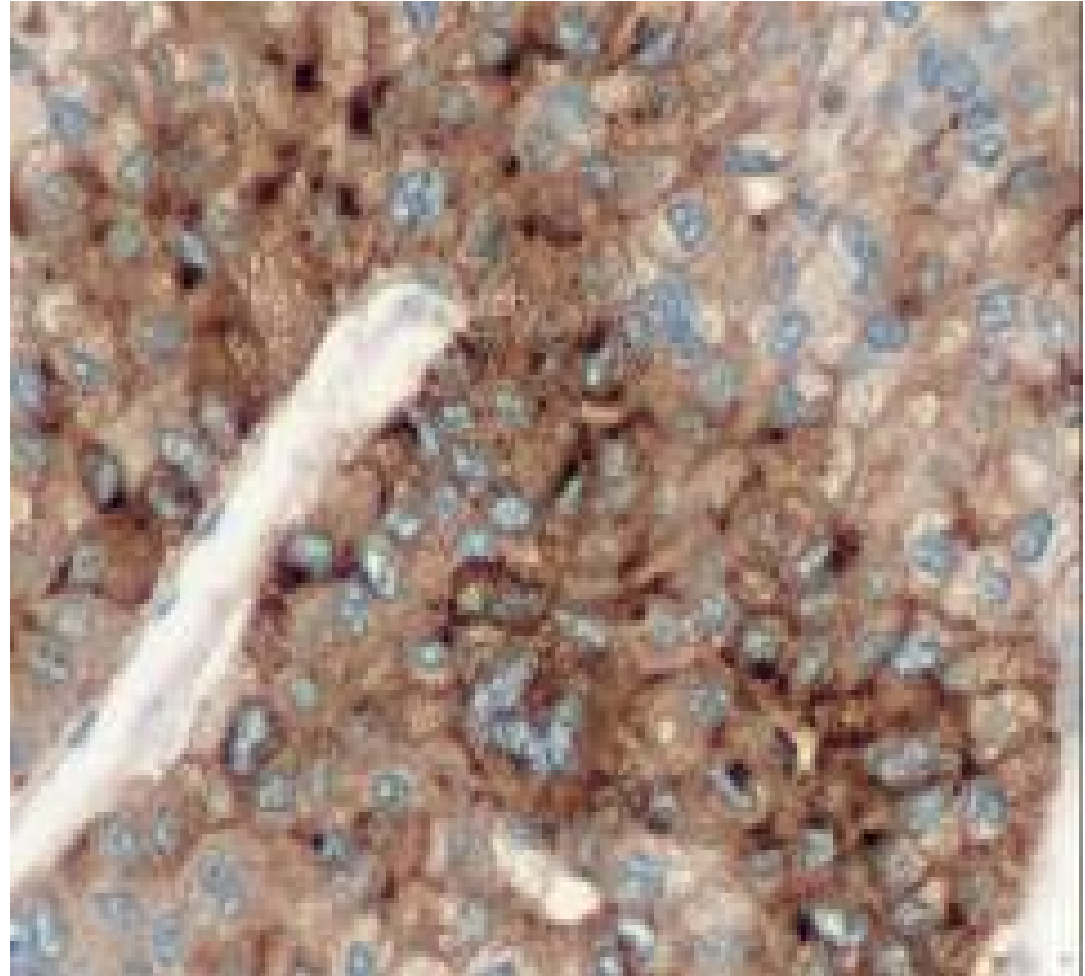
1. The H Score itself
2. Percent positivity (cut off between 0 and 1)
3. High expresser positivity (cut off between 1 and 2)
4. Majority subset
5. Highest intensity subset

