The British Association of Gynaecological Pathologists

Masterclass with the Experts in Collaboration with ISGyP
4th June 2025

- PD-L1 immunohistochemistry in Cervical Cancer
- Dr Matt Evans
- University Hospitals Birmingham NHS Foundation Trust, Central and South Genomic Laboratory Hub, Black Country Pathology Services
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The British Association of Gynaecological Pathologists

Speaker disclosures

- I have the following to disclose:

I have received honoraria from Agilent Technologies, Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Incyte Biosciences, Jazz Pharmaceuticals, Merck Sharp & Dohme, Pfizer, Roche and Servier

PD-L1 immunohistochemistry in cervical cancer

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Overview



What is the *biological* rationale for PD-L1 testing?



What is the *clinical* rationale for PD-L1 testing?



How do I interpret PD-L1 staining?



What are some of the key technical considerations?

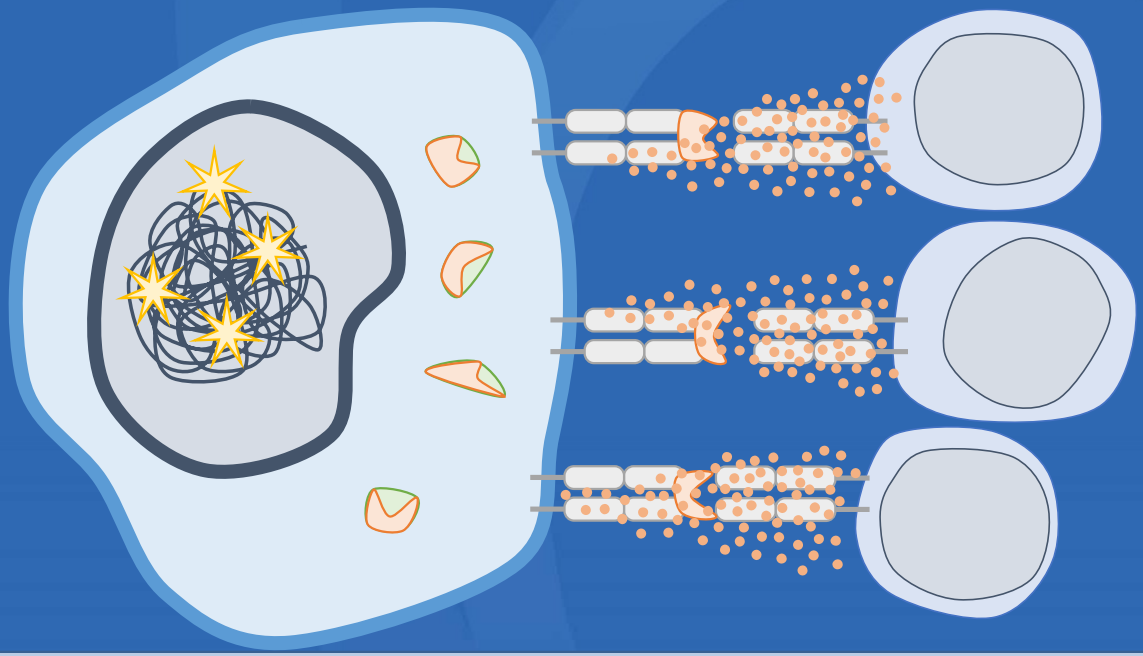


Put it to the test!

What is the *biological* rationale for PD-L1 testing?

What's the biological rationale?

