Practical Molecular Pathology of the GI Tract
Part 2

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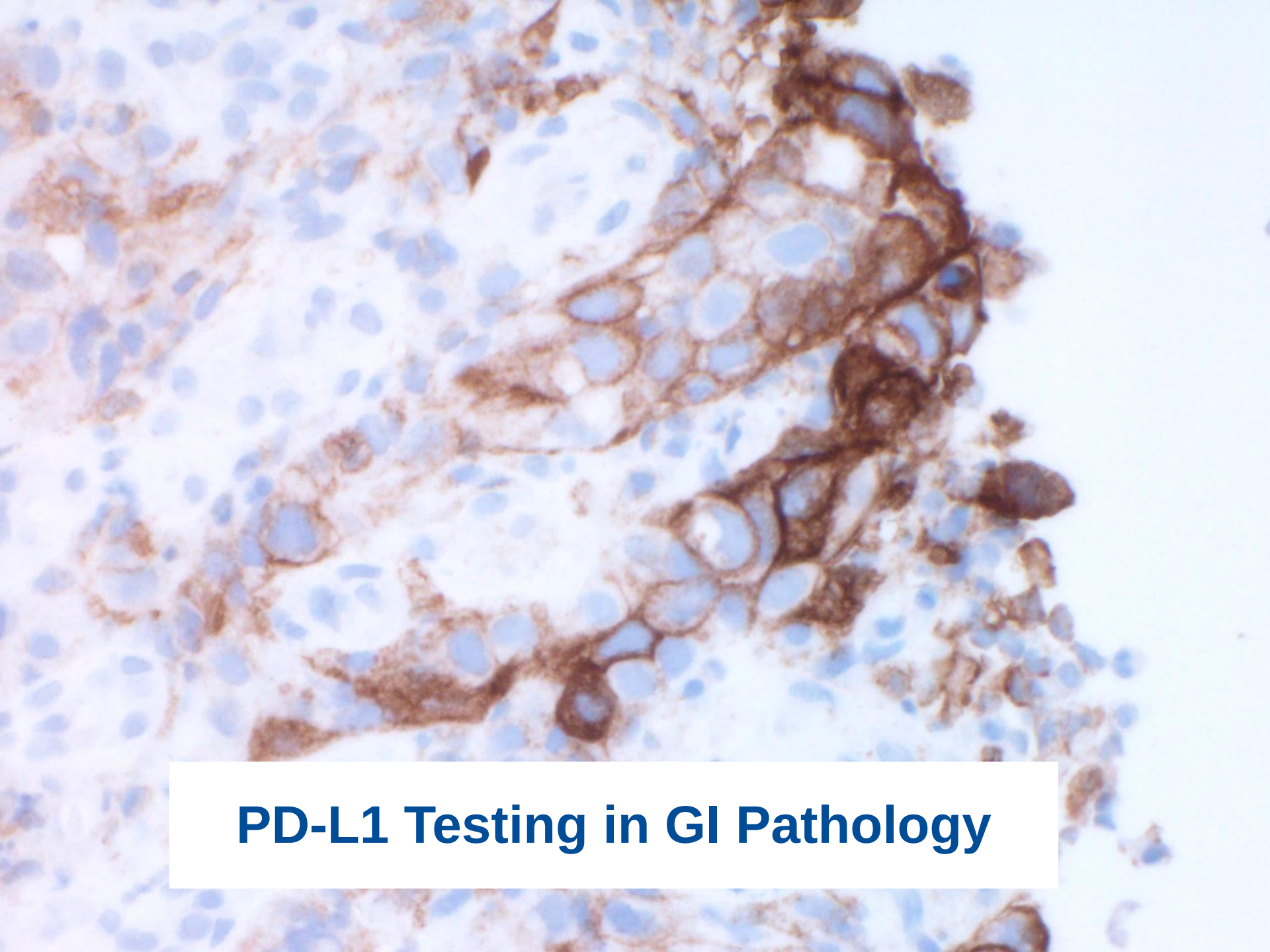
Disclosures

- Nothing to disclose

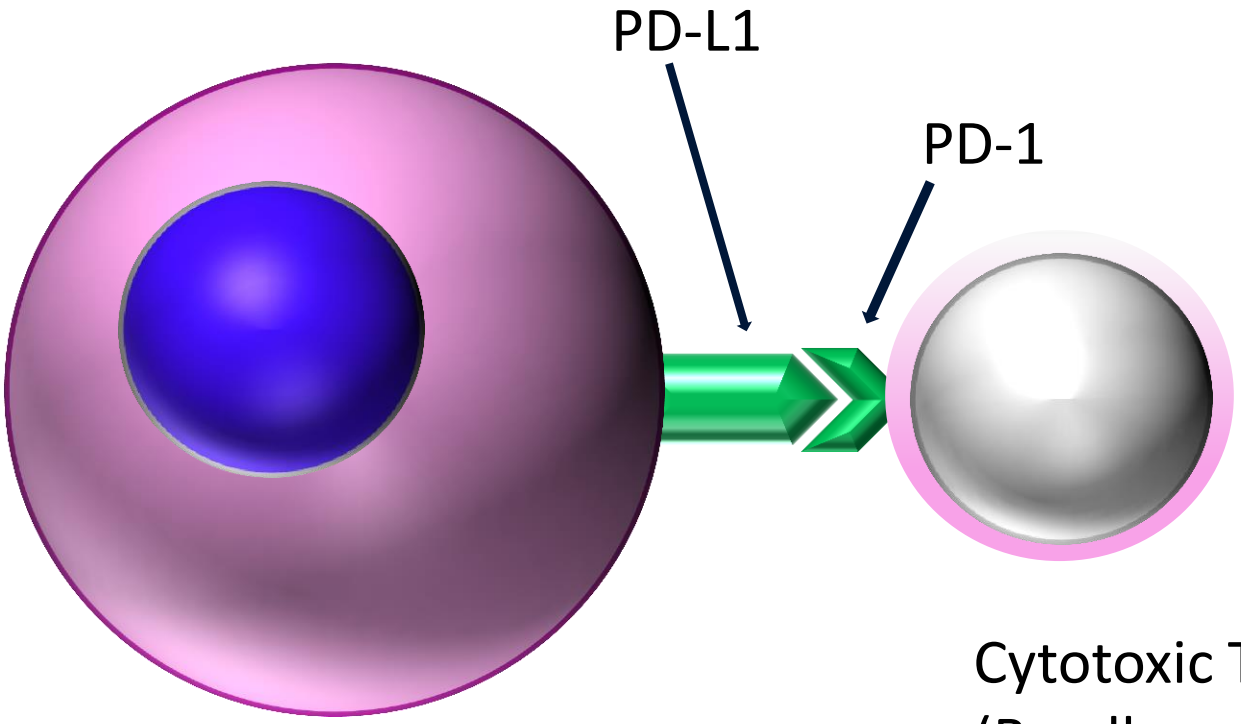
Overview

- PD-L1 testing in gastrointestinal pathology
  - Gastric and GEJ adenocarcinomas
- Molecular Pathology of Gastrointestinal Stromal Tumors (GISTs)
- Her2/Neu testing in gastrointestinal pathology
  - Gastric and GEJ adenocarcinomas

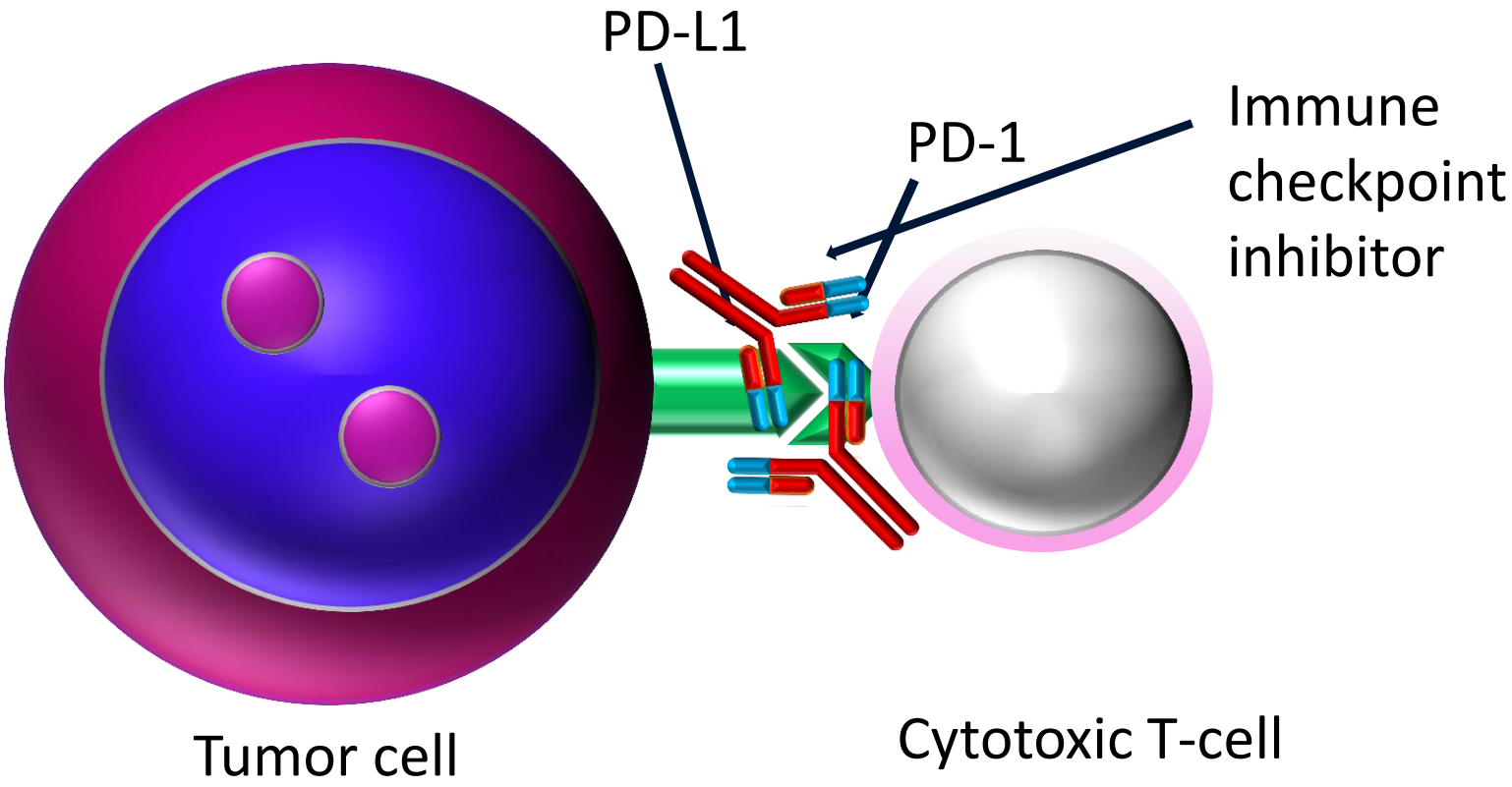


PD-L1 Testing in GI Pathology

PD-1/PD-L1 Interaction in Normal Immunomodulation



PD-1/PD-L1 Interaction in Cancer



Commercially Available PD-L1 IHC Clones

- FDA Approved **COMPANION** Diagnostic
  - **PD-L1 22C3** pharmDx, Dako, Agilent Technologies, Santa Clara, CA

- FDA Approved **COMPLEMENTARY** Diagnostic
  - **PD-L1 28-8** pharmDx, Dako, Agilent Technologies, Santa Clara, CA
  - **PD-L1 SP142**, Roche Ventana, Tucson, AZ
  - **PD-L1 SP263**, Roche Ventana, Tucson, AZ

- Non-FDA Approved
  - **PD-L1 E1L3N**, Cell Signaling Technology, Danvers, MA

Companion vs. Complementary

- **COMPANION**
  - SHOWN TO BE **PREDICTIVE** of patient response to specific immunotherapy for the specific type of tumor, on a specific platform
  - Testing is **REQUIRED** in order for specific immunotherapy to be prescribed

- **COMPLEMENTARY**
  - "**MAY BE PREDICTIVE**" of patient response to specific immunotherapy for the specific type of tumor, on a specific platform
  - Testing is **NOT REQUIRED** for specific immunotherapy to be prescribed

Indications for nivolumab (Opdivo®) Treatment Dako 28-8 pharmDx

Clone	28-8 rabbit anti-PD-L1 monoclonal antibody
Platform	• EnVision FLEX visualization system • Autostainer Link 48
Melanoma	FDA approved (COMPLEMENTARY) for treatment with nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY) • ≥1% tumor proportion score (TPS) • 28-8 IHC OPTIONAL
Non-squamous NSCLC 2nd line treatment	FDA approved (COMPLEMENTARY) for treatment with nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY) • ≥1% tumor proportion score (TPS) • 28-8 IHC OPTIONAL
Head and neck squamous cell carcinoma (HNSCC) 2nd line treatment	FDA approved (COMPLEMENTARY) for treatment with nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY) • ≥1% tumor proportion score (TPS) • 28-8 IHC OPTIONAL • September 2017

Indications for nivolumab (Opdivo®) Treatment Dako 28-8 pharmDx

Indication	Comment
Urothelial carcinoma	FDA approved (**COMPLEMENTARY**) for treatment with nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY) • ≥1% tumor proportion score (TPS) • 28-8 IHC OPTIONAL • September 2017
Classical Hodgkin lymphoma 2nd line treatment	FDA approved • NO IHC TESTING REQUIRED
Renal cell carcinoma (RCC) 2nd line treatment	FDA approved • NO IHC TESTING REQUIRED
dMMR/MSI colorectal carcinoma 2nd line treatment	• FDA approved • NO 28-8 IHC TESTING REQUIRED • MMR IHC or MSI TESTING REQUIRED

Dako 22C3 pharmDx

Clone	**22C3 mouse anti-PD-L1 monoclonal antibody**
Platform	• EnVision FLEX visualization system • Autostainer Link 48
NSCLC 1st line treatment	FDA approved (**COMPANION**) diagnostic for **high expression PD-L1 tumors**, for treatment with pembrolizumab (Keytruda®, Merck, Kenilworth, NJ)
NSCLC 2nd line treatment	FDA approved (**COMPANION**) diagnostic for **low expression PD-L1 tumors**, for treatment with pembrolizumab (Keytruda®, Merck, Kenilworth, NJ)
Gastric/GEJ carcinoma 3rd line treatment	FDA approved (**COMPANION**) September 2017 for tumors expressing PD-L1, for treatment with pembrolizumab (Keytruda®, Merck, Kenilworth, NJ)

Indications for pembrolizumab (Keytruda®) treatment

Indication	Comment
NSCLC 1st line treatment	FDA approved with PD-L1 22C3 • ≥50% tumor proportion score (TPS)
NSCLC 2nd line treatment	FDA approved with PD-L1 22C3 • ≥1% tumor proportion score (TPS)
NSCLC 1st treatment, in combination with chemotherapy	FDA approved • NO 22C3 IHC TESTING REQUIRED
Head and neck squamous cell carcinoma (HNSCC) 2nd line treatment	FDA approved • NO 22C3 IHC TESTING REQUIRED
Melanoma 2nd line treatment	FDA approved • NO 22C3 IHC TESTING REQUIRED
Classical Hodgkin lymphoma 2nd line treatment	FDA approved • NO 22C3 IHC TESTING REQUIRED
Colorectal and other solid dMMR/MSI tumors 2nd line treatment	FDA approved • NO 22C3 IHC TESTING REQUIRED • dMMR IHC or MSI TESTING REQUIRED