Glypican-3 Staining in Hepatocellular Carcinoma



Glypican-3 H-Score in HCC

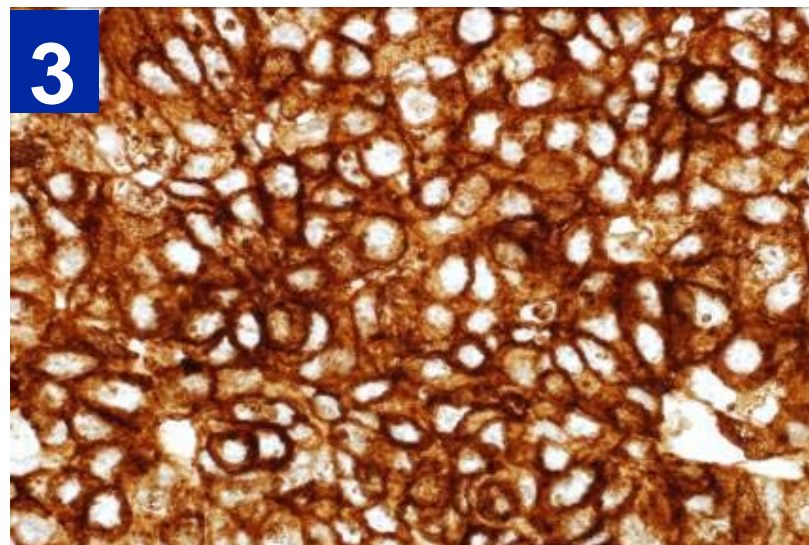
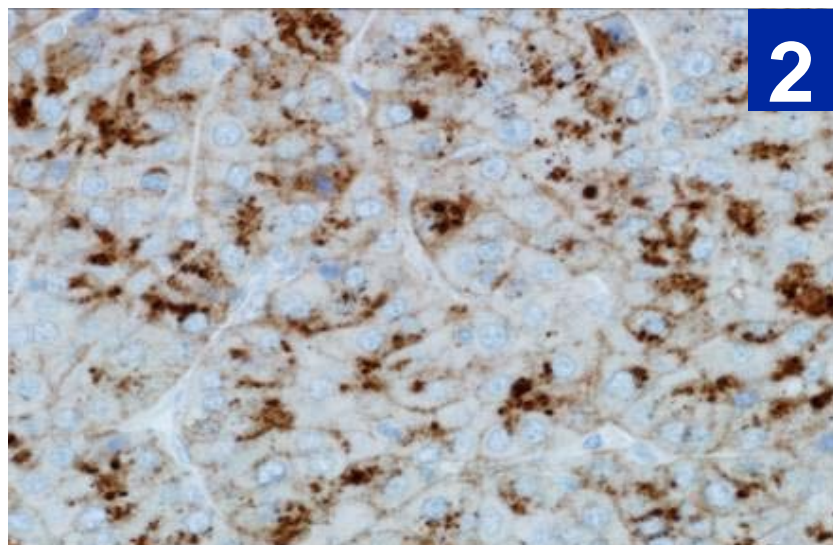
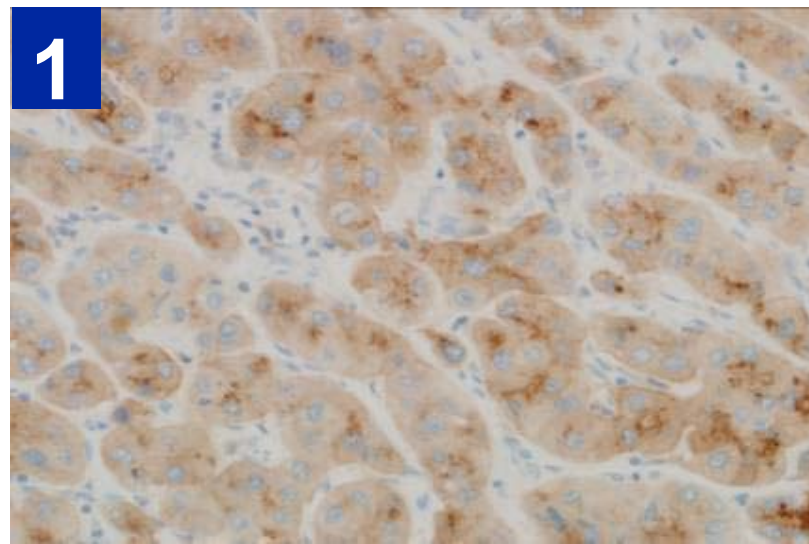
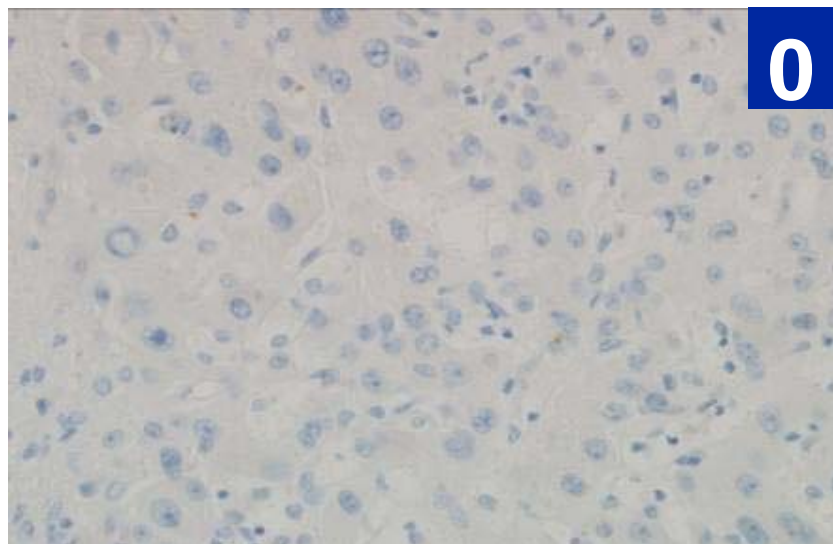
- **Cytoplasmic:**
 - 0, 15%
 - 1+, 45%
 - 2+, 40%
 - H-score = 125
- **Membrane:**
 - 0, 10%
 - 1+, 35%
 - 2+, 45%
 - 3+, 10%
 - H-score = 155