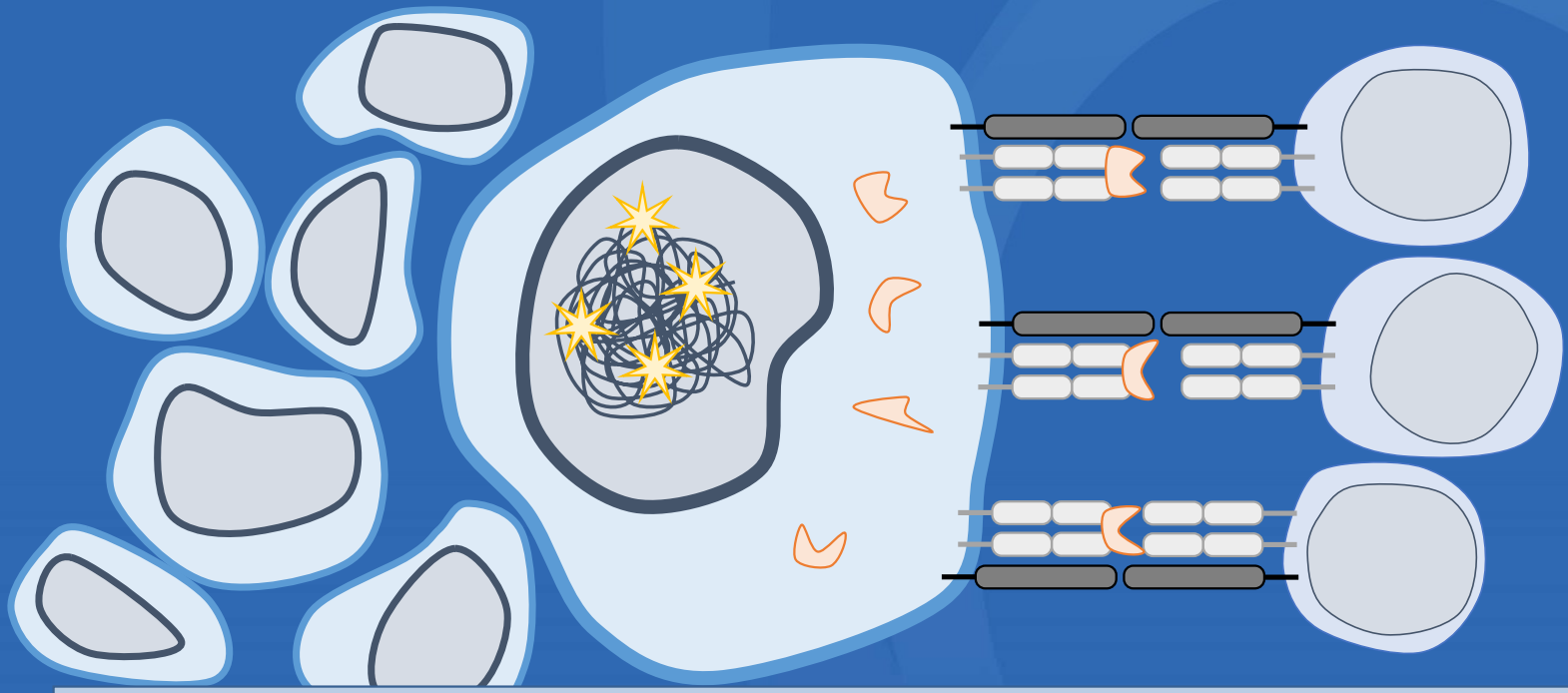
Tumour mutation burden (TMB)



The high mutation burden of cancer cells predisposes them to immune-mediated destruction

What's the biological rationale?