Indications for pembrolizumab (Keytruda®) treatment- September 2017 UPDATE

Indication	Comment
Gastric/GEJ carcinoma 3rd line treatment	FDA approved with 22C3: September 2017 • ≥1% Combined positive score (CPS)

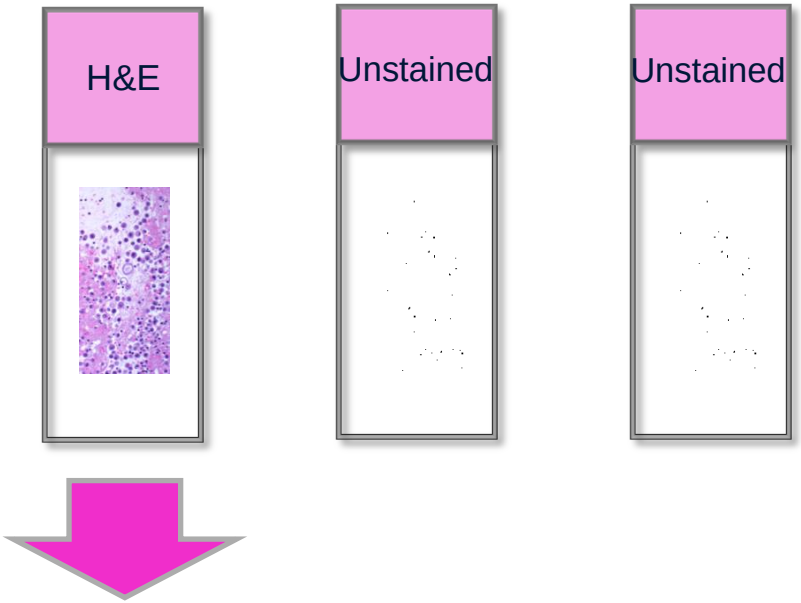
KEYNOTE-059 Study (NTC02335411)

- 257 patients
  - 148 (58%) showed PD-L1 22C3 expression (CPS ≥1)
- Among 143 patients with PD-L1 expression, 13.3% ORR
  - 1.4% complete response, 11.9% partial response
- Among the 19 responders, duration of response ranged from 2.8+ to 19.4+ months
  - 11 patients with 6+ month response
  - 5 patients with 12+ month response

Accepting Specimens for PD-L1 IHC Testing

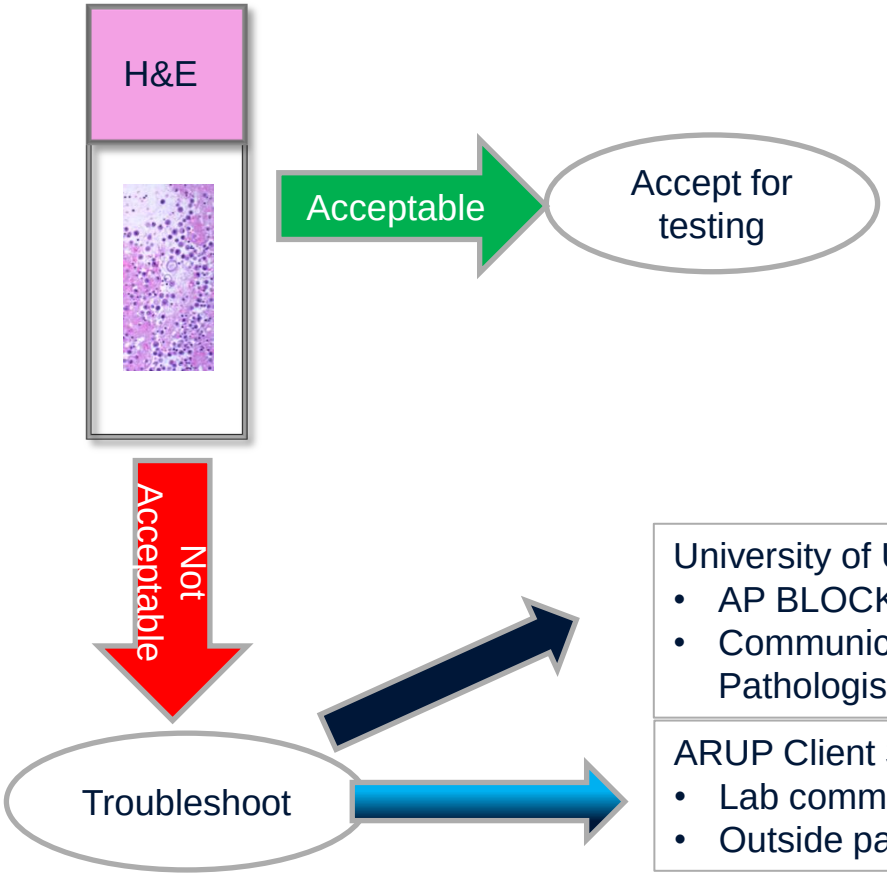
OR

Initial Processing of Specimens



Adequacy assessment: ≥100 viable tumor cells

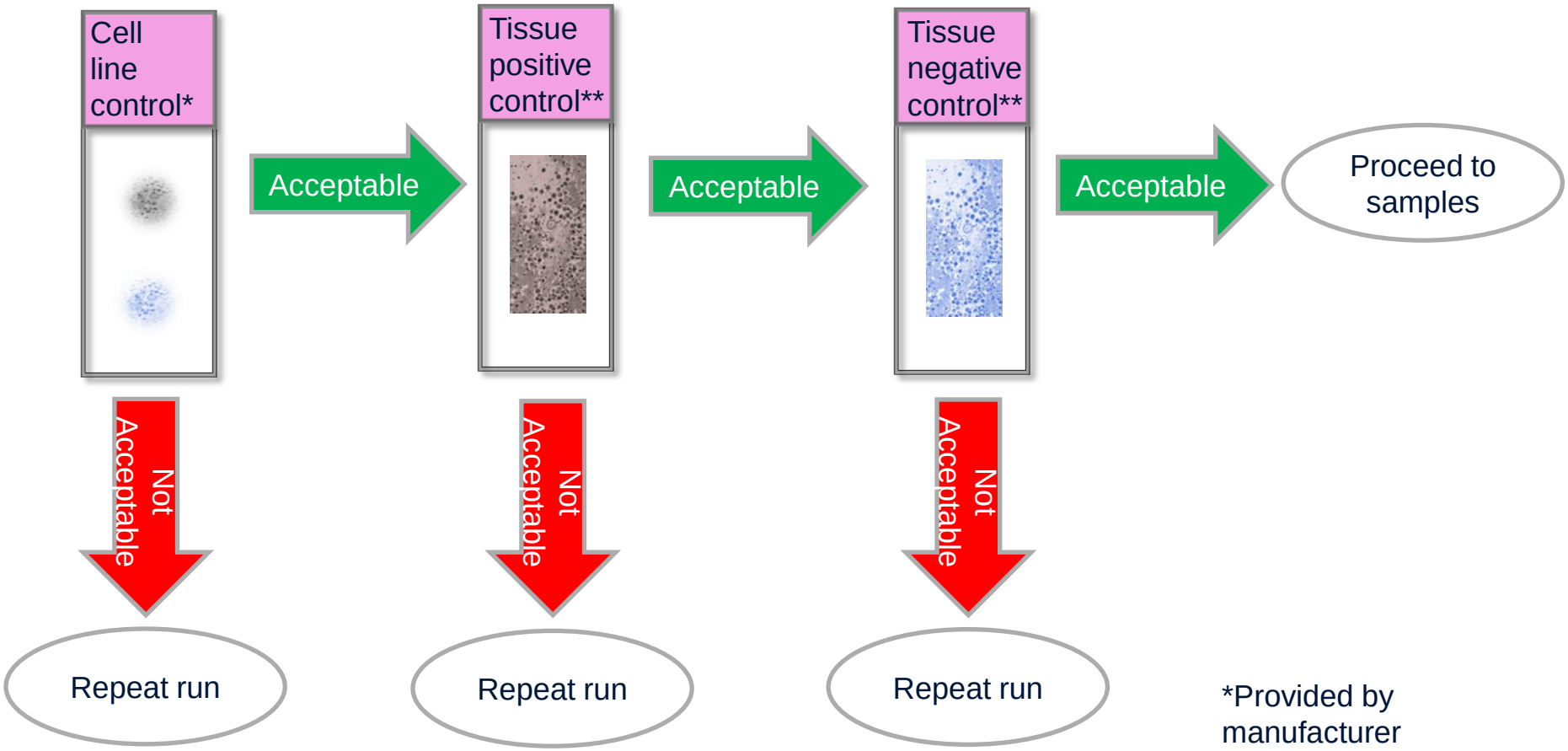
Initial Processing of Specimens



University of Utah/ARUP Specimens:
- AP BLOCK SELECT
- Communication with Surgical Pathologist/Oncologist

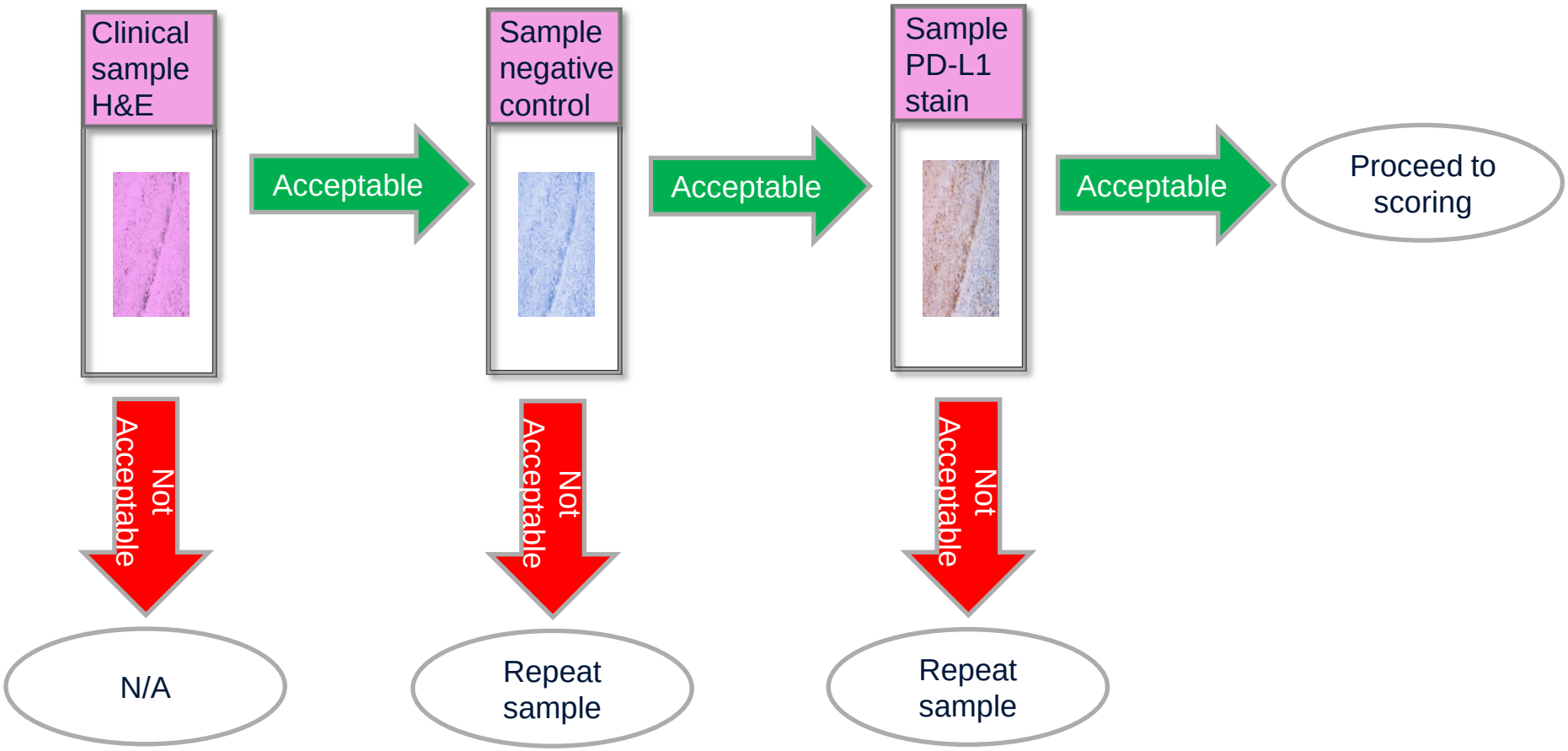
ARUP Client Specimens:
- Lab communication with ordering client
- Outside pathologist and/or oncologist

PD-L1 Run Quality Control – Controls



*Provided by manufacturer
**In-house tissue control

PD-L1 Run Quality Control - Samples



Scoring PD-L1 in NSCLC: Tumor Proportion Score (TPS)

$$TPS = \frac{\#\ of\ PD{-}L1\ positive\ tumor\ cells}{Total\ \#\ of\ PD{-}L1\ positive\ and\ PD{-}L1\ negative\ tumor\ cells} \times 100$$

What to score?

- Score partial or complete cell membrane staining.
  - Exclude cytoplasmic staining from scoring.
- Score only viable tumor cells
  - Exclude infiltrating immune cells, normal cells, necrotic cells, debris.
- Staining intensity not important.

Scoring PD-L1 in GEA: Combined Proportion Score (CPS)

$$CPS = \frac{\#\ of\ PD{-}L1\ positive\ cells*}{Total\ \#\ of\ PD{-}L1\ positive\ and\ PD{-}L1\ negative\ tumor\ cells} \text{ x } 100$$

*: tumor cells, tumor associated lymphocytes/macrophages

NOT a percentage, but a SCORE

Scoring PD-L1: Combined Proportion Score (CPS)

Element	Included in Scoring	Excluded from Scoring
Tumor cells	Convincing partial or complete linear membrane staining (at any intensity) of viable tumor cells	Tumor cells with only cytoplasmic staining
Immune cells	Membrane and/or cytoplasmic staining (at any intensity of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma* • Lymphocytes (including lymphocyte aggregates) • Macrophages Only MICs directly associated with response to tumor	• MICs associated with adenoma, dysplasia, CIS • MICs associated with ulcers, chronic gastritis and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils, eosinophils and plasma cells
Other	Not included	• Normal cells (including ganglion cells) • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris
Morphology patterns	Invasive adenocarcinoma (including diffuse adenocarcinoma)	• Adenoma, dysplasia and CIS • Gastric ulcers/chronic gastritis

*Adjacent MICs are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response to the tumor should be excluded.
Adapted from: SK00621-5 PD-L1 IHC 22C3 pharmDx interpretation manual (Dako)