Glypican 3 Assay Staining Interpretation

Score	Description
0	<ul style="list-style-type: none"> Absent membrane staining. Cytoplasmic staining of any intensity in <10% of tumor cells
1	<ul style="list-style-type: none"> Membrane staining of any intensity in <10% of tumor cells <p>AND/OR</p> <ul style="list-style-type: none"> Cytoplasmic staining of any intensity >10% of tumor cells (note that strong cytoplasmic staining, if present, must be in <50% of tumor cells)
2	<ul style="list-style-type: none"> Presence of weak to moderate membrane staining in ≥10% of tumor cells (note that strong membrane staining, if present, must be in <10% of tumor cells) <p>WITH OR WITHOUT:</p> <ul style="list-style-type: none"> Cytoplasmic staining of any intensity >10% of tumor cells (note that strong cytoplasmic staining, if present, must be in <50% of tumor cells)
3	<ul style="list-style-type: none"> Strong membrane staining in >10% of tumor cells with or without cytoplasmic staining <p>OR</p> <ul style="list-style-type: none"> Strong cytoplasmic staining in >50% of tumor cells

Examples of GPC3 IHC Scores



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Positive/Negative System

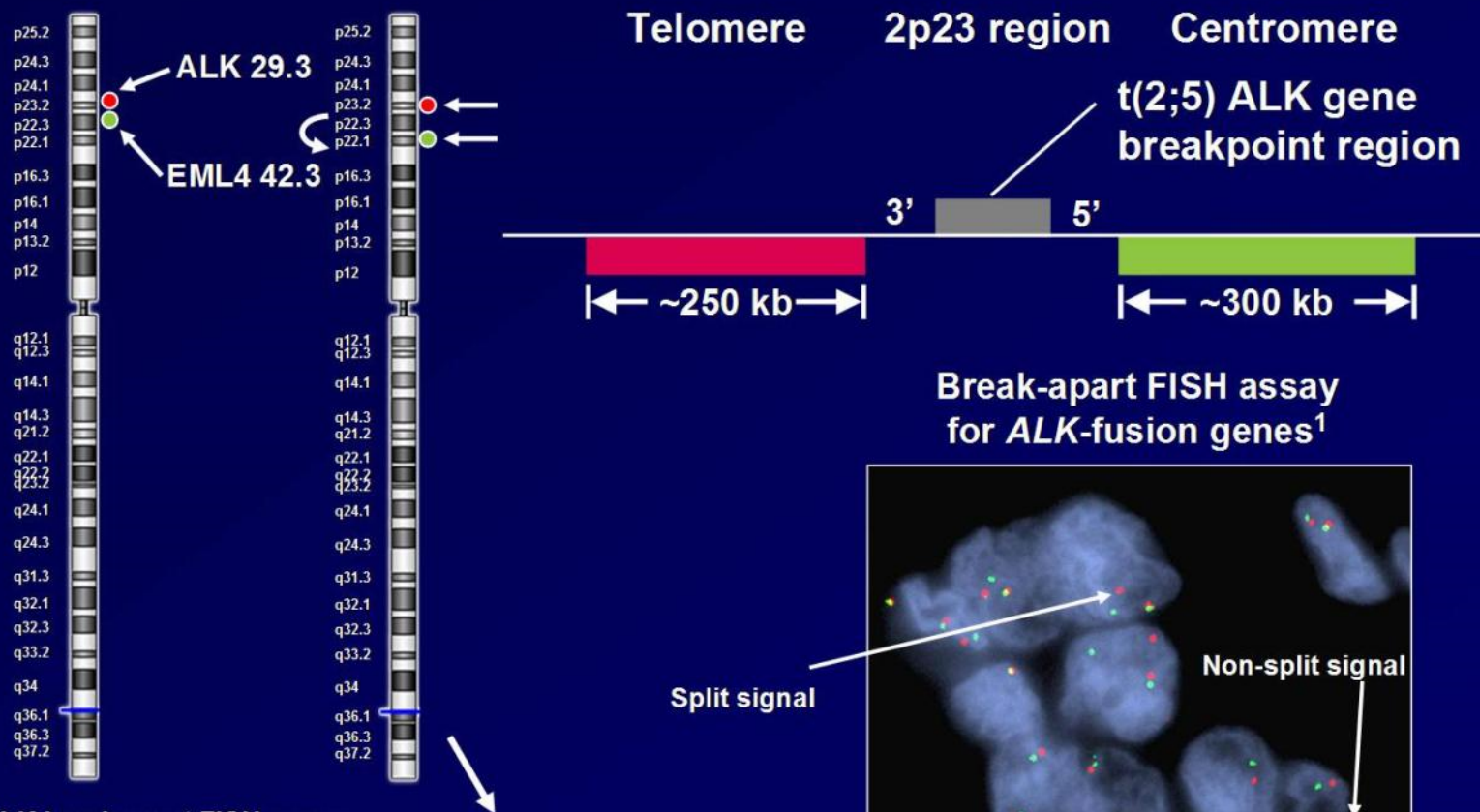
Positive:

Presence of staining of any intensity above background in tumor cells (any percentage of positive tumor cells).