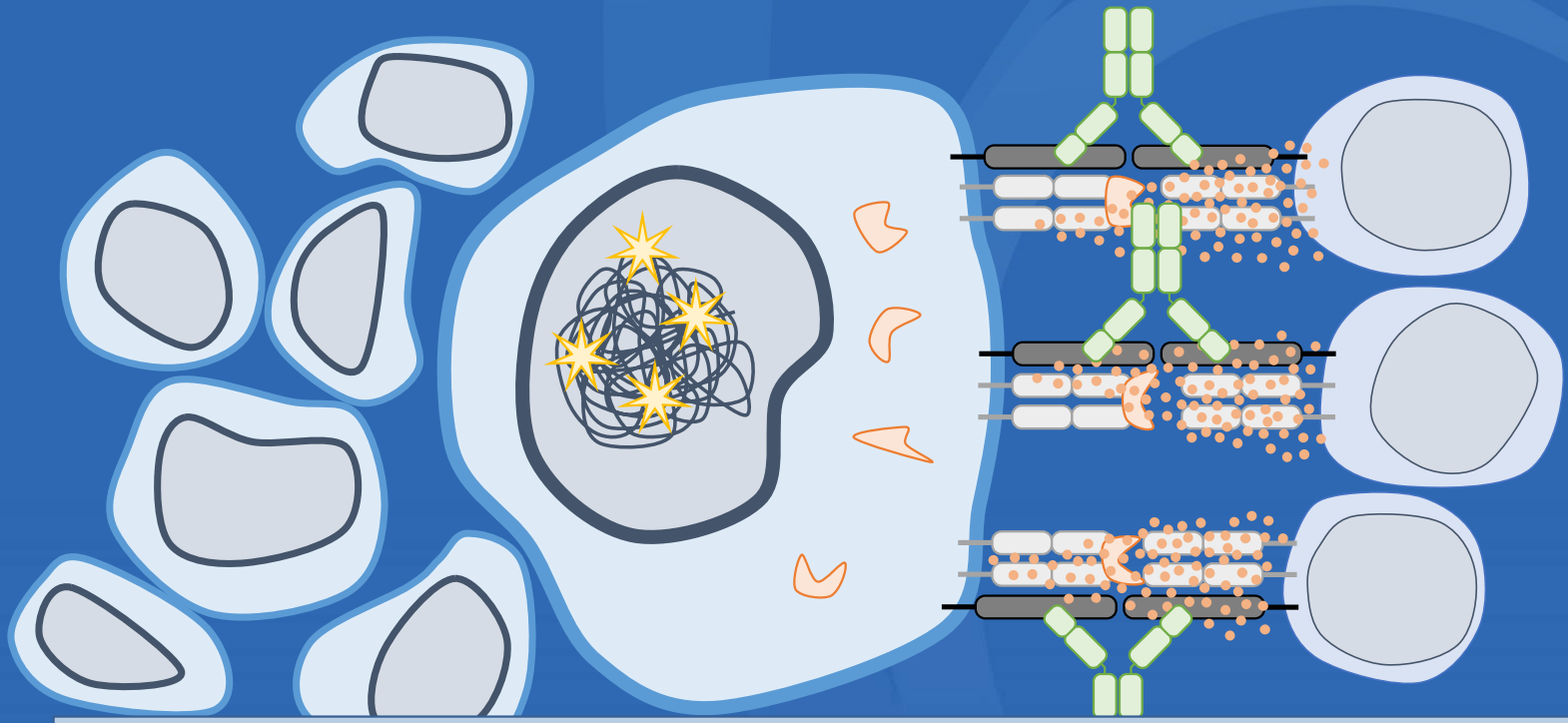
Immune checkpoint expression



Expression of immune checkpoints allows cancer cells to evade the immune response

What's the biological rationale?

Immune checkpoint inhibition



Immune checkpoint inhibitors make cancer cells visible to the immune system

What can help to predict who will respond to immune checkpoint inhibition?

Having a more heavily mutated genome

More neoantigen to target, once immune checkpoints are inhibited

Inability to repair DNA damage:
MMR defects, *POLE* variants impairing proofreading function

Having more immune checkpoint to inhibit

More target to inhibit

PD-L1 expression



What is the *clinical* rationale for PD-L1 testing?

Why are we interested in PD-L1 expression in advanced cervical cancer?



Cervical Cancer

- in combination with chemoradiotherapy, for the treatment of patients with FIGO 2014 Stage III-IVA cervical cancer. (1.12)
- in combination with chemotherapy, with or without bevacizumab, for the treatment of patients with persistent, recurrent, or metastatic cervical cancer whose tumors express PD-L1 (CPS ≥1) as determined by an FDA-approved test. (1.12, 2.1)
- as a single agent for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS ≥1) as determined by an FDA-approved test. (1.12, 2.1)



Cervical cancer

KEYTRUDA, in combination with chemoradiotherapy (external beam radiation therapy followed by brachytherapy), is indicated for the treatment of FIGO 2014 Stage III - IVA locally advanced cervical cancer in adults who have not received prior definitive therapy.

KEYTRUDA, in combination with chemotherapy with or without bevacizumab, is indicated for the treatment of persistent, recurrent, or metastatic cervical cancer in adults whose tumours express PD-L1 with a CPS ≥ 1.



Cervical cancer

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If you want to prescribe pembrolizumab (KEYTRUDA®) without radiotherapy in advanced cervical cancer, you need PD-L1 CPS ≥ 1

U.S. Food and Drug Administration. (2025, April). *KEYTRUDA® (pembrolizumab) injection, for intravenous use. Prescribing information*. Retrieved May 25, 2025, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2025/125514s174lbl.pdf