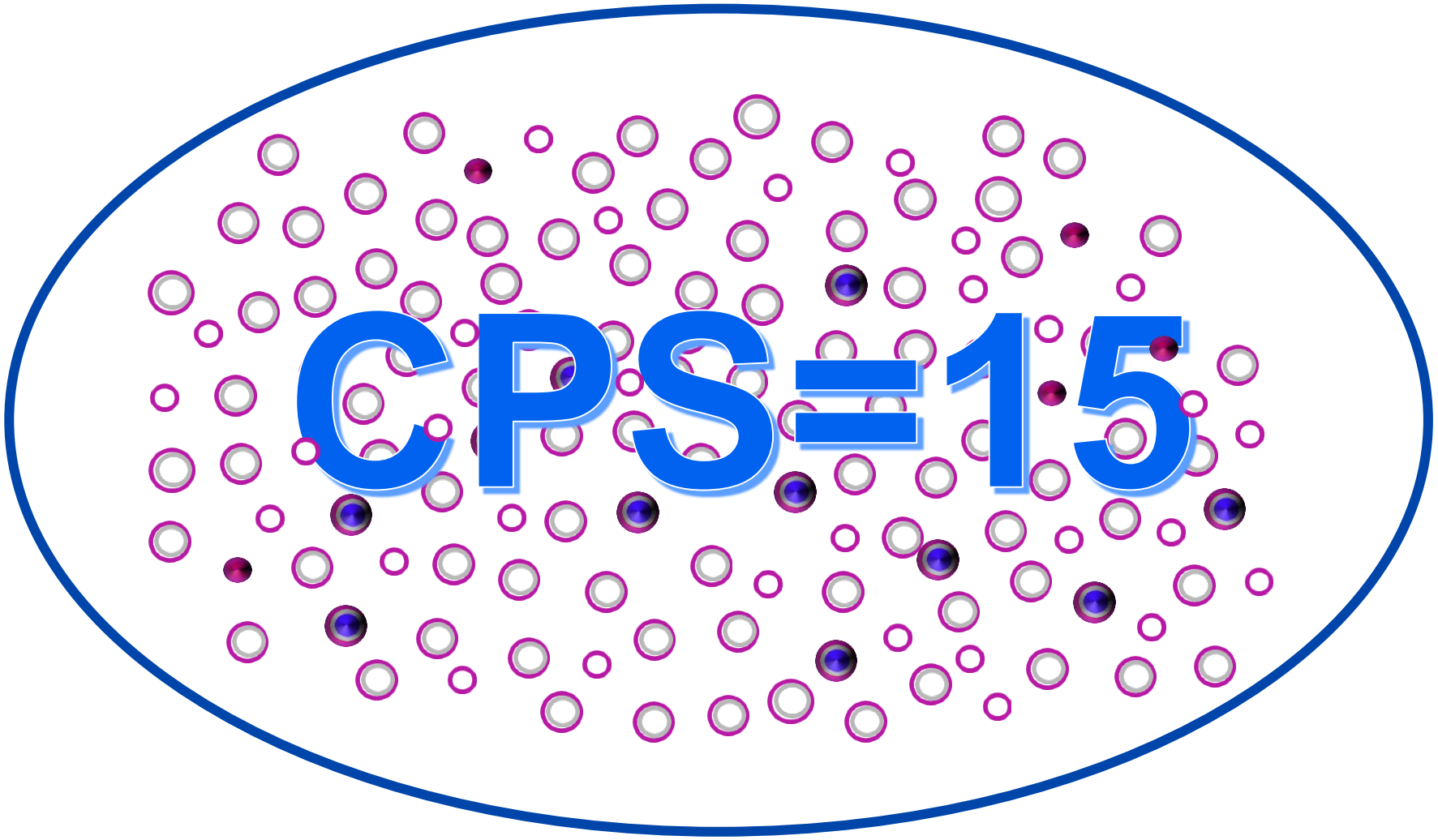
PD-L1 CPS Explained

: PD-L1 positive tumor cells

: PD-L1 negative tumor cells

: PD-L1 positive MICs

: PD-L1 negative MICs



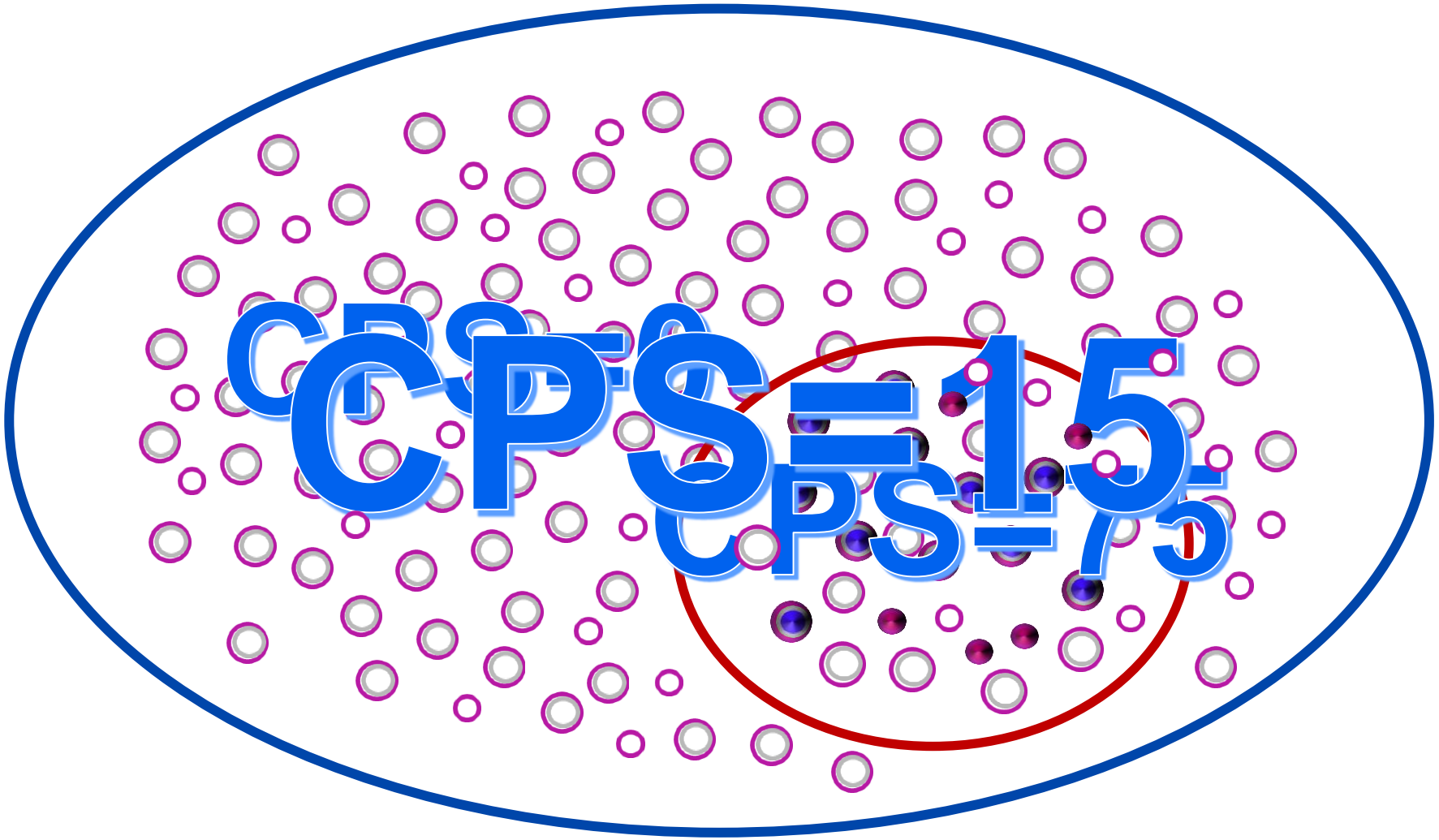
PD-L1 CPS Explained

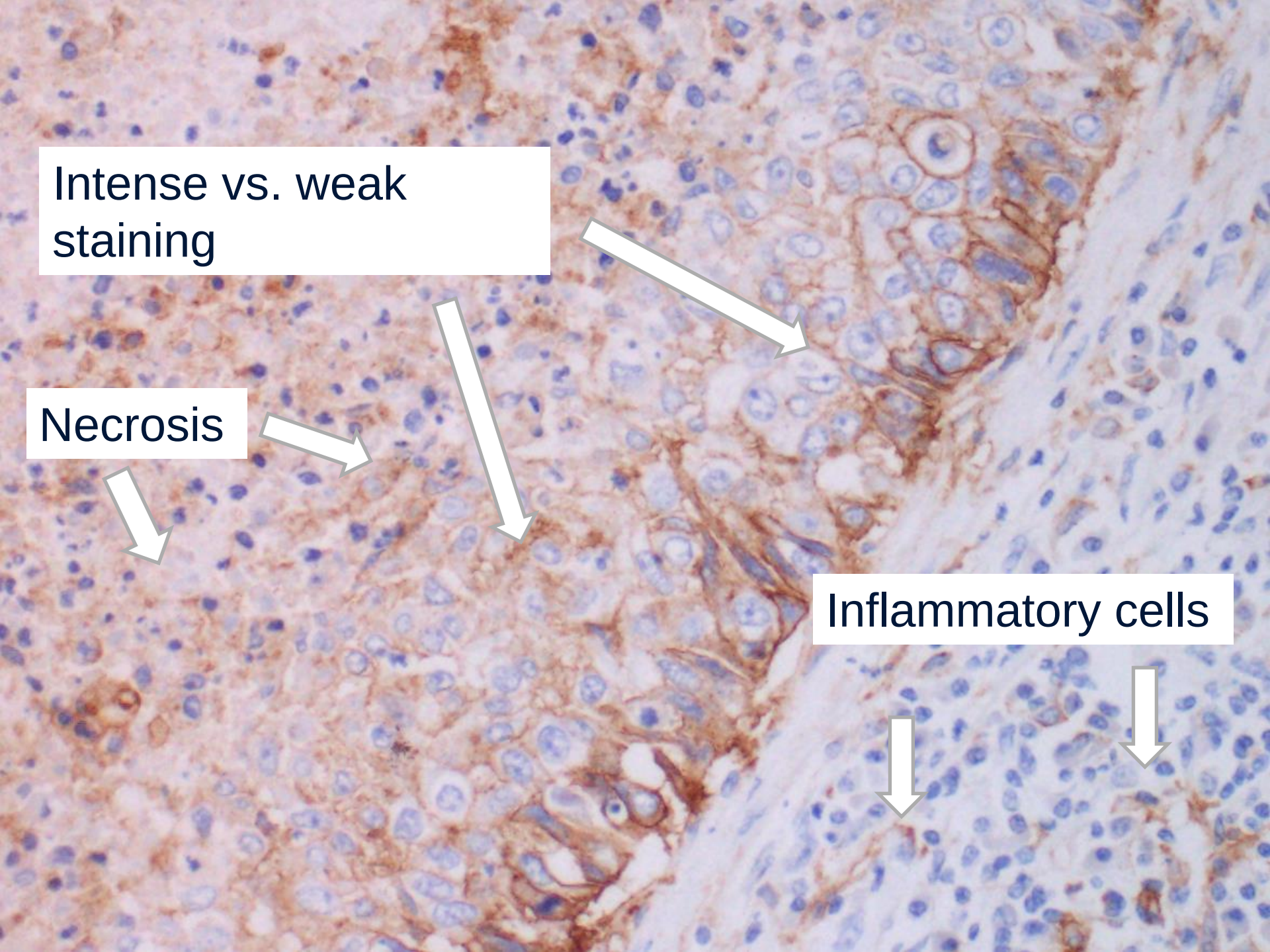
: PD-L1 positive tumor cells

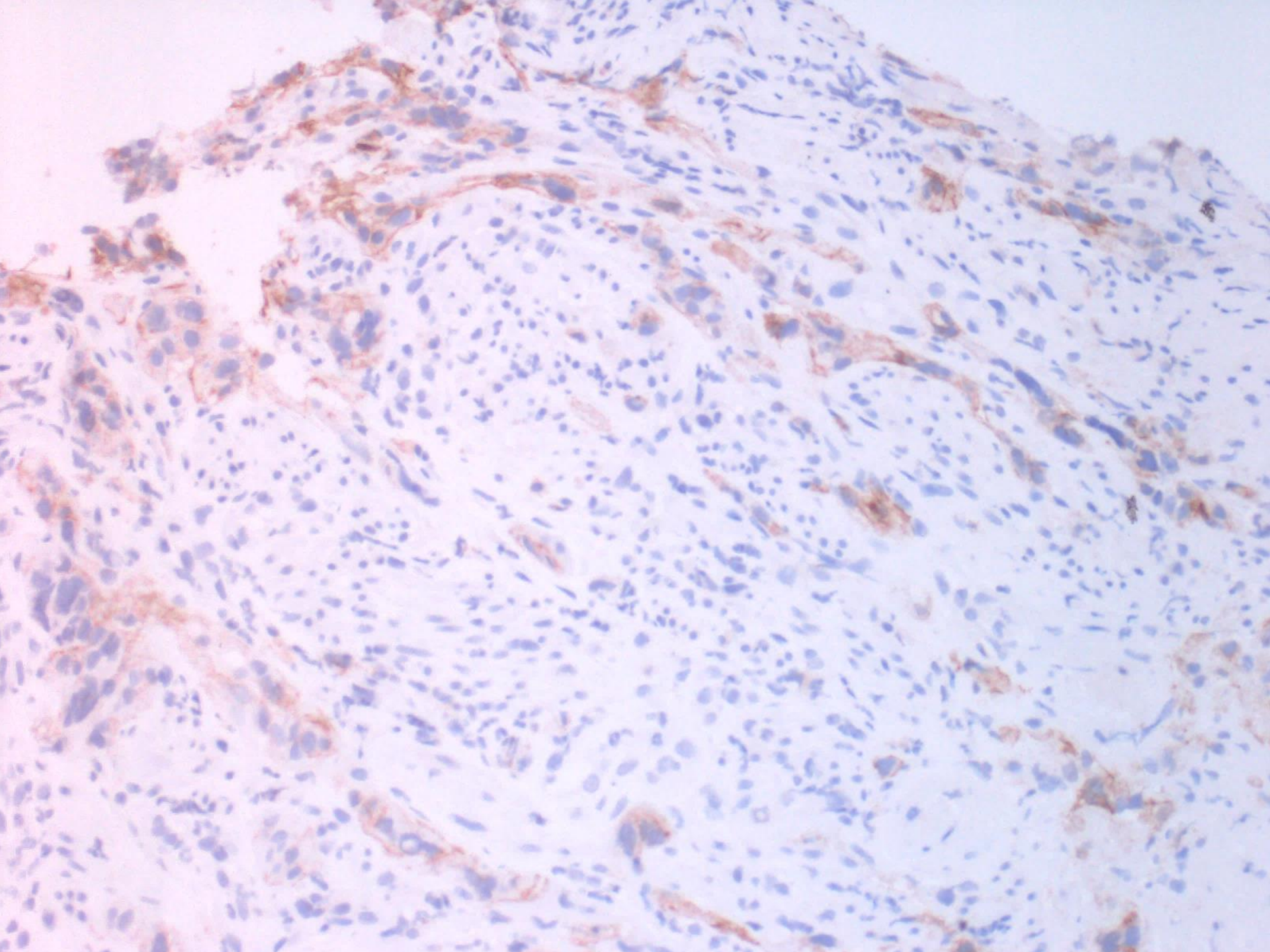
: PD-L1 negative tumor cells

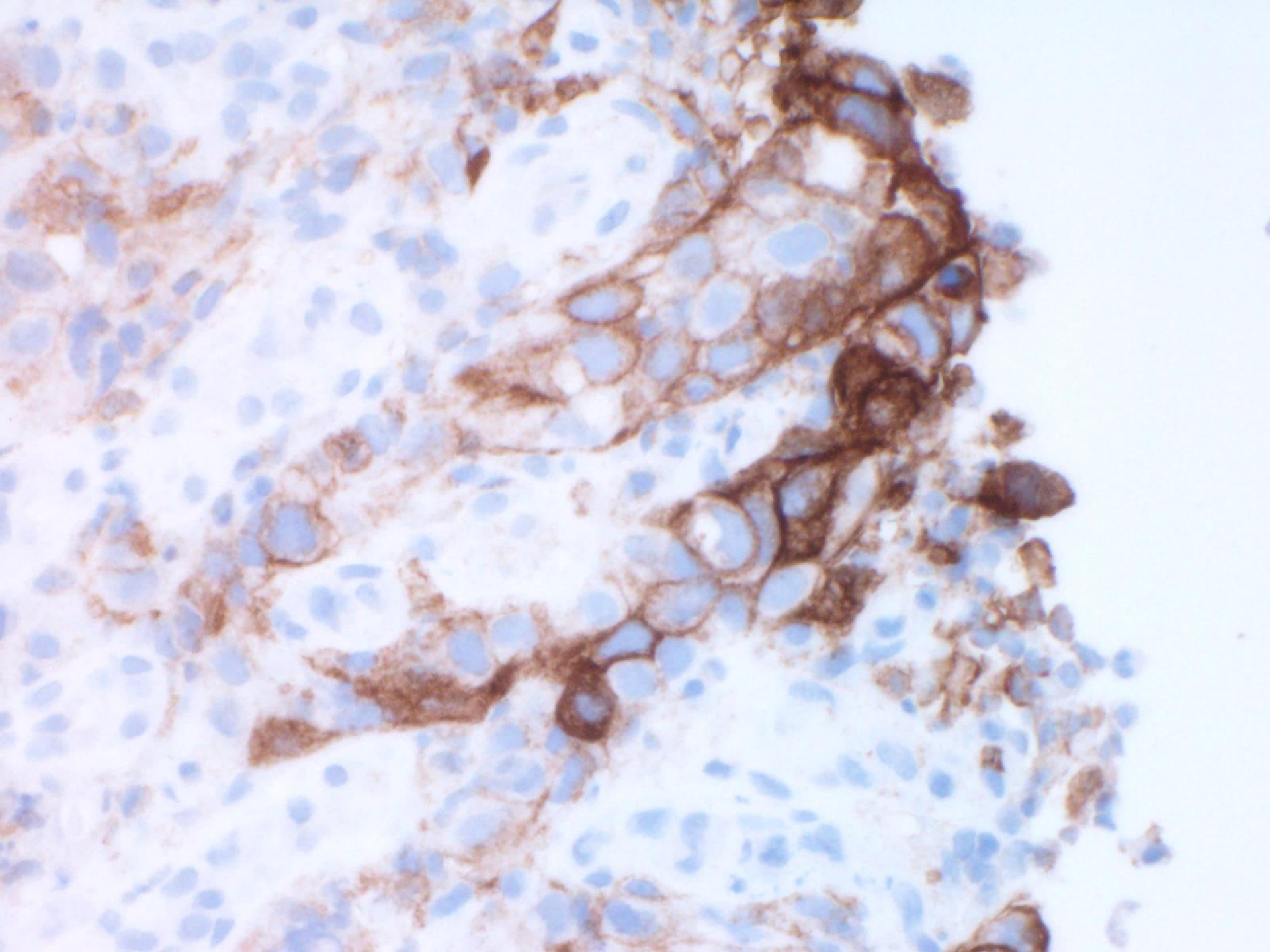
: PD-L1 positive MICs

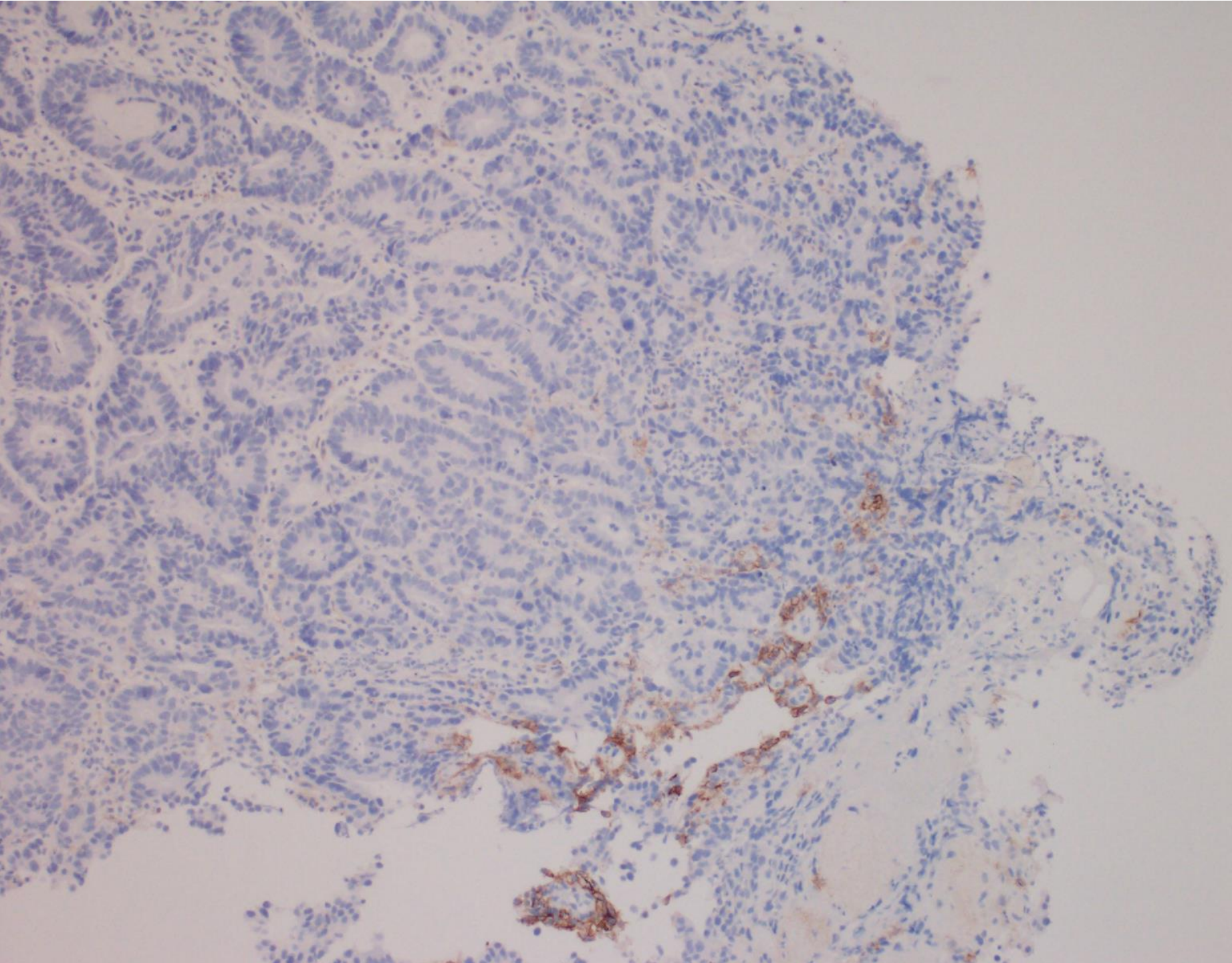
: PD-L1 negative MICs

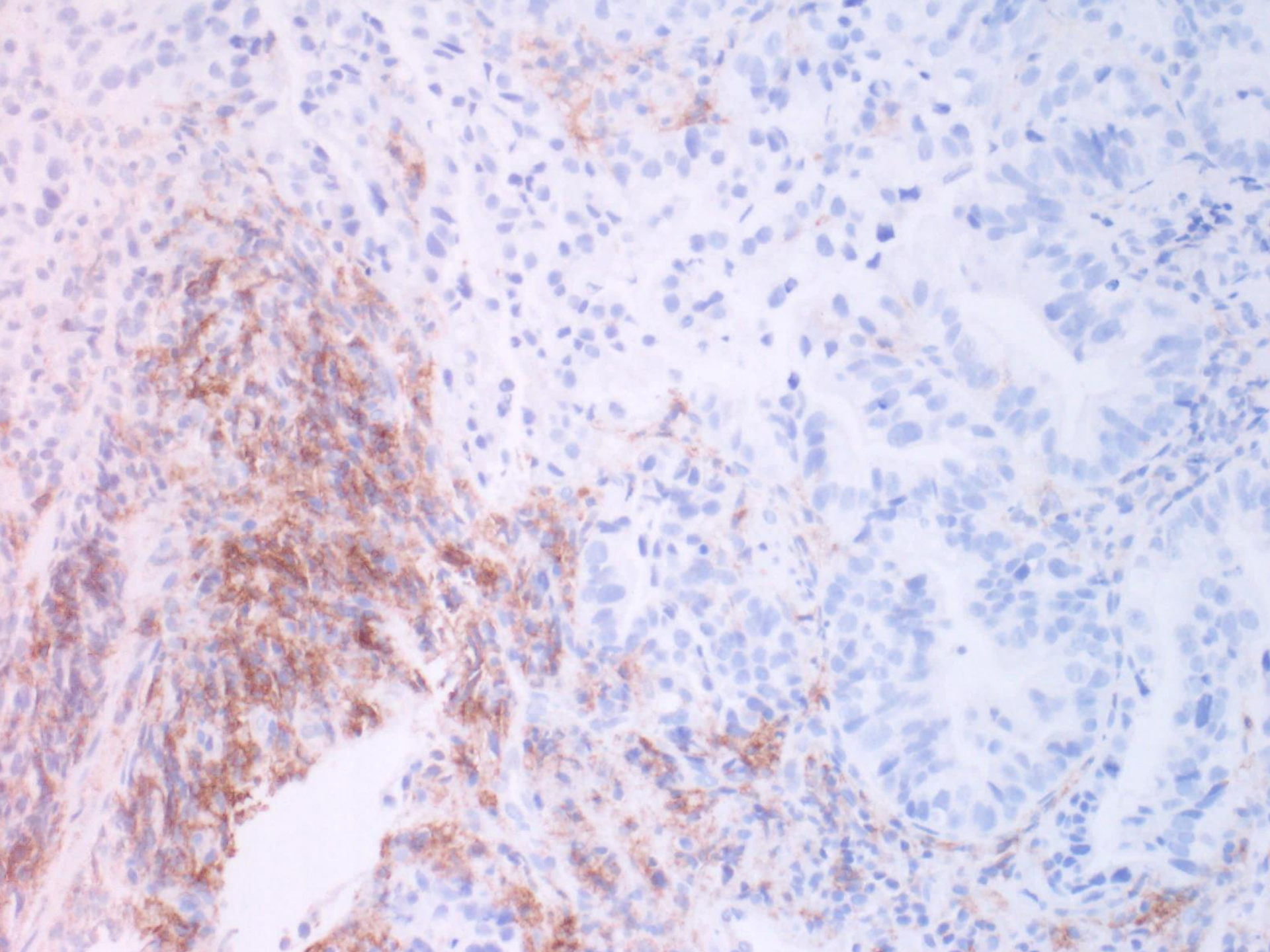


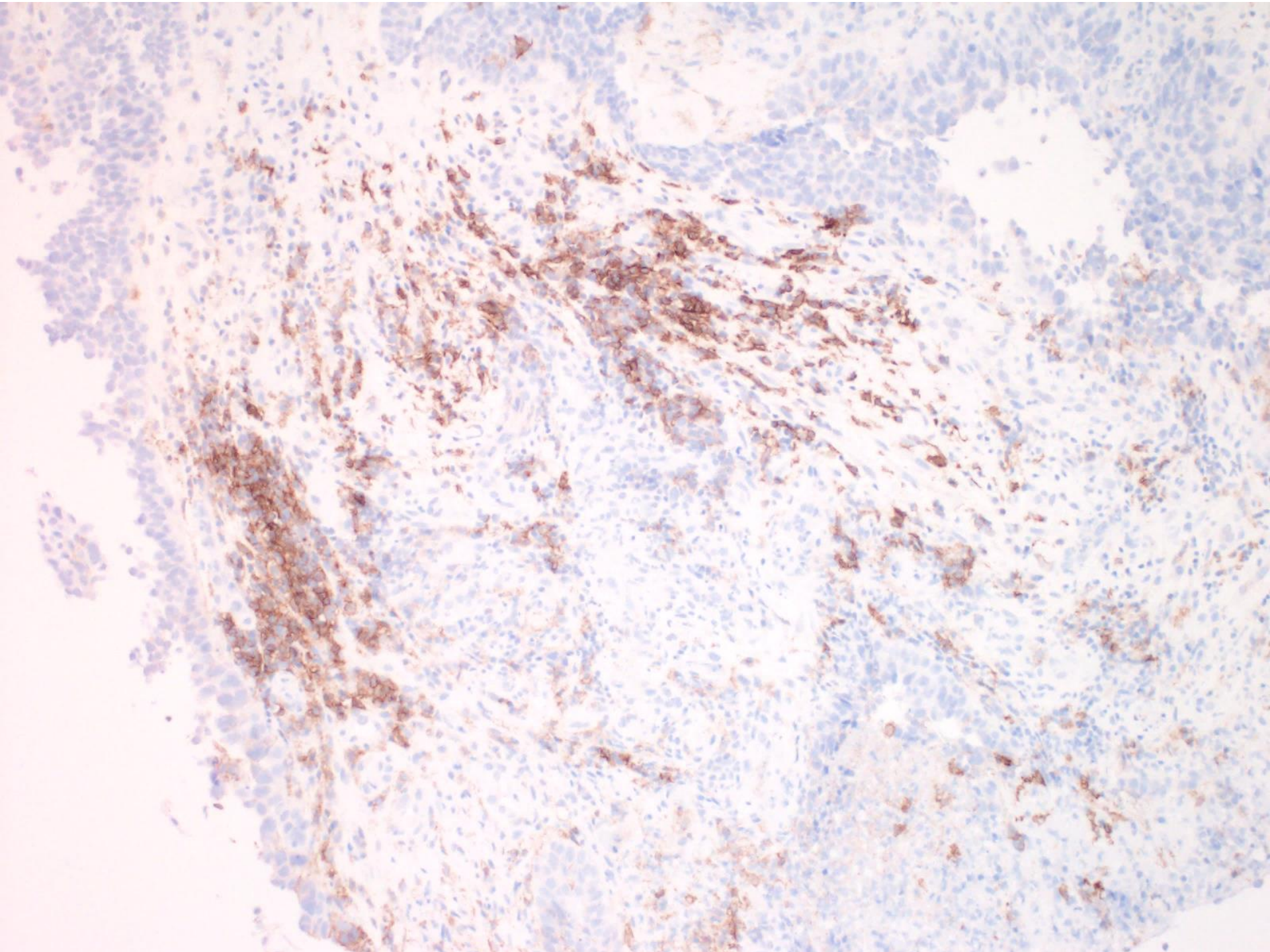

Intense vs. weak staining
Necrosis
Inflammatory cells

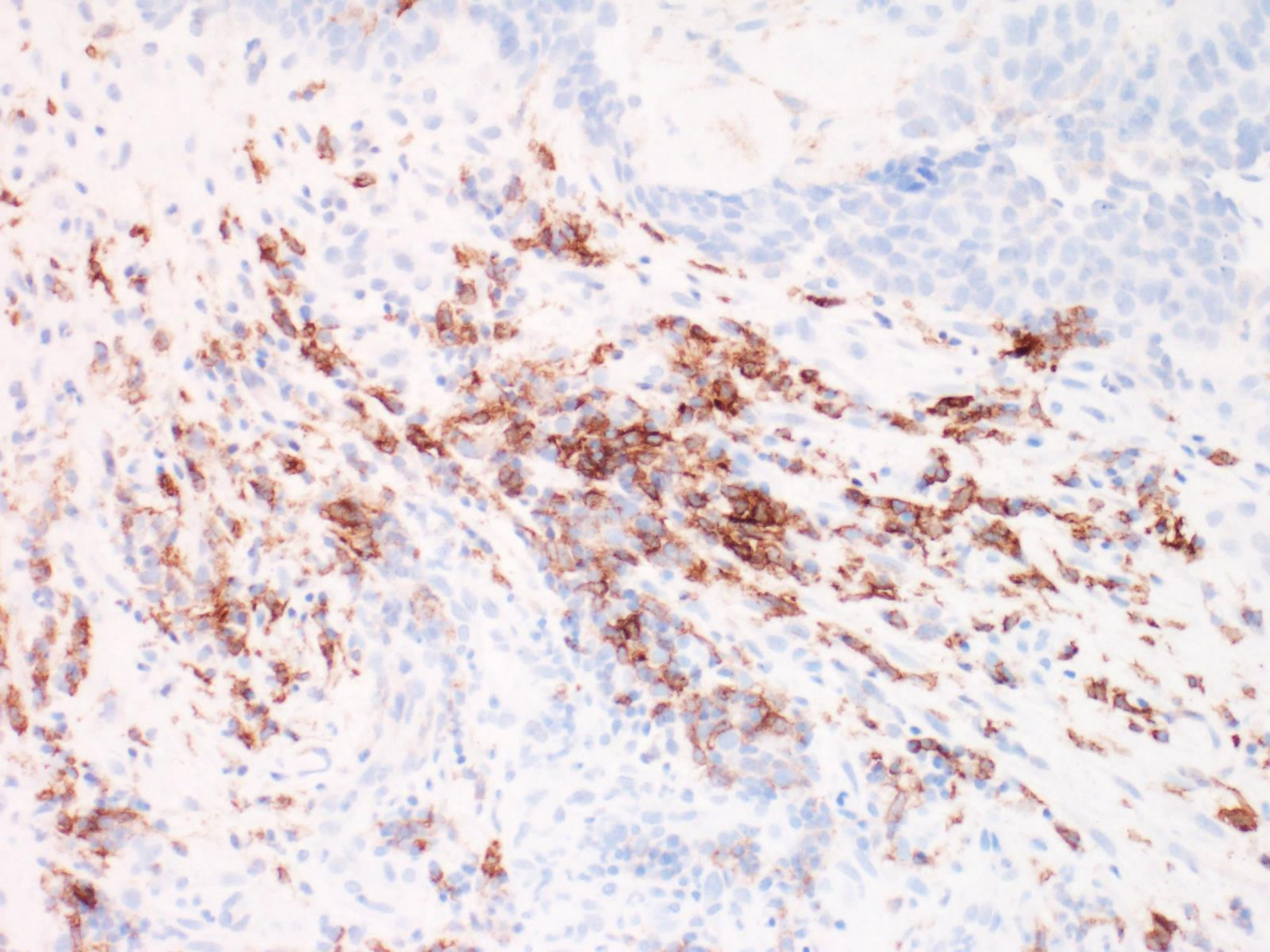












Gastric/GEJ PD-L1 22C3 Interpretation

- Gastric and GEJ adenocarcinomas are divided into two categories based on Combined Proportion Score (CPS):
  - CPS < 1: No PD-L1 expression
  - CPS ≥ 1: PD-L1 expression

- At ARUP, also CPS tiers reported
  - No expression: <1
  - Expression: 1, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100

PD-L1 Verification and Implementation Summary

- FDA-cleared assays require verification by the laboratory prior to implementation
- FDA-cleared kits = total test approach
- Engagement of clinical laboratory early in the verification process ensures successful implementation
- Ongoing quality control measures are crucial for insuring quality of results and adequate performance of the assay

Gastric/GEJ Adeno PD-L1 22C3 and Specimen Age

Tumor Tissue	PD-L1 Expression (CPS ≥1), n (%)	No PD-L1 Expression (CPS <1), n (%)
Overall Study, n=257	148 (58%)	109 (42%)
Archival Tissue*, n=167	82 (49%)	85 (51%)
Newly Obtained Tissue*, n=90	66 (73%)	24 (27%)

- KEYNOTE-059 Study (NTC02335411) data
- * Archival tissue ≤42 days between biopsy/excision and PD-L1 22C3 testing; newly obtained tissue: >42 days between biopsy/excision and PD-L1 22C3 testing