Negative:

Absence of staining in tumor cells

FISH Assay for ALK Rearrangement*

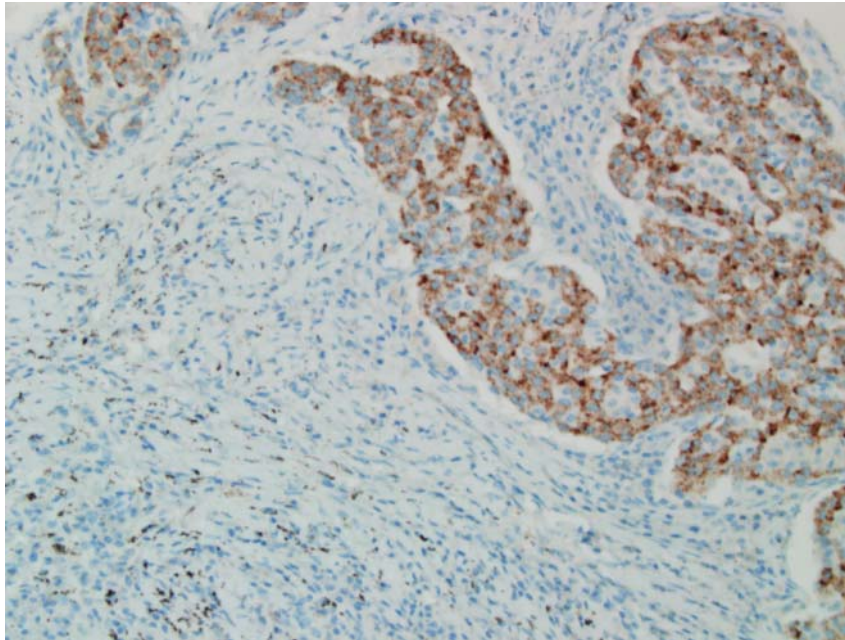


ALK break-apart FISH assay
[Courtesy John Iafrate, Massachusetts General Hospital]

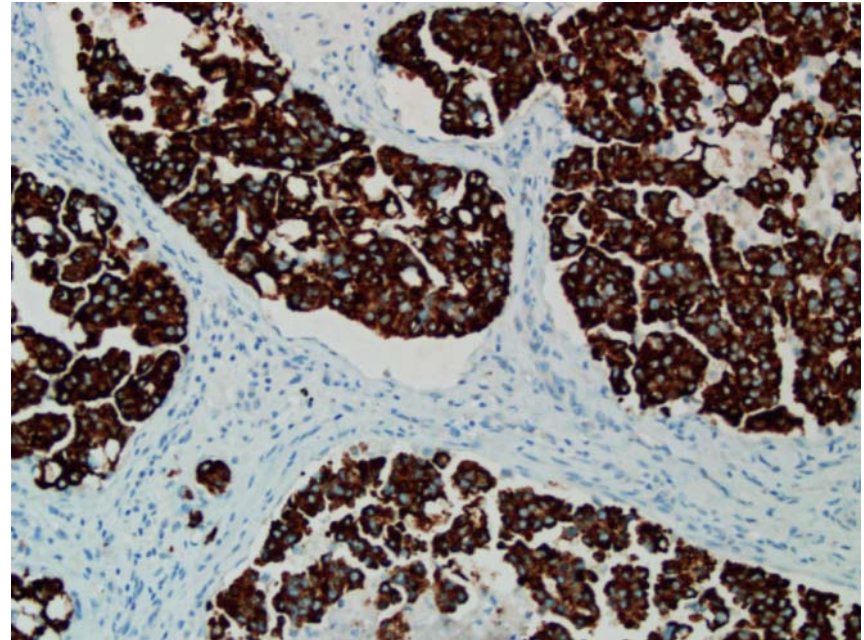
*Assay is positive if rearrangements can be detected in $\geq 15\%$ of cells
FISH = fluorescence in situ hybridization

¹Shaw AT et al. J Clin Oncol
2009;27:4247-4253

Binary Scoring Algorithm for Determining ALK Status (Positive or Negative)