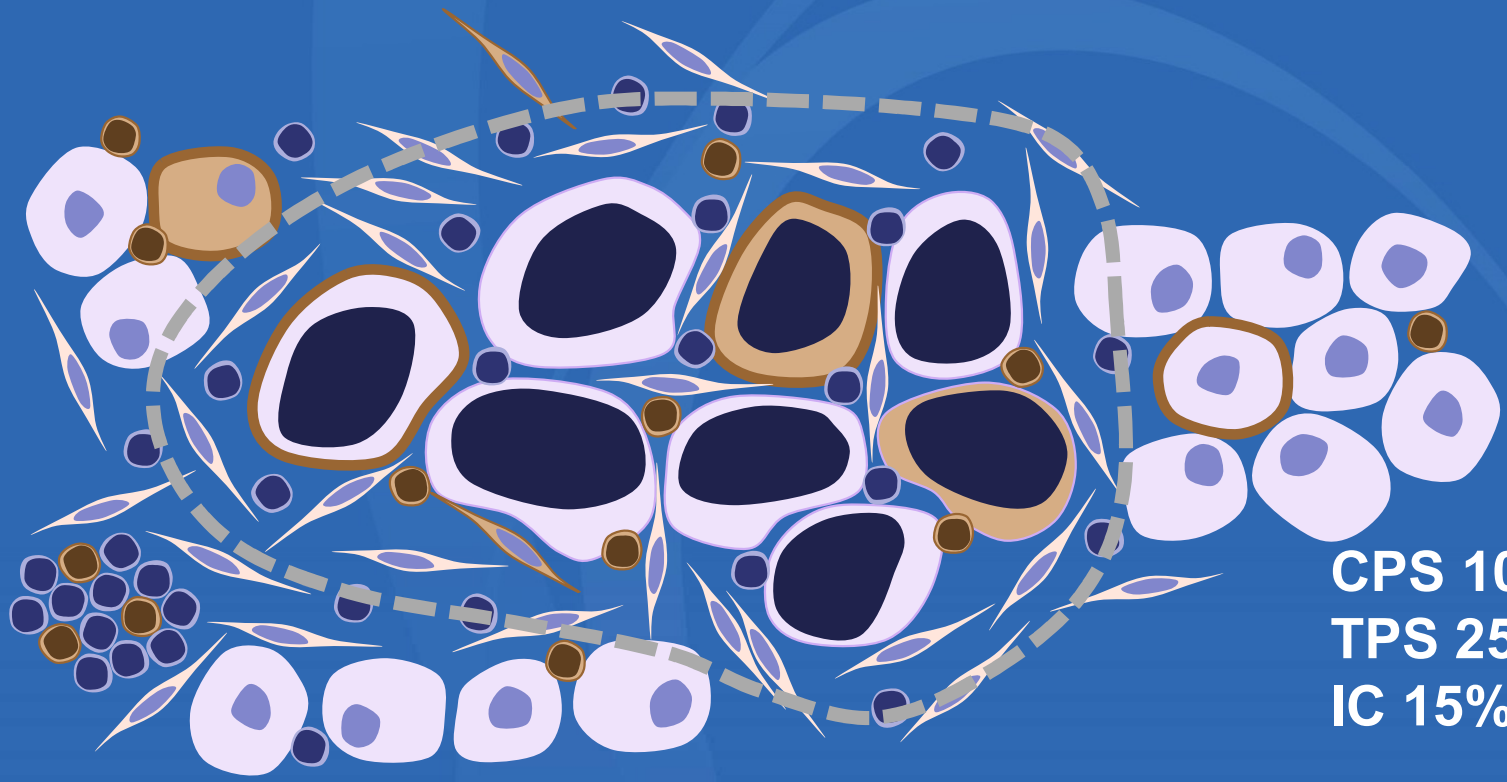
European Medicines Agency. (2025, April). *Keytruda (pembrolizumab): EPAR – Product information*. Retrieved May 25, 2025, from https://www.ema.europa.eu/en/documents/product-information/keytruda-epar-product-information_en.pdf

Merck Sharp & Dohme (UK) Limited. (2025, May 23). *KEYTRUDA 25 mg/mL concentrate for solution for infusion: Summary of Product Characteristics (SmPC)*. Retrieved May 25, 2025, from https://www.medicines.org.uk/emc/product/2498/smpc



How do I interpret PD-L1 staining?

How do we calculate a PD-L1 score?



CPS 100
TPS 25%
IC 15%

$$\text{CPS} = \frac{\text{TC}^{+} + \text{IC}^{+}}{\text{TC}^{\text{total}}} \times 100 = \frac{2+6}{8} \times 100 = 100$$

$$\text{TPS} = \frac{\text{TC}^{+}}{\text{TC}^{\text{total}}} \times 100 = \frac{2}{8} \times 100 = 25\%$$

IC = area of tumour occupied by positive inflammatory cells ≈ 15%

A few caveats
All my opinion…

Pathologists are reliable when it comes to recognising patterns
…not so much when it comes to quantification

I treat PD-L1 scoring as my best estimate of PD-L1 staining
Based on a mixture of approximate quantification and pattern recognition

Our job is to accept that the way the trial assessors interpreted PD-L1 staining is predictive of response, to learn how the trial assessors scored patterns of PD-L1 staining, and try to imitate that as closely as possible in our daily work
…not to try to find sense in the scoring!

I do not count!

Step 1

Is this sample suitable for PD-L1 interpretation?