- Reference: SK00621-5 PD-L1 IHC 22C3 pharmDx interpretation manual (Dako)

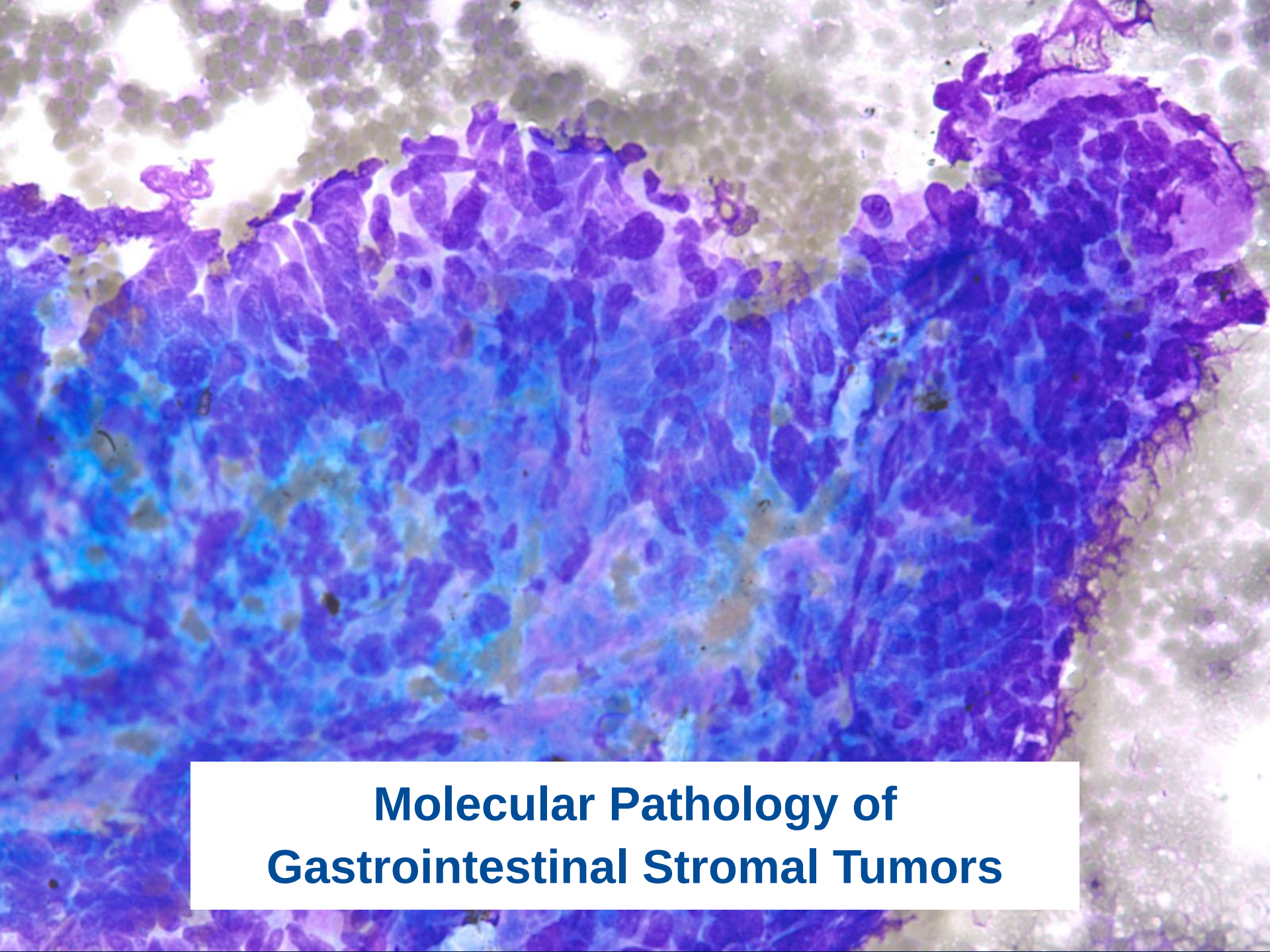
GI PD-L1 22C3 Summary: Gastric GEJ Adenocarcinomas

- 3rd line treatment with pembrolizumab (Keytruda®) in gastric/GEJ adenocarcinomas
  - NOT esophageal squamous cell carcinomas
- Graded with CPS
  - DIFFERENT TEST ORDER THAN NSCLC 22C3
  - Different scoring system from TPS used in NSCLC
  - NOT a percentage, but a SCORE
  - Includes tumor cells and MICs
- Dako recommends using specimens obtained within 42 days of 22C3 testing
  - All specimens eligible
  - Consider repeating CPS <1 (no expression) specimens for archival specimens

GI PD-L1 22C3 Summary: Other GI Malignancies

- MMR-deficient/MSI colorectal carcinomas approved for 2nd line treatment with pembrolizumab (Keytruda®) and nivolumab (Opdivo®)
  - No PD-L1 testing needed

- Other MMR-deficient/MSI solid tumors approved for 2nd line treatment with pembrolizumab (Keytruda®)
  - No PD-L1 testing needed



Molecular Pathology of Gastrointestinal Stromal Tumors

Gastrointestinal Stromal Tumors (GISTs)

- Believed to derive from the interstitial cells of Cajal
  - **Myenteric** Interstitial cells of Cajal serve as a pacemaker which creates the bioelectrical slow wave potential that leads to contraction of the smooth muscle
  - **Intramuscular** Interstitial cells of Cajal are involved in the stimulation of smooth muscle cells, neurotransmitters act through them.

Gastrointestinal Stromal Tumors (GISTs)

- Represent approximately 1% (0.1-3%) of GI malignancies
- Incidence of 0.32 per 100,000 people win the US
- Relatively simple from a molecular pathology perspective
- Hirota et al. (1998) the first to describe how many GISTs derive from activating mutations of KIT gene

Incidence of GISTs by Anatomic Location

- Stomach: 50%
- Small intestine: 25%
- Esophagus, colon, rectum: 10%
- Extra-intestinal (mesentery, omentum, retroperitoneum): 10%