Positive by FISH



Positive by FISH

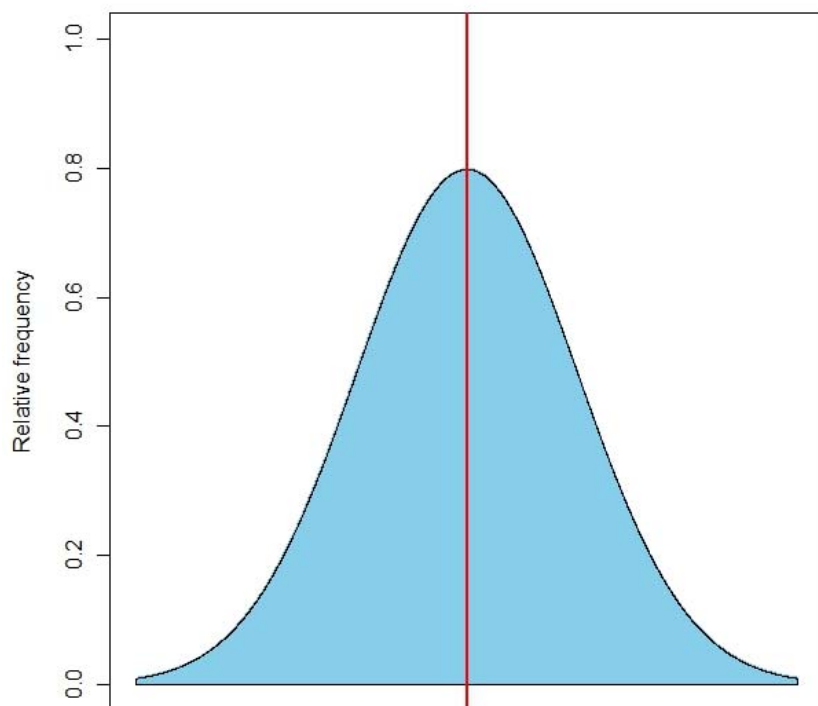
Intra-Reader & Inter-Reader Reproducibility

- **Intra-Reader**
 - Precision of the same pathologist reading the same cases (n=30-50, 3X, 2 wk wash-out period)
- **Inter-Reader**
 - Precision of 3-4 different pathologists reading the same cases (n~100)
 - Strongly influenced by distribution of cases around the cut-off for positive vs. negative



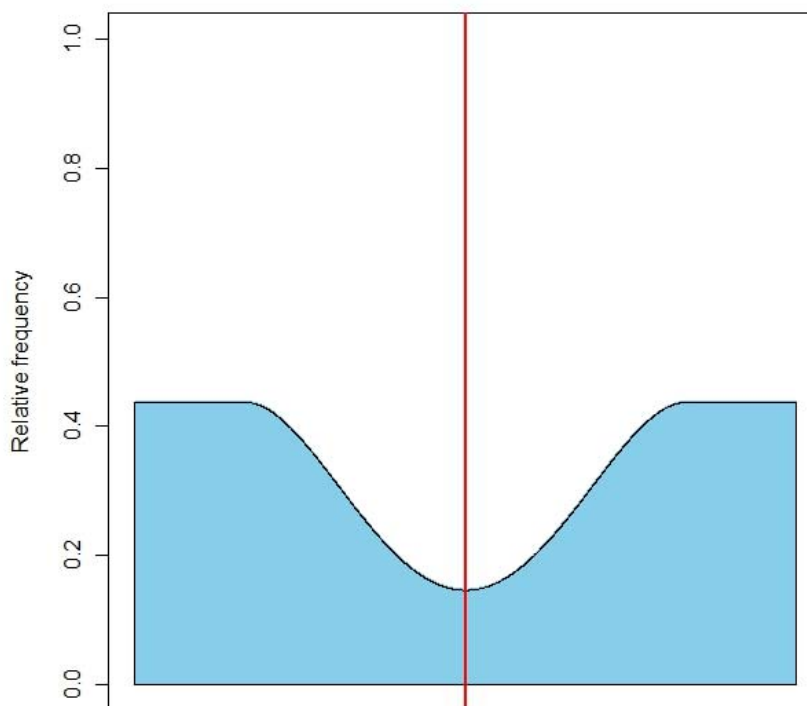
Distribution of Cases Around Cut-Off Point for Biomarker Positive vs Negative