You need at least 100 tumour cells

They must be fully viable
(not necrotic or on the verge of necrotic)

They must be invasive
(not in situ)

Their morphology must be well preserved
(not autolytic, crushed, subject to heavy heat artefact)

You must have intact fragments of tumour with attached stroma

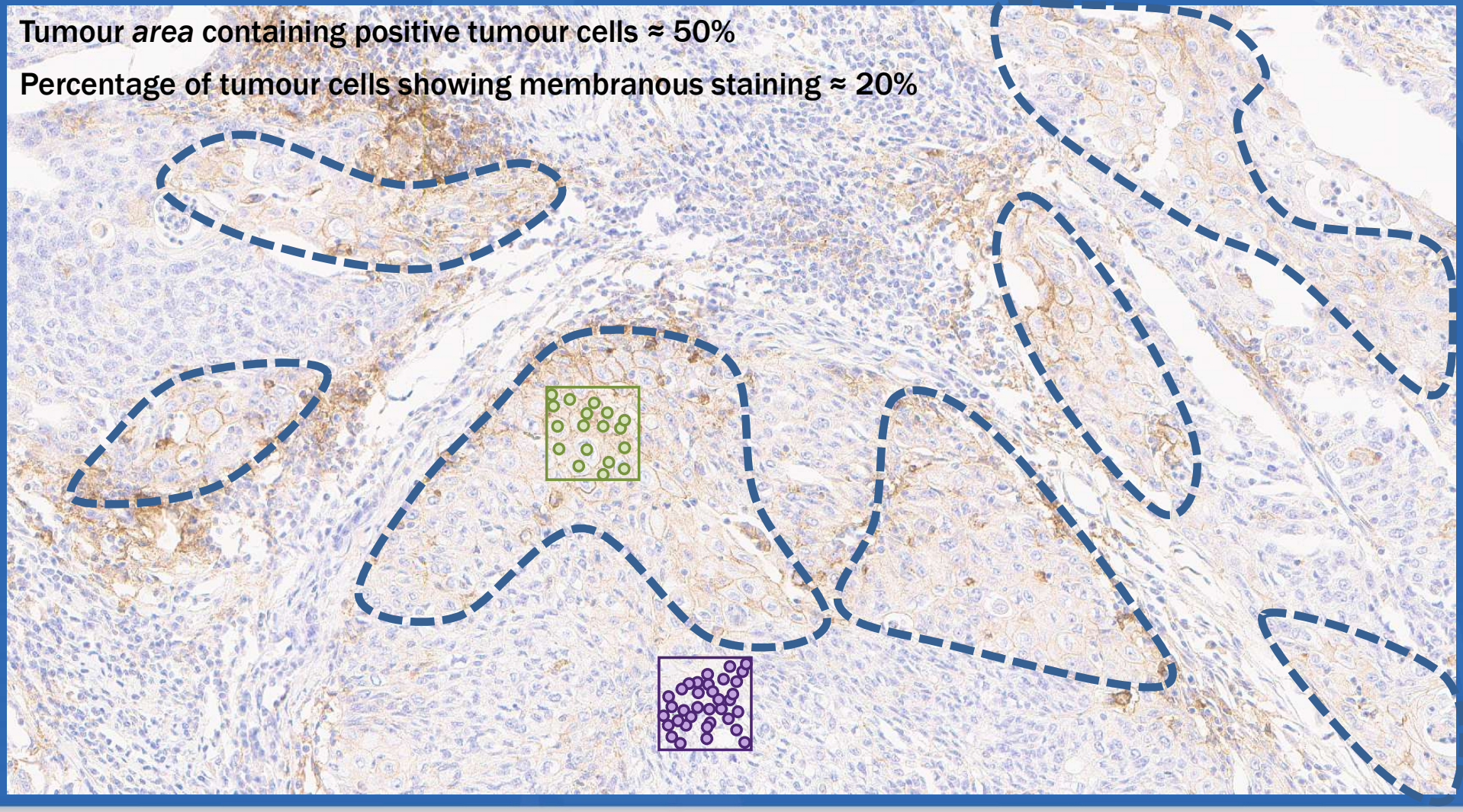
Cytological samples are generally not suitable
(even if processed as cell blocks)

Step 2

What percentage of tumour cells show membranous staining?

$$\text{CPS} = \frac{\text{TC}^{+} + \text{IC}^{+}}{\text{TC}^{\text{total}}} \times 100$$

Which tumour cells should be included in the calculation at all?	When is a tumour cell positive?
They must be fully viable (not necrotic or on the verge of necrotic)	Membranous staining (cytoplasmic alone doesn't count)
They must be invasive (not in situ)	Any degree of completeness
	"Perceptible and convincing" at up to 20 × magnification



Microscopic images provided by Agilent/Dako

Step 3
Add in inflammatory cell staining

$$CPS = \frac{\cancel{TC^+} + IC^+}{\cancel{n}\text{total}} \times 100$$

Which inflammatory cells should be included in the calculation?