Immunohistochemistry of GIST

- The most commonly used IHC markers are CD117 (C-KIT), DOG-1, and CD34
- 90-95% of overall GISTs show strong cytoplasmic CD117 staining
- 70% overall GISTs show staining for CD34
- DOG1 useful in tumors that are morphologically consistent with GIST, but are CD117-negative
- Janeway et al. (2011) suggested that staining for SDH-B has been shown useful in gastric tumors
  - Loss of SDHB staining is correlated with KIT/PDGFRA non-mutant tumors
  - SDH family gene mutations/altered methylation → loss of SDH

Mutations in GISTs

- Around 80-85% contain mutations in KIT
- Another 5-10% contain mutations in PDGFRA
- 10-15% KIT/PDGFRA-negative GISTs
  - More than half shown to have defects in the Krebs circle family of enzymes: succinate dehydrogenase (SDH)
  - Due to SDH mutation or altered methylation (rare germline mutations)
  - SDH-deficient GIST
- Rare cases, typically in the small bowel, with BRAF or NRAS mutations
- GIST in the context of NF1 can (inconsistently) have KIT/PDGFRA somatic mutations

Mutational Status and Risk Stratification of GISTs

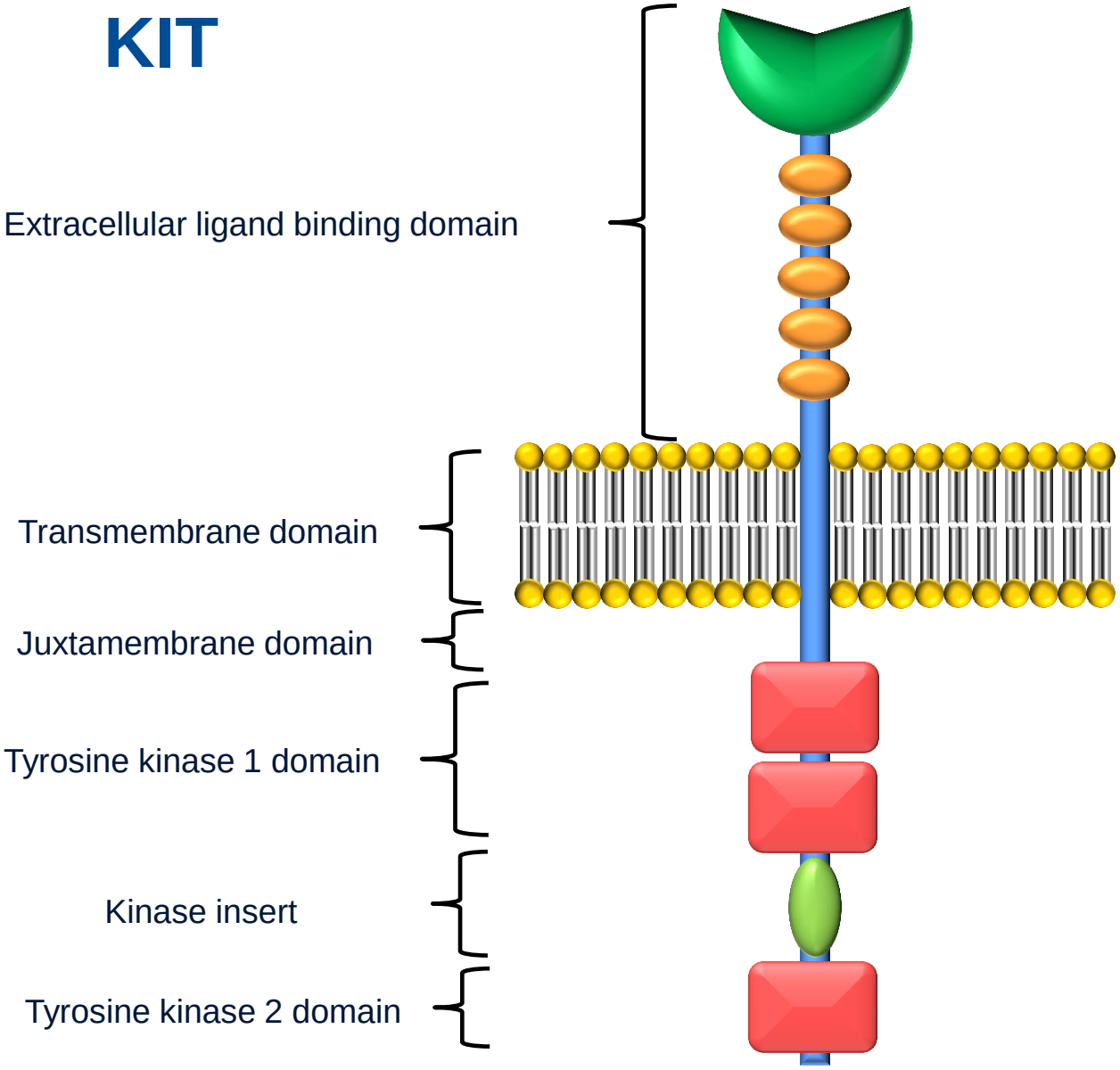
- Mitotic rate and size are used to estimate risk of progression
  - Very low risk, low risk, intermediate risk, high risk (Fletcher, Hum Pathol 2002;33:459-65)
  - Doesn't apply to succinate dehydrogenase deficient GISTs
- Location
  - Gastric generally does better than small intestine or rectum (Miettinen, Semin Diagn Pathol 2006; 23:70-83)
- Mutation status non included in risk stratification
    - About 15% are homozygous for somatic KIT mutations → worse prognosis

KIT - PDGFRA

- The KIT gene (4q12) encodes for a 145 kDA tyrosine kinase (TK) receptor, which is in the type III TK family, along with PDGFRA and PRDGFB TKs, among others

- This PDGFRA gene (also 4q12) encodes for a 170 kDA tyrosine kinase (TK) receptor for members of the platelet-derived growth factor family (type III)