Bell-shaped distribution



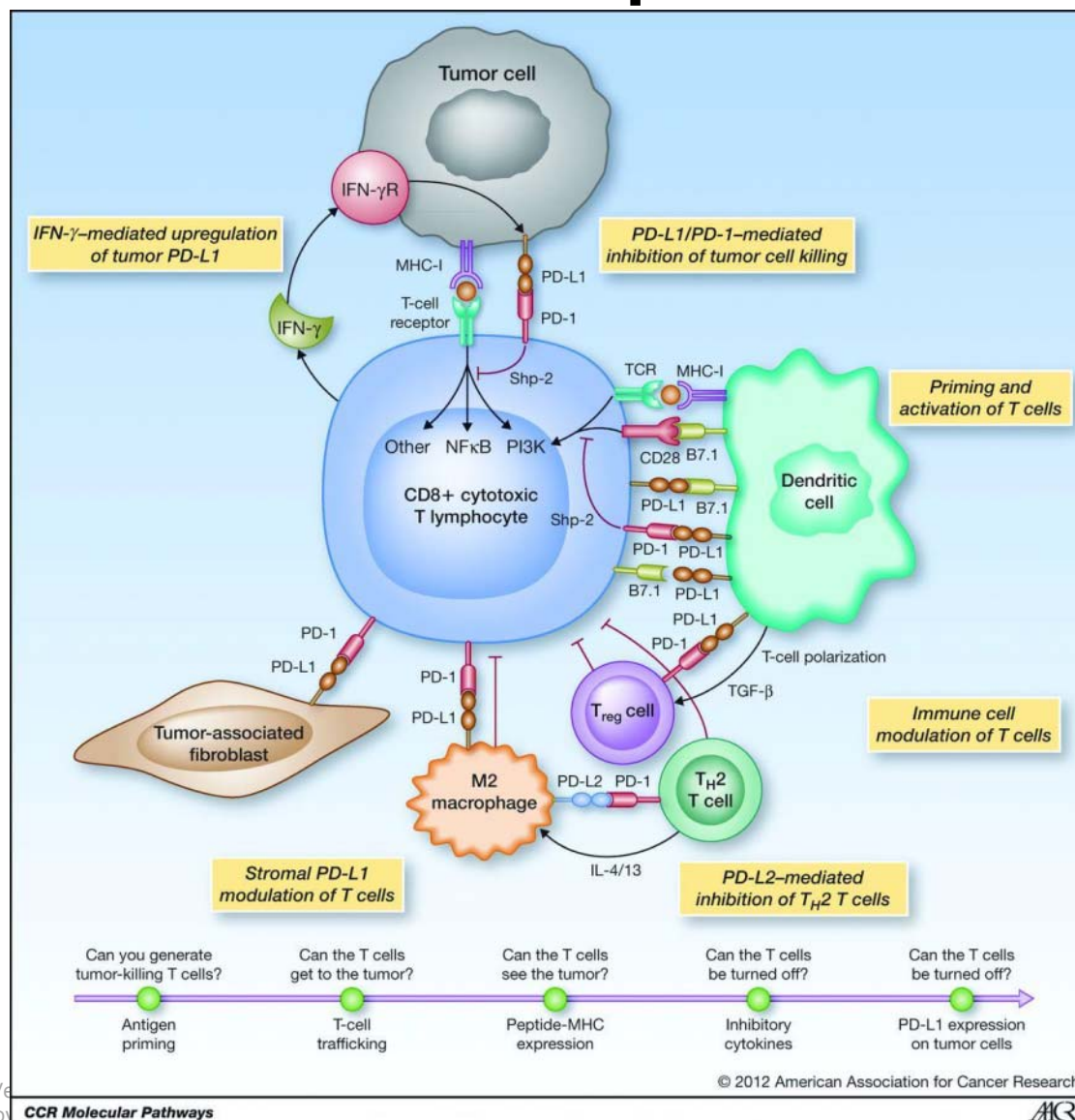
Staining scores

U-shaped distribution