They must be lymphocytes or mononuclear macrophages
(not granulocytes, plasma cells or multinucleate cells)

They can show any type of staining, but it must be “perceptible and convincing” at up to 20 × magnification

They must be tumour-associated, representing a response to viable, invasive tumour cells

Step 3
Add in inflammatory cell staining

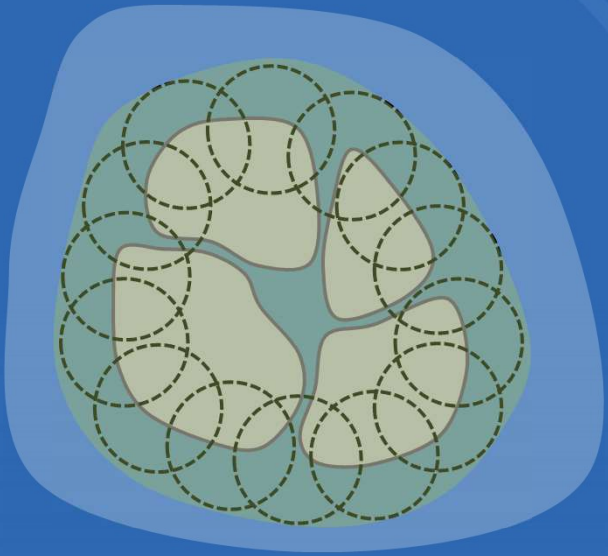
When should inflammatory cells *not* be considered tumour-associated?

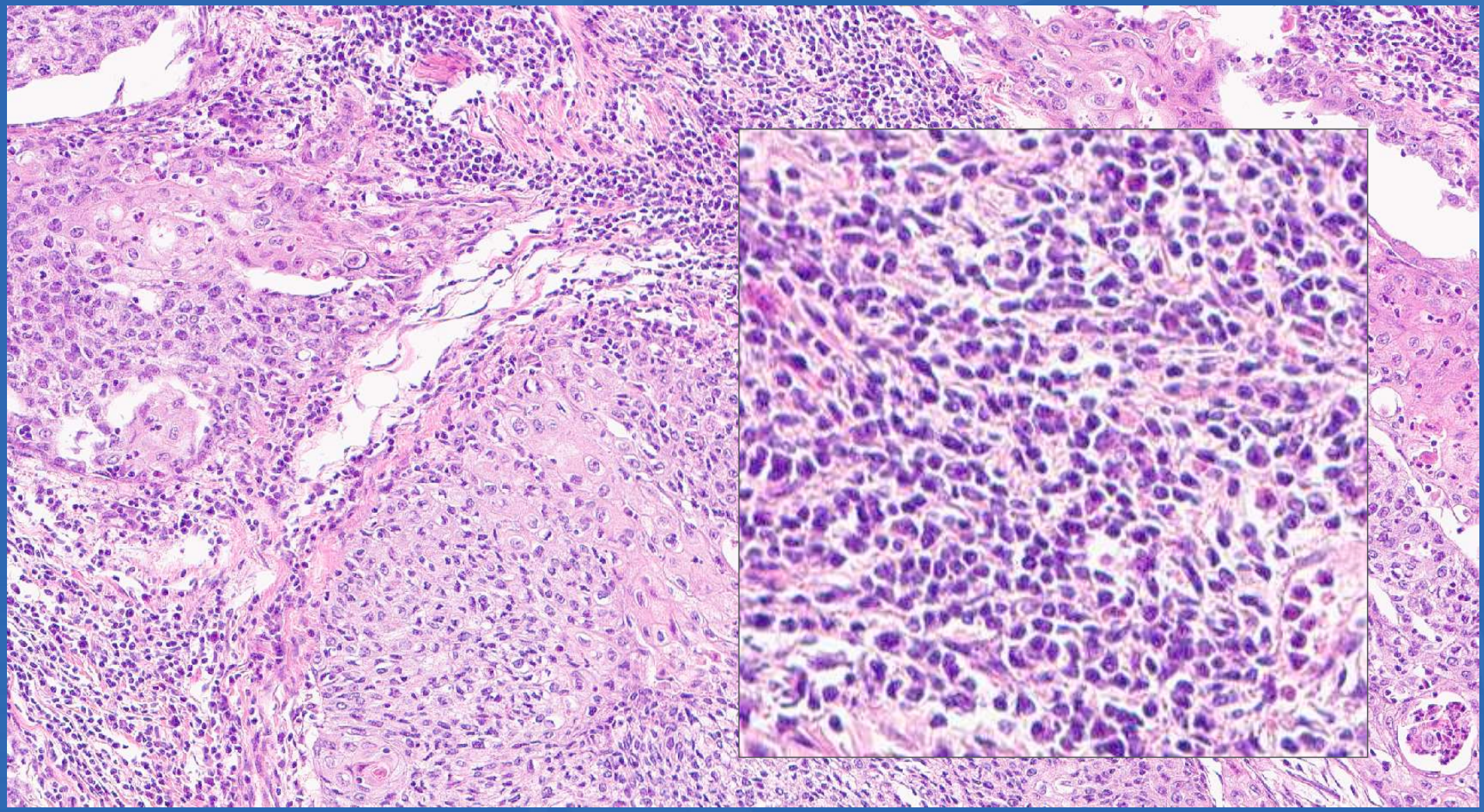
Inflammatory cells which you think are a reflection of cervicitis