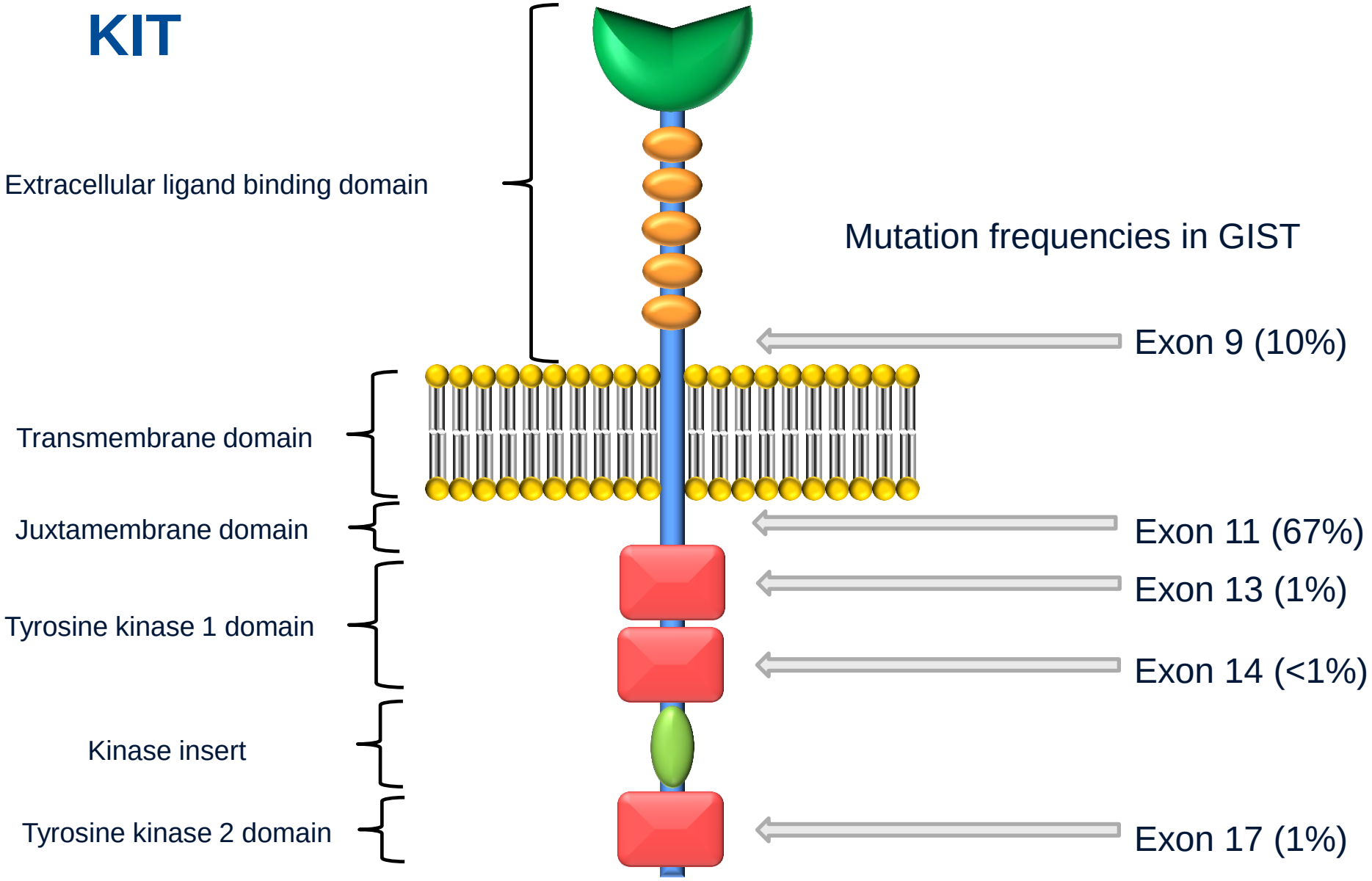
KIT


Extracellular ligand binding domain
Transmembrane domain
Juxtamembrane domain
Tyrosine kinase 1 domain
Kinase insert
Tyrosine kinase 2 domain

How are kinases activated in tumors?

- Tyrosine kinase domain mutations (EGFR)
  - Results in a constitutively active TK activity
- Ligand independent receptor dimerization (KIT)
  - Receptors dimerize even in the absence of a ligand, resulting in activation
- Translocations fusing the tyrosine kinase domain to another gene (EML4-ALK)
  - Results in constant activation of TK domains
- Amplification (Her2)
  - Increase in the overall TK activity

KIT


Extracellular ligand binding domain
Transmembrane domain
Juxtamembrane domain
Tyrosine kinase 1 domain
Kinase insert
Tyrosine kinase 2 domain
Mutation frequencies in GIST
Exon 9 (10%)
Exon 11 (67%)
Exon 13 (1%)
Exon 14 (<1%)
Exon 17 (1%)

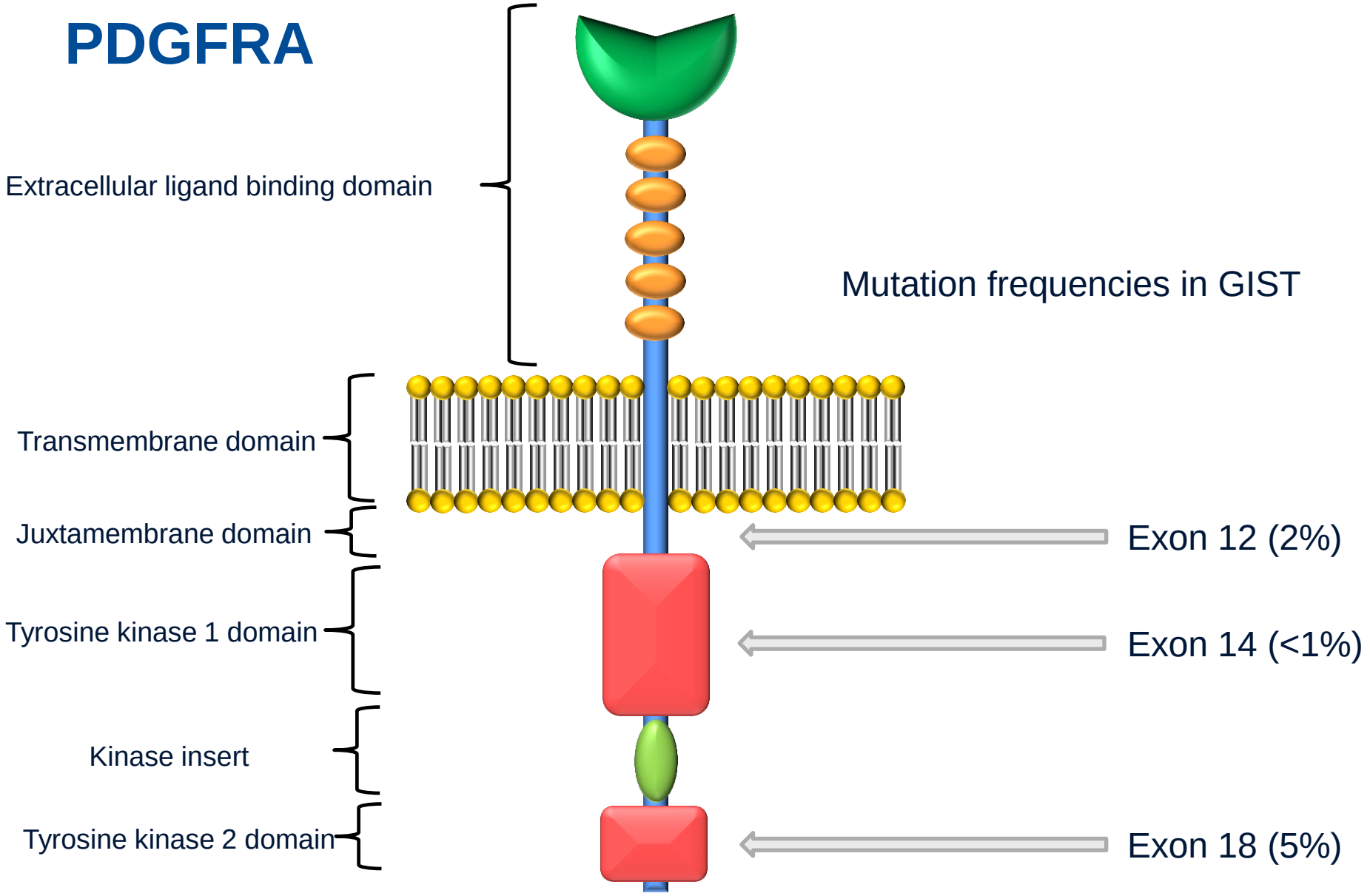
How do KIT mutations cause tumors?

- Mutations in extracellular or juxtamembrane domains (exons 9 and 11) lead to ligand independent receptor dimerization and activation

- Primary TK2 (exon 17) mutations stabilize activation loop in active configuration

- Unclear how primary TK1 (exon 13) mutations are oncogenic; maybe interfere with juxtamembrane domain inhibition of activation loop

PDGFRA


Extracellular ligand binding domain
Transmembrane domain
Juxtamembrane domain
Tyrosine kinase 1 domain
Kinase insert
Tyrosine kinase 2 domain
Mutation frequencies in GIST
Exon 12 (2%)
Exon 14 (<1%)
Exon 18 (5%)

Types of KIT exon 11 mutations

- Around 100 exon 11 mutations reported
- Deletions for the most part
- p.Trp557_Lys558del (p.W557_L558del) most common (stomach)
- p.Tyr568del (p.Y568del), p.Tyr570del (p.Y570del) (small intestine)
- Deletions in general, (especially codons 557, 558) associated with worse prognosis

Types of KIT exon 9 mutations

- Small intestine and colon, more aggressive
- Requires higher dose imatinib
- p.Ala502_Tyr503dup (p.A502_Y503dup) most common

Tyrosine Kinase (TK) Domain KIT mutations

- Substitutions (point mutations) more common than deletions or insertions, indels

- Exon 13 (TK1)
  - p.Lys642Glu (p.L642E) most common mutation

- Exon 17 (TK2)
  - Codon 822 substitutions most common

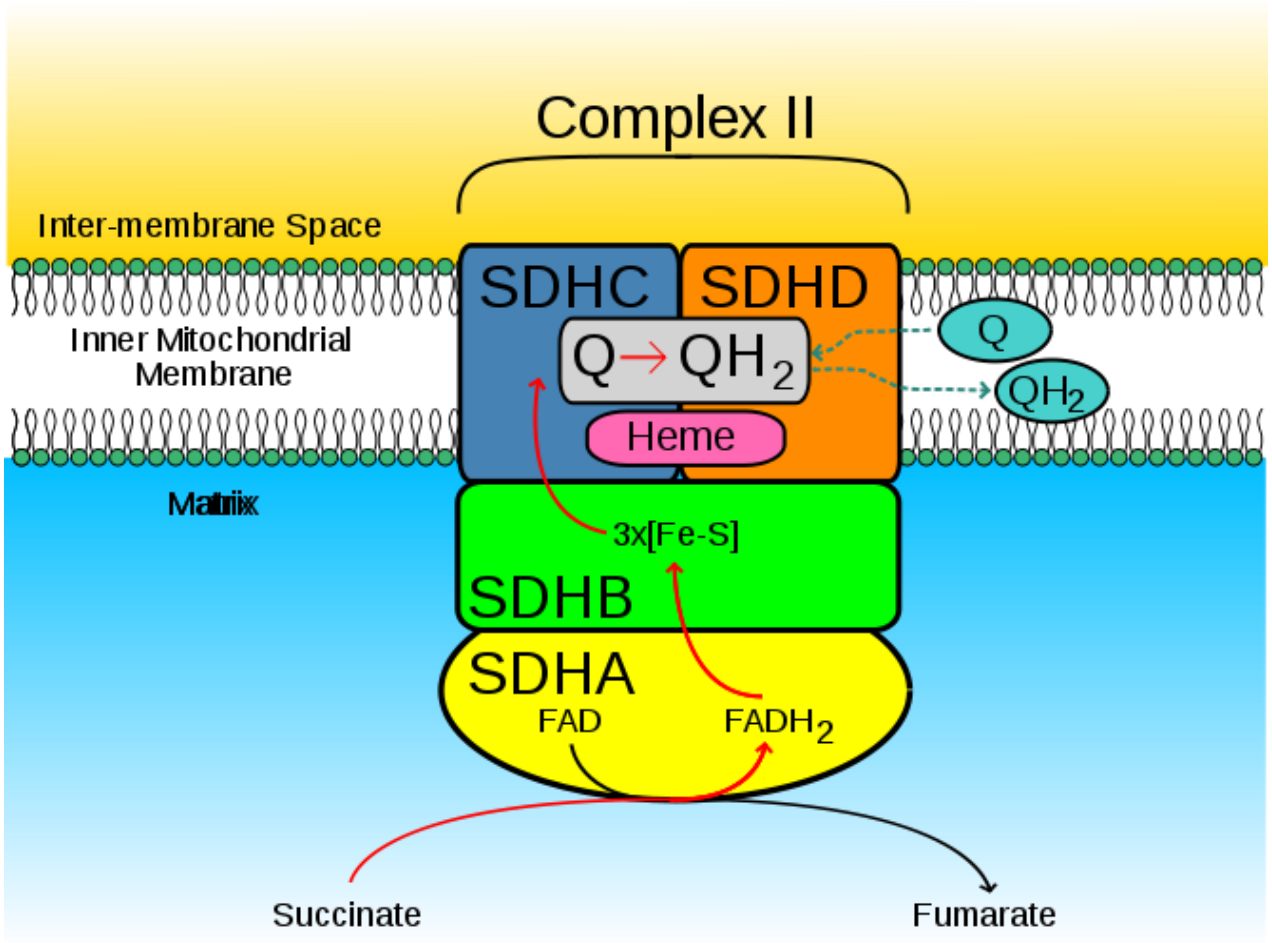
PDGFRA mutated GIST's

- Epithelioid morphology
- Gastric and extra-GI location
- KIT negative (or weakly positive) by IHC
- May be less aggressive
- p.Asp842Val (p.D842V) in TK2 is most common mutation

SDH-Deficient GIST

- More than half of wild type GISTs
- More common (7.5%) in stomach
- Majority of pediatric GISTs
- Despite lymph node (unusual for GISTs) and distant metastases, indolent behavior
- Accepted GIST risk factors don’t apply
- Distinctive morphology: epithelioid, multinodular/plexiform
- Nearly all KIT and DOG-1 positive
- Do not respond to imatinib

Succinyl Dehydrogenase (SDH) Complex



Source: Wikimedia

How Do SDH Mutations Cause Disease?