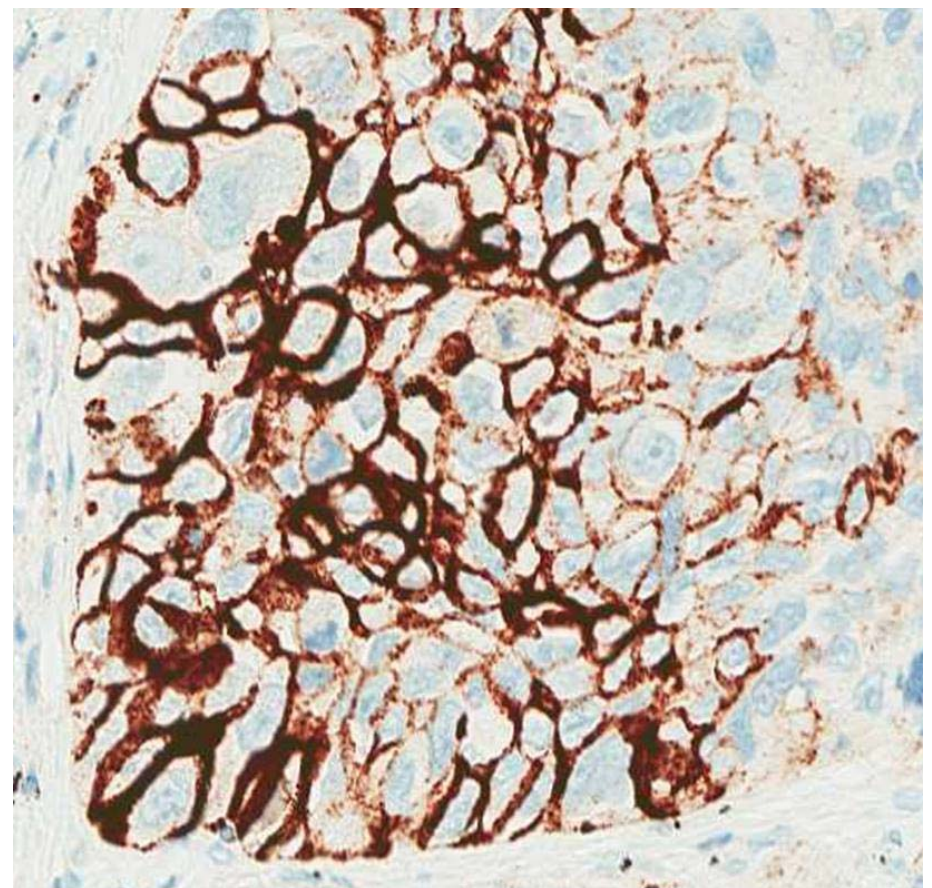
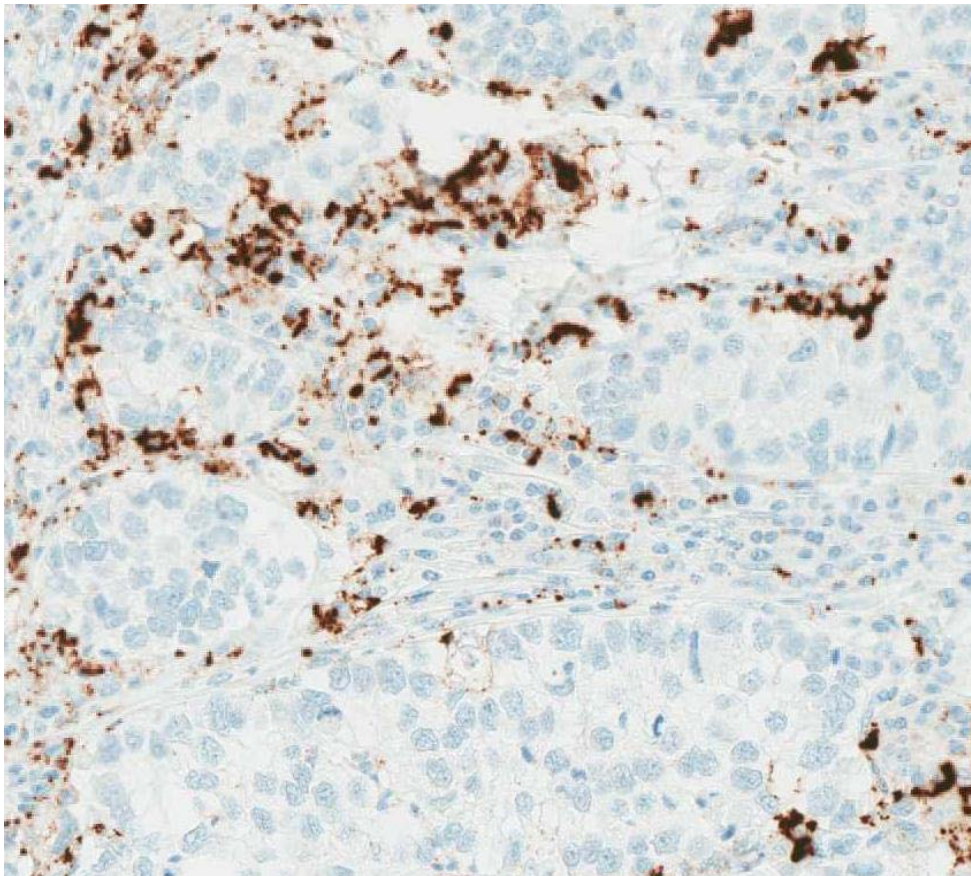
Staining scores