Inflammatory cells which you think are a reaction to ulceration or necrosis

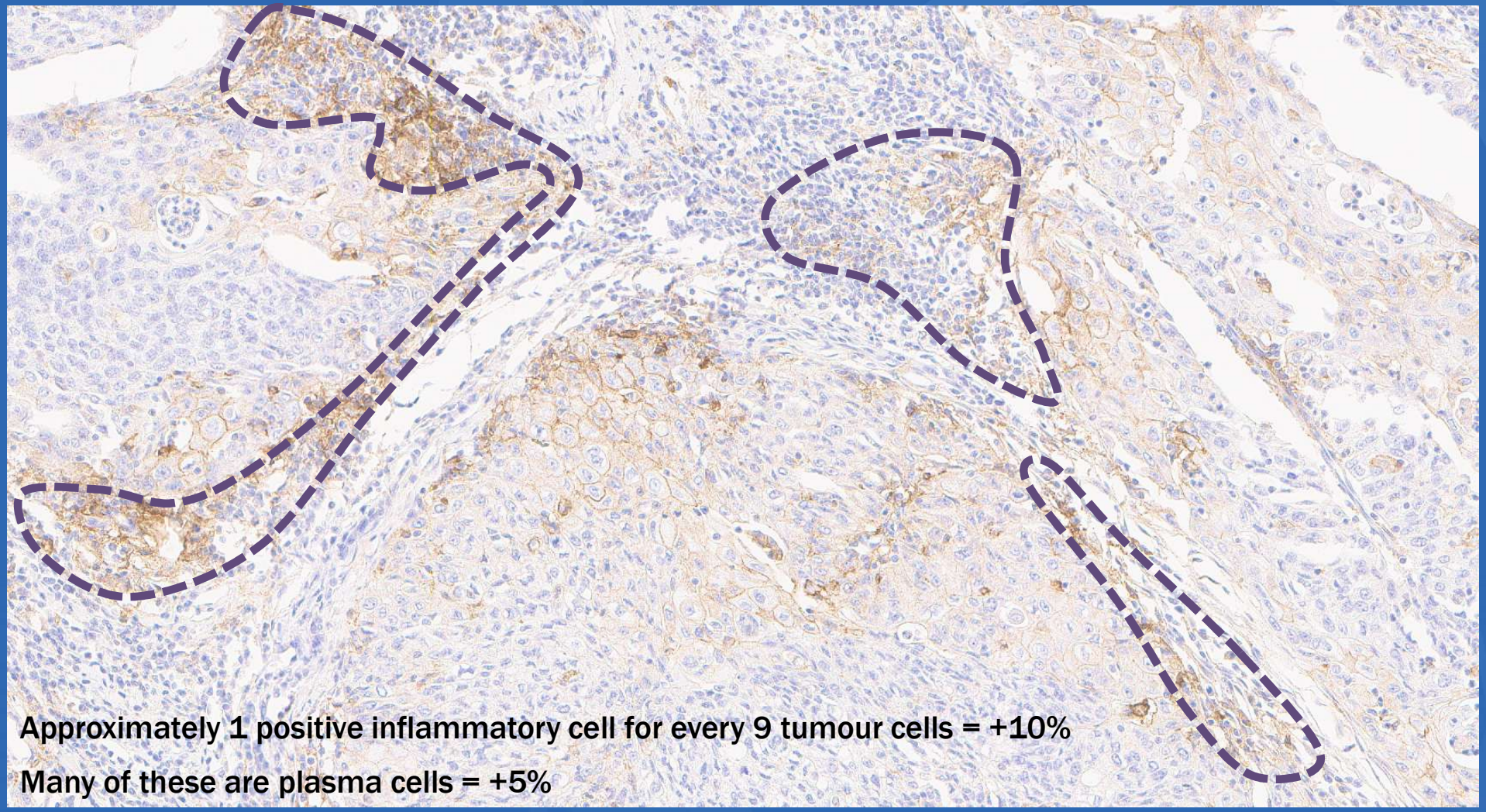
Inflammatory cells which are associated with in situ disease