- Loss of any of these subunits through gene mutation or post-transcriptional downregulation (e.g. methylation) destabilizes the complex
- Accumulation of succinate
- Increased transcription of HIF1a-regulated genes
- Decreased DNA demethylation
- SDH-deficient GISTs show global increase in DNA methylation
  - Similar to that in IDH1/IDH2 mutated gliomas and leukemias

SDH-deficient GIST's

- Regardless of subunit mutation, tumor will show loss of cytoplasmic staining for SDHB (good screening tool for SDH-deficient)
- Majority will have SDH gene (A, B, C, D) mutations
  - Including germline mutations
- Small percentage due to germline mutations of SDH B, C, D (Carney-Stratakis syndrome)
  - Paragangliomas
- Rest mostly due to epigenetic/post-transcriptional (e.g. methylation) silencing
- SDHA has 3 pseudogenes

GIST Related Syndromes/Complexes

- **Familial GIST**: Multiple tumors, diffuse hyperplasia of interstitial cells of Cajal, mastocytosis
  - KIT and PDGFRA **germline** mutations
- **NF1**: 7% multiple small intestinal GIST's, do not metastasize, no KIT or PDGFRA mutations
- **Carney's triad** (not inherited)
  - Pulmonary chondroma, extra-adrenal paraganglioma and epithelioid gastric GIST, mostly young women
  - No KIT or PDGFRA mutations
  - Succinate dehydrogenase deficient (but no mutations)
- **Carney-Stratakis syndrome**
  - Multifocal gastric GISTs and paragangliomas
  - **Germline** mutations in SDH subunits

Carney-Stratakis Syndrome

- **Familial paraganglioma and GIST**
  - Autosomal dominant
- Germline mutations in succinate dehydrogenase genes SDHB, SDHC or SDHD
  - No germline or somatic KIT or PDGFRA mutations
- Mean age 23
  - Males and females affected
- Nearly all GISTs occur in the stomach
  - Frequently multiple and multinodular
- GIST may metastasize to lymph nodes
  - Usually protracted, indolent course (e.g. 15 years) in most cases even with metastasis or recurrence
- Paragangliomas frequently aggressive
- Presentation (except for the triad features), pathology and behavior are essentially the same as Carney Triad and sporadic SDHB deficient (pediatric type) GIST
  - Must exclude NF1 and Carney Triad

Treatment

- Surgery usually first line
- Imatinib competes with ATP for binding site
  - Action against non-TK KIT and PGFRA mutations
  - Used for metastatic disease
    - If surgery is not an option
    - After surgery with high risk of recurrence
- KIT exon 11 mutated tumors more likely to respond to imatinib than exon 9 mutated or wild type
- KIT exon 9 mutated tumors respond better to higher dose of imatinib

Imatinib resistance

- Primary Resistance associated with:
  - KIT wild type
  - KIT exon 9 mutants (possible dosage effect)
  - PDGFRA p.Asp842Val (p.D842V)

Imatinib resistance

- Secondary resistance associated with:
  - Secondary mutations in KIT exons 13, 14 (TK1) which interfere with drug binding
  - Secondary mutations in KIT exons 17,18 (TK2) which stabilize TK2 in active conformation

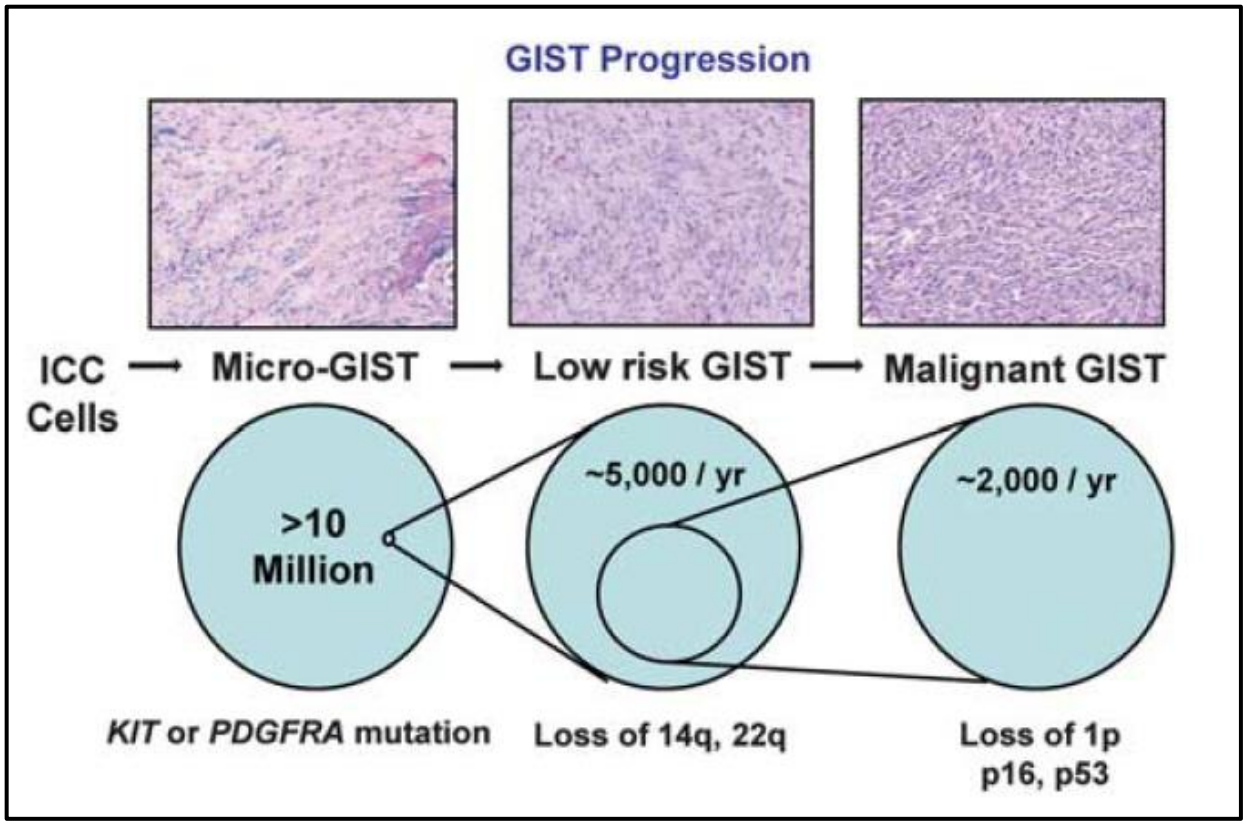
Imatinib resistance

- Secondary resistance:
  - Secondary mutations more likely to occur in exon 11 mutated tumors than exon 9 (possible dosage related)
  - Secondary mutations not seen in wild type tumors
  - Usually single nucleotide substitutions
  - Occur on same allele as original mutation (*cis*)

Treatment for Imatinib-Resistant GIST

- Sunitinib (second generation TKI) used for those who fail imatinib, active against ATP binding pocket mutations
- Many alternative TKIs target VEGF
- PDGFRA p.Asp842Val (p.D842V) is resistant to both TKIs
  - May be sensitive to dasatinib

Chromosomal Abnormalities and GIST Progression



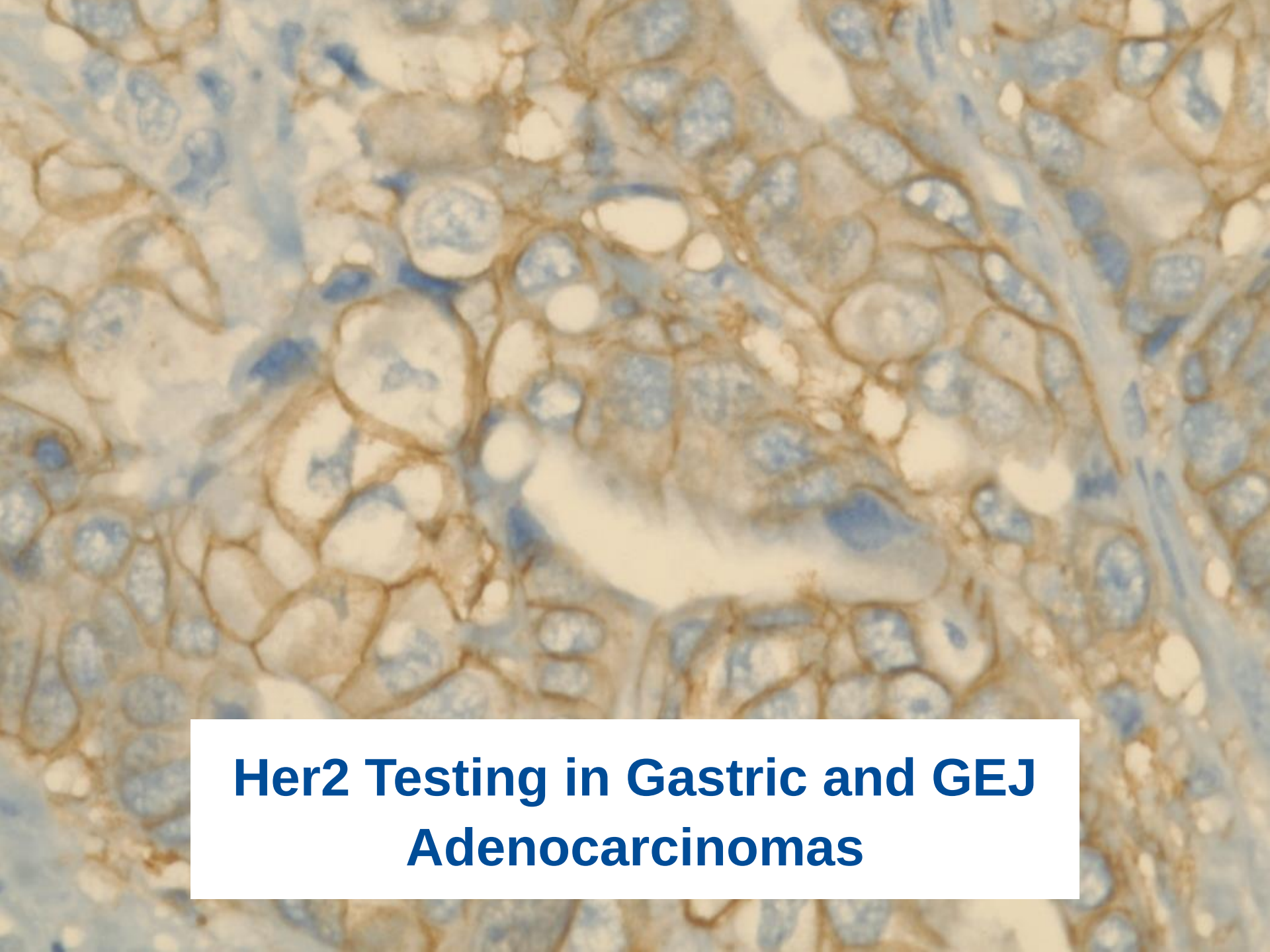
Yearly GIST incidence/progression in the US. Corless, Mod Pathol 2014;27:S1-S16.

- 2/3 of both WT and KIT/PDGFRA mutant GISTs present monosomy 14 or partial 14q loss
- 14q11.2 deletions include PARP2, APEX1, NDRG2
- 14q32 include SIVA gene
- Loss of 22q seen in ~50% of GISTs
- Losses of 1p, 9p, 11p and 17p are less common, but more significantly associated with malignancy

GIST - Summary

	Pathologic Marker	Associations
IHC	KIT	Positive in most tumors, exception may be PDGFRA-mutated GIST
	DOG-1	Positive in almost all tumors
	SDHB Loss	• Loss seen in tumors with mutations in SDH family of enzyme genes or with altered methylation; associated with gastric primaries and more indolent behavior • GIST may be part of the Carney triad or Carney-Stratakis dyad • Limited response to imatinib but better disease control with VEGFR targeting TKIs
Mutations	KIT	• Exon 11: best and longest duration of response with imatinib in advanced disease • Exon 9: shorter duration of response with imatinib and overall poorer survival in advanced disease; most commonly in small bowel GIST
	PDGFRA	Similar response and outcomes as KIT mutations with the exception of PDGFRA D842V that has very limited response to standard kinase therapy; most commonly in gastric GIST
	SDH family of enzymes	Referral for genetic counseling to assess for Carney-Stratakis dyad
	NF-1	• GIST can also contain a somatic KIT or PDGFRA mutation • May present with multifocal small volume disease, often indolent in nature • Referral to genetic counseling if not previously known to be NF-1 carrier
	BRAF, NRAS	Very rare, usually of small bowel location

von Mehren, 2016



Her2 Testing in Gastric and GEJ Adenocarcinomas

Her2/Neu Testing in Gastric and Esophageal Carcinomas

- Her2 initially discovered as an overexpressed transmembrane tyrosine kinase receptor in approximately one-third of breast cancer patients
  - In breast cancer associated with decreased survival, Her2, or c-erb-B2, proto-oncogene quickly became an important tumor marker and target for therapy

- Approximately 30% of gastroesophageal junction adenocarcinomas and 20% of gastric cancers overexpress Her2, and early studies showed wide variability of overexpression depending on the specific method of testing
  - Rates for Her2 overexpression in squamous cell carcinoma of the esophagus has been found to range from 5% to almost 40%

Her2/Neu Testing in Gastric and Esophageal Carcinomas

- International, randomized, Phase III Trastuzumab on GAstric (ToGA) cancer study
  - Survival benefit with trastuzumab plus chemotherapy (capecitabine or 5-fluorouracil and cisplatin) in patients with Her2-positive locally advanced, recurrent and/or metastatic gastric or GEJ tumors that overexpress Her2 (Bang et al. Lancet 2010;376:687–97)
  - Patients with high Her2-expressing tumors derived the greatest benefit from trastuzumab therapy.
- Her2 positivity as defined in the ToGA cancer study was:
  - Immunohistochemistry 3+ and/or
  - FISH Her2/CEP17 ratio ≥2.0
- Of the 3,803 patients originally screened for eligibility, 810 patients had IHC or FISH Her2-positive tumors, but only 594 patients were randomly assigned to treatment
- The Her2 positivity rate was 22.1%, with similar rates between European and Asian patients (23.6% vs 23.9%)

Her2/Neu Testing in Gastric and Esophageal Carcinomas

- On the basis of the ToGA study findings it is recommended that all patients with gastric cancer should have their tumors tested for Her2 status at the time of initial diagnosis
  - European Medicines Agency: patients with Her2-positive metastatic disease whose tumors are 3+ by IHC or positive by FISH or positive by silver ISH (SISH) are eligible for trastuzumab therapy
  - US FDA: Approval for trastuzumab granted in October 2010 for patients with metastatic adenocarcinoma of the stomach or gastro–esophageal junction whose tumors were Her2-positive as determined using approved testing methods

ToGA Trial Findings

- The median overall survival was 13.8 months for patients receiving trastuzumab plus chemotherapy, compared with 11.1 months for those receiving chemotherapy alone (hazard ratio [HR], 0.74; 95% confidence interval [CI], 0.60-0.91; p=0.0038)
- Patients with IHC of 3+ derived more benefit than those with IHC of 2+ (and concurrent Her2 amplification by ISH)
- Upon further follow-up of these patients, reanalysis demonstrated considerable reduction in patient benefit from the addition of trastuzumab (HR, 0.8; 95% CI, 0.67-0.97; p=0.019). The difference in the median survival diminished to 1.4 months

Her2 Expression in Gastric/GEJ Adenocarcinomas

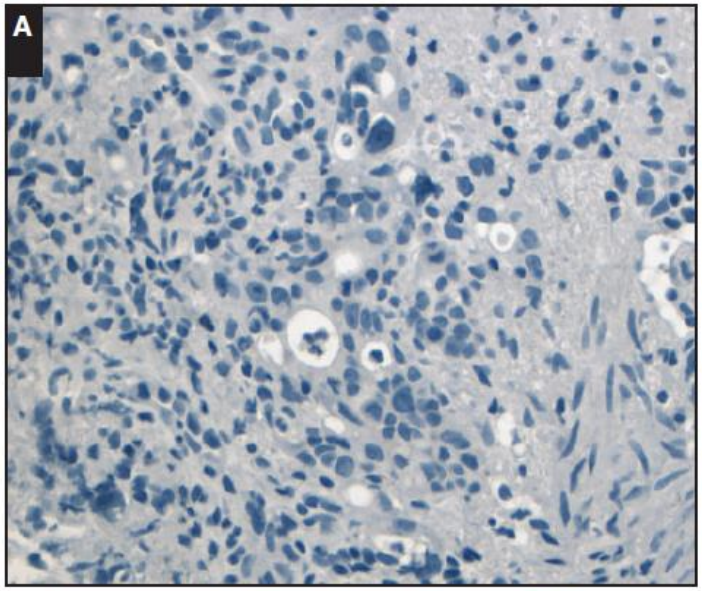
- Gastric cancer exhibits unique immunostaining characteristics compared with breast cancer, including
  - Up to 30% incidence of tumor heterogeneity (≤30% of tumor cells staining positive or only focal staining of tumor cells)
- Her2-positive gastric carcinomas are usually of the gland-forming intestinal type and may show incomplete, basolateral, or lateral staining
  - All these are considered as a positive result with IHC
- ToGA study data demonstrated that patients with tumors that had high levels of Her2 protein expression (3+ by IHC or 2+ by IHC and positive ISH derived the greatest benefit from treatment with trastuzumab
  - Consequently immunohistochemistry should be the initial testing method

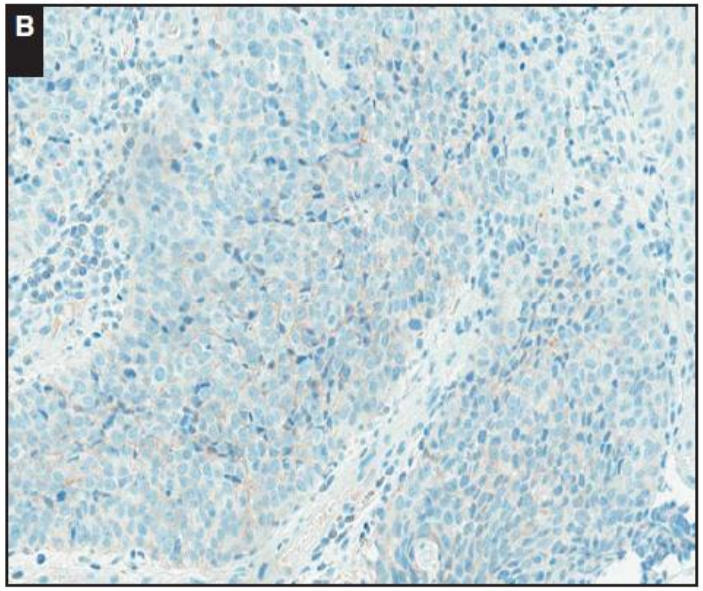
Her2 IHC Scoring in Gastroesophageal Adenocarcinomas

Adapted from Ruschoff et al. Mod Pathol 2012;25:637–50.