Immunotherapeutics



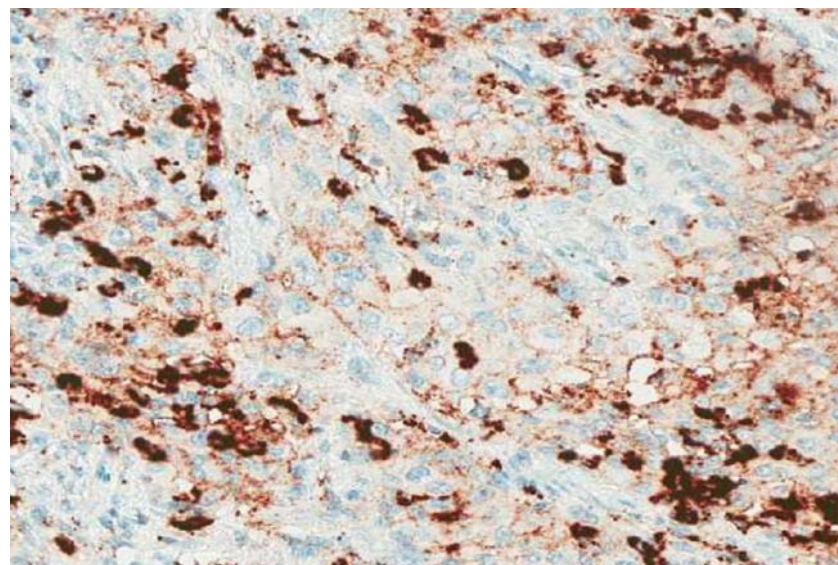
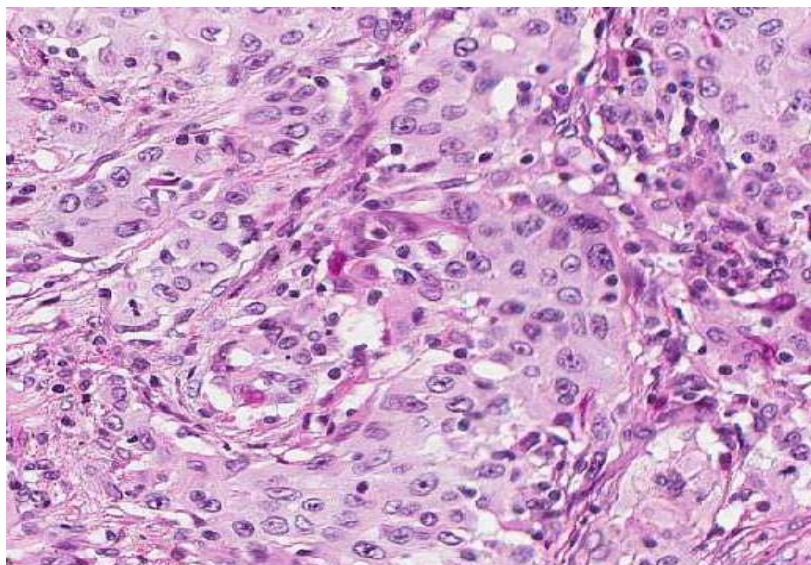
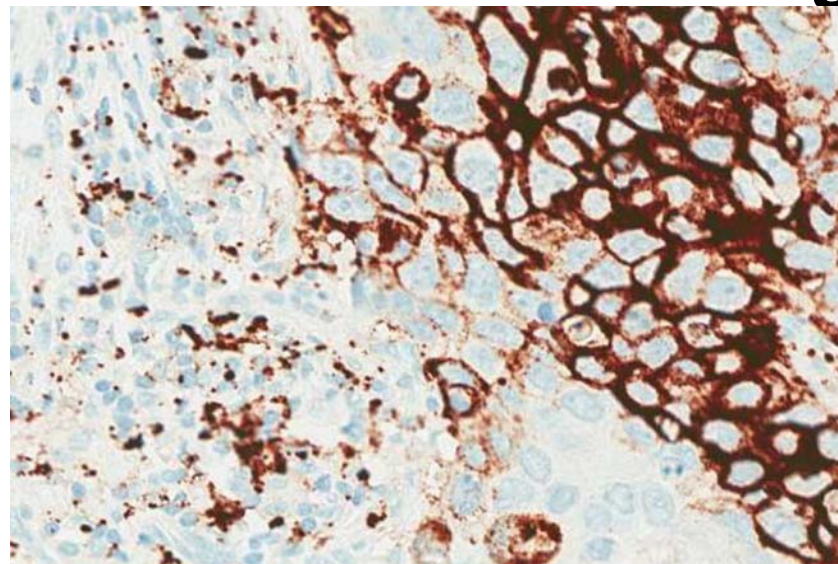
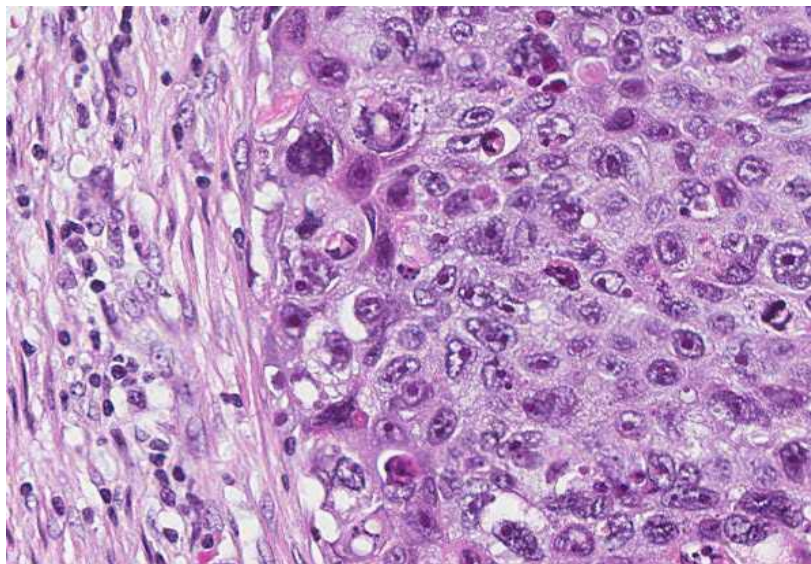
Tumor Cell versus Immune Cell Infiltrate

New Paradigm



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Combination of Tumor and Immune Cell Scoring



Doing now what patients need next