When does an inflammatory cell stop being tumour-associated?





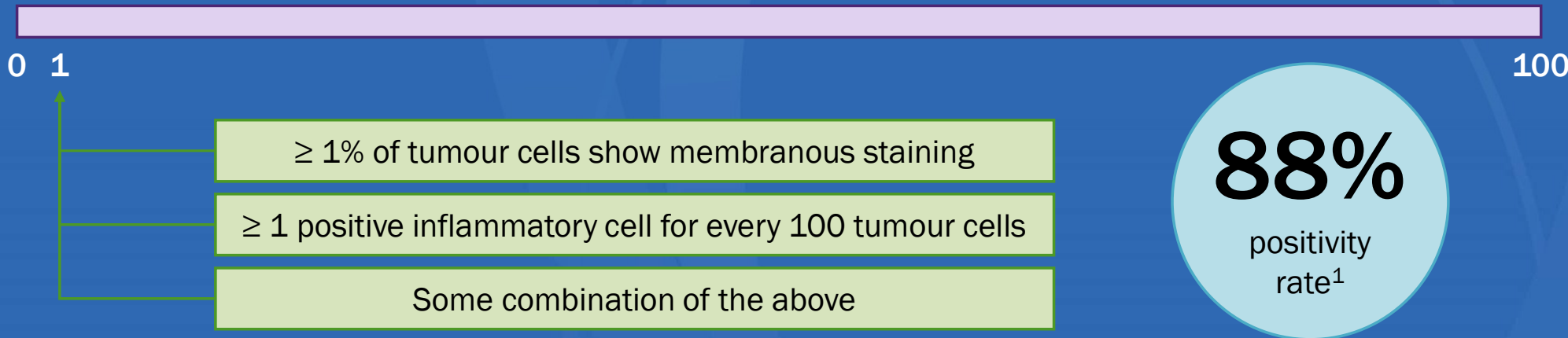
Microscopic images provided by Agilent/Dako



Microscopic images provided by Agilent/Dako

Step 3
Put it all together

CPS = Percentage of tumour cells showing membranous staining + Correction factor based on density of inflammatory cell staining, reduced to reflect the plasma cell/granulocyte content



88% positivity rate[1]

1: Colombo, N., Dubot, C., Lorusso, D., Caceres, M. V., Hasegawa, K., Shapira-Frommer, R., Salman, P., Yañez, E., Gümüş, M., Olivera, M., Samouëlian, V., Castonguay, V., Arkhipov, A., Li, K., Toker, S., Tekin, C., Tewari, K. S., & Monk, B. J. (2021). Pembrolizumab for persistent, recurrent, or metastatic cervical cancer. *New England Journal of Medicine, 385*(20), 1856–1867.

Step 3
Put it all together

Your assessment must be based on all the tissue on the slide – not just representative areas or hotspots



What are some of the key technical considerations?

How should I be doing my staining?

Agilent/Dako PD-L1 IHC **22C3** pharmDx	on	Agilent/Dako Autostainer Link 48
Agilent/Dako PD-L1 IHC **22C3** pharmDx	on	Agilent/Dako Omnis
VENTANA PD-L1 (**SP263**) Assay	on	VENTANA BenchMark ULTRA IHC/ISH System
Agilent/Dako PD-L1 IHC **22C3** pharmDx	on	VENTANA BenchMark ULTRA IHC/ISH System

Colombo, N., Dubot, C., Lorusso, D., Caceres, M. V., Hasegawa, K., Shapira-Frommer, R., Salman, P., Yañez, E., Gümüş, M., Olivera, M., Samouëlian, V., Castonguay, V., Arkhipov, A., Li, K., Toker, S., Tekin, C., Tewari, K. S., & Monk, B. J. (2021). Pembrolizumab for persistent, recurrent, or metastatic cervical cancer. *New England Journal of Medicine, 385*(20), 1856–1867.



Put it to the test!