Score	Surgical specimen staining pattern	Biopsy specimen staining pattern	Her2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	Negative
1+	Faint/barely perceptible membranous reactivity in ≥10% if tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
2+	Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells.	Tumor cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3+	Strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a strong complete, basolateral, or lateral membranous reactivity, irrespective of percentage of tumor cells stained	Positive

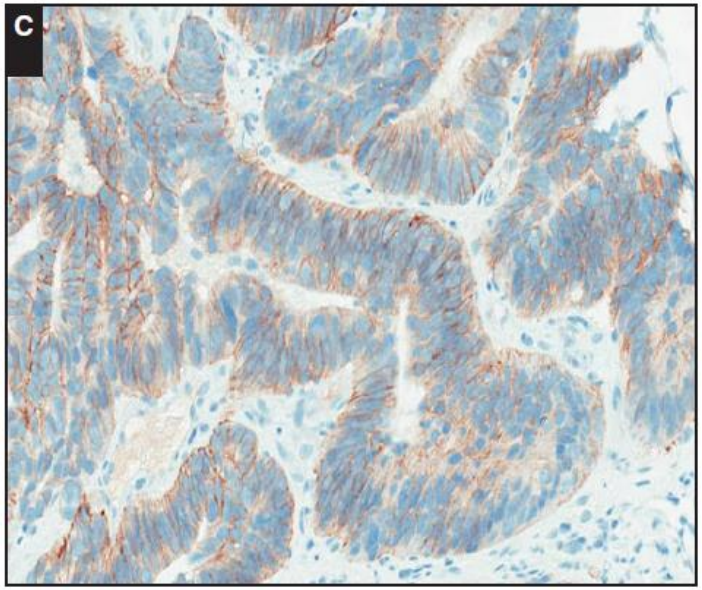
0

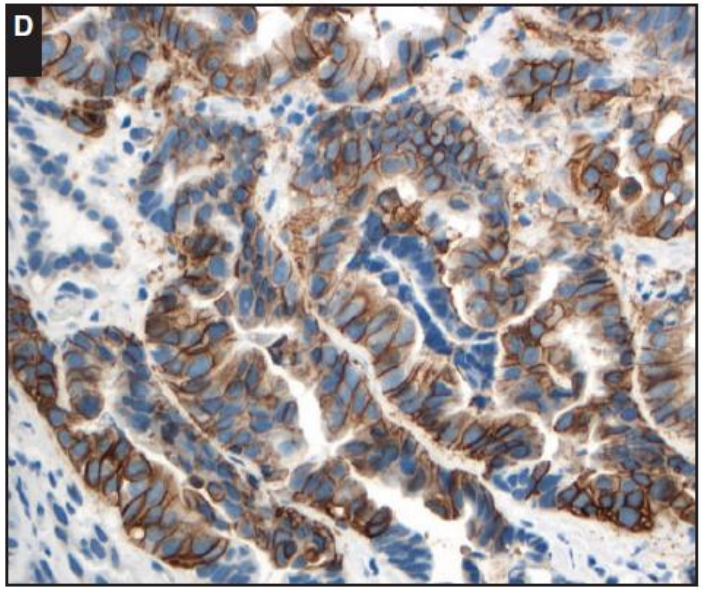




1+

2+





3+

Bartley et al. Am J Clin Pathol 2016;146:647-69

HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma

Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology

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Am J Clin Pathol December 2016;146:647-669

CAP-ASCP-ASCO Guidelines: 1. Strong Recommendation

- In patients with advanced gastroesophageal adenocarcinoma (GEA) who are potential candidates for Her2-targeted therapy, the treating clinician should request Her2 testing on tumor tissue
- Her2 status provides little additional value such as prognostic or predictive information
- Currently, there is no evidence of benefit of Her2-directed therapy in patients without advanced GEA

Types of Specimens for Testing: Resection vs Biopsy

Study	Design	Findings
MAGIC trial	115 patient biopsy or resection specimens tested for Her2	92.9% (145 of 156) concordance between the two types of specimens
ToGA trial	2,596 (68%) patients' tumors were acquired by a biopsy, and 1,199 (32%) from the surgical specimens	Overall positive rate was 23.2% for biopsy specimens and 19.7% for the surgical specimens
Janjigian et al.	381 patients with advanced GEA.	No difference in Her2 positivity between resections/biopsies of primary (biopsies 21% vs resection 19%, p=0.791) or metastatic disease and no association with prognosis

Types of Specimens for Testing: Resection vs Biopsy

Study	Design	Findings
Yoshida et al.	207 surgically resected tumors and paired biopsy specimens from 158 patients with intestinal-type gastric cancers were analyzed for Her2 IHC/FISH	• Her2 overexpression in 17% of cases • Amplification was detected in 31% of resections and 32% of biopsies • 90.9% IHC/FISH concordance in resections and 90.2% in biopsies • 72.7% FISH concordance rate of FISH between the surgical and biopsy specimens
Grillo et al.	103 patients with matched specimens	The concordance of IHC and FISH between biopsy and surgical samples was 80% and 95%, respectively.
Pirelli et al.	61 consecutive pairs of biopsy specimens and surgical specimens	Concordance of Her2 status of 91.8%

Types of Specimens for Testing: Primary vs Metastasis

Study	Design	Findings
Qiu et al.	100 gastric cancers, both primary and lymph node metastases by IHC	• Her2 2+/3+ was noted in 33% of primary specimens and 39.4% of the nodes • When comparing in two or more nodes, there was 25.3% discordance
Selcukbiricik et al.	Compared primary and metastasis by SISH	92.5% concordance
Cho et al.	Compared 41 primary tumors vs synchronous metastases	97.6% concordance
Bozzetti et al.	68 paired samples	98.5% FISH concordance (n=68) and 94.9% IHC concordance (n=39)

Types of Specimens: Cytology of Primary or Metastatic Tumor

Study	Design	Finding
Bozzetti et al.	compared metastatic FNA and histology specimens by FISH	• Amplification in 21% of histology specimens and in 9% of cytology specimens • Likely that the discrepancy observed may be related to the small sample size
Wong et al.	Assessed Her2 status on effusions by IHC and SISH. Cell blocks from 46 effusions examined	• 15% showed 2+/3+ by IHC • 7% showed Her2 amplification on SISH • In 39% of cases, Her2 status was compared with histologic specimens, showing 100% concordance

CAP-ASCP-ASCO Guidelines: 2. Recommendation

- Treating clinicians or pathologists should request Her2 testing on tumor tissue in the biopsy or resection specimens (primary or metastasis)

- Her2 testing on fine needle aspiration (FNA) specimens (cell blocks) is an acceptable alternative

CAP-ASCP-ASCO Guidelines:

3. Strong Recommendation. Treating clinicians should offer combination chemotherapy and Her2-targeted therapy as the initial treatment for appropriate patients with Her2-positive tumors who have advanced GEA

4. Strong Recommendation. Laboratories/pathologists must specify the antibodies and probes used for the test and ensure that assays are appropriately validated for Her2 IHC and ISH on GEA specimens

Validation/Verification of Her2 Assays

- If using a method other than the FDA-approved kit, pathologists and laboratories should carefully validate both IHC and ISH for Her2, and validation should be performed in the laboratory in which the assay will be used
- The cases used for validation should be predominantly GEA cases as opposed to other tumors (e.g. breast carcinomas) to allow those scoring to develop and maintain expertise with the different GEA tumor types and appearances
- CAP and/or CLIA guidelines should be followed for assay validation

Validation/Verification of Her2 Assays

- The CAP Laboratory Accreditation Program (ANP.22978) for Her2 validation for breast carcinomas proposes validation using 20 positive and 20 negative specimens for an FDA-approved test and 40 positive/40 negative cases if the test is a laboratory-developed test (LDT)
- If using a brightfield ISH assay kit, initial validation should be done by comparison to an FDA-approved FISH assay
- Records of validation must be maintained as per the CAP Laboratory Accreditation Program (ANP.22750, ANP.22978, and ANP.22956)

CAP-ASCP-ASCO Guidelines:

5. Strong Recommendation. When GEA Her2 status is being evaluated, laboratories/pathologists should perform/order IHC testing first followed by ISH when the IHC result is 2+ (equivocal). Positive (3+) or negative (0 or 1+) Her2 IHC results do not require further ISH testing

6. Strong Recommendation. Pathologists should use the Ruschoff/Hofmann method (previous table) in scoring Her2 IHC and ISH results for GEA

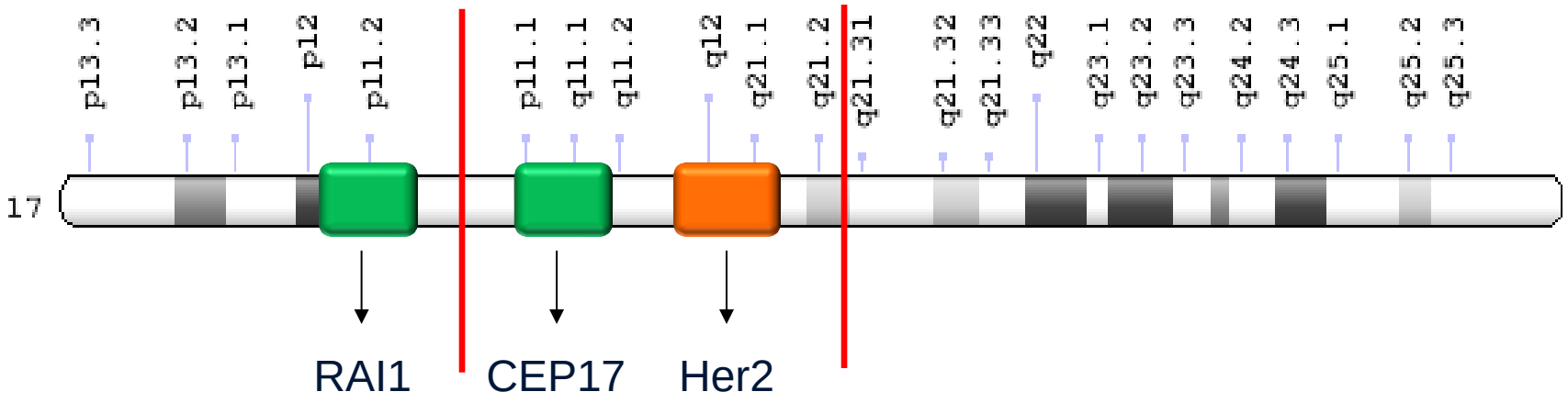
Criteria for Her2 FISH

- At least 20 non-overlapping nuclei of tumor cells are evaluated for Her2 probe and CEP17 probe signal enumeration
- A ratio of Her2 signal to CEP17 signal ≥2.0 is considered positive
- A ratio of Her2 signal to CEP17 signal <2.0 is considered negative
- If IHC is 2+ and there are three or more CEP17 signals, on average, with a ratio below 2, then the presence of more than six Her2 signals, on average, is interpreted as positive for Her2 amplification by ISH/FISH
- Fewer than four Her2 signals, on average, is interpreted as negative for Her2 amplification
- Four to six signals, on average, indicates another 20 cells should be scored in a different target area

Criteria for Her2 FISH

- If additional scoring does not allow a definitive result to be rendered, then multiple options are feasible:
  1. Consultation between scorer and pathologist regarding selection of malignant cells or tumor areas for scoring
  2. Switching out CEP17 for an alternative chromosome 17 probe in a retest to calculate the ratio with a new probe
     - RAI1 (17p11.2) used at ARUP
  3. Selecting a different tumor block for Her2 testing
  4. Using genomics or an alternative analytic method to evaluate Her2 amplification

Chromosome 17



Chromosome 17 map: US National Library of Medicine, National Institutes of Health

CAP-ASCP-ASCO Guidelines:

7. Recommendation. Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than one tissue block may be selected if different morphologic patterns are present

8. Strong Recommendation. Laboratories should report Her2 test results in GEA specimens in accordance with the CAP “Template for Reporting Results of Her2 (ERBB2) Biomarker Testing of Specimens From Patients With Adenocarcinoma of the Stomach or Esophagogastric Junction.”

9. Strong Recommendation. Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of Her2 expression by IHC in GEA specimens for subsequent ISH scoring when required

CAP-ASCP-ASCO Guidelines:

10. Strong Recommendation. Laboratories must incorporate GEA Her2 testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to ensure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing GEA Her2 testing should participate in a formal proficiency testing program, if available, or an alternative proficiency assurance activity

11. No Recommendation. There is insufficient evidence to recommend for or against genomic testing in patients with GEA at this time

CAP-ASCP-ASCO Guidelines: Specimen Considerations

- Biopsy or resection specimens used for Her2 testing are rapidly placed in fixative ideally within 1 hour (cold ischemic time)
- Fixed in 10% neutral buffered formalin for 6 to 72 hours
- Validation studies must address preanalytic factors supporting the stated range of acceptable tissue preparations (e.g. 10% neutral buffered formalin, alcohol fixatives, decalcification, air-dried smears, formalin post-fixation)
- Laboratories should test a sufficient number of GEA cases to ensure that assays consistently achieve expected results.

CAP-ASCP-ASCO Guidelines: Turnaround Time

- The panel recommends a benchmark of 90% of reports available within 10 working days from the date of procedure or specimen acquisition
- Laboratories that require send out of tests for Her2 testing in GEA should process and send specimens to reference laboratories in a timely manner
- The panel suggests that a benchmark of 90% of specimens be sent to the reference laboratory within 3 working days of tissue processing

Thank you!

- Comments - questions

- Comments – questions after the seminar:

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