PD-L1 Immunostaining in Cervical & Vulvar Squamous Cell Carcinoma

PRESENTED BY
Anne M. Mills, MD
University of Virginia Department of Pathology

USCAP 109TH ANNUAL MEETING
2020 EYES ON YOU
USCAP

Important Information Regarding CME/SAMs

The **Online CME/Evaluations/SAMs claim** process will only be available on the USCAP website until **September 30, 2020**

No claims can be processed after that date!

After **September 30, 2020** you will NOT be able to obtain any CME or SAMs credits for attending this meeting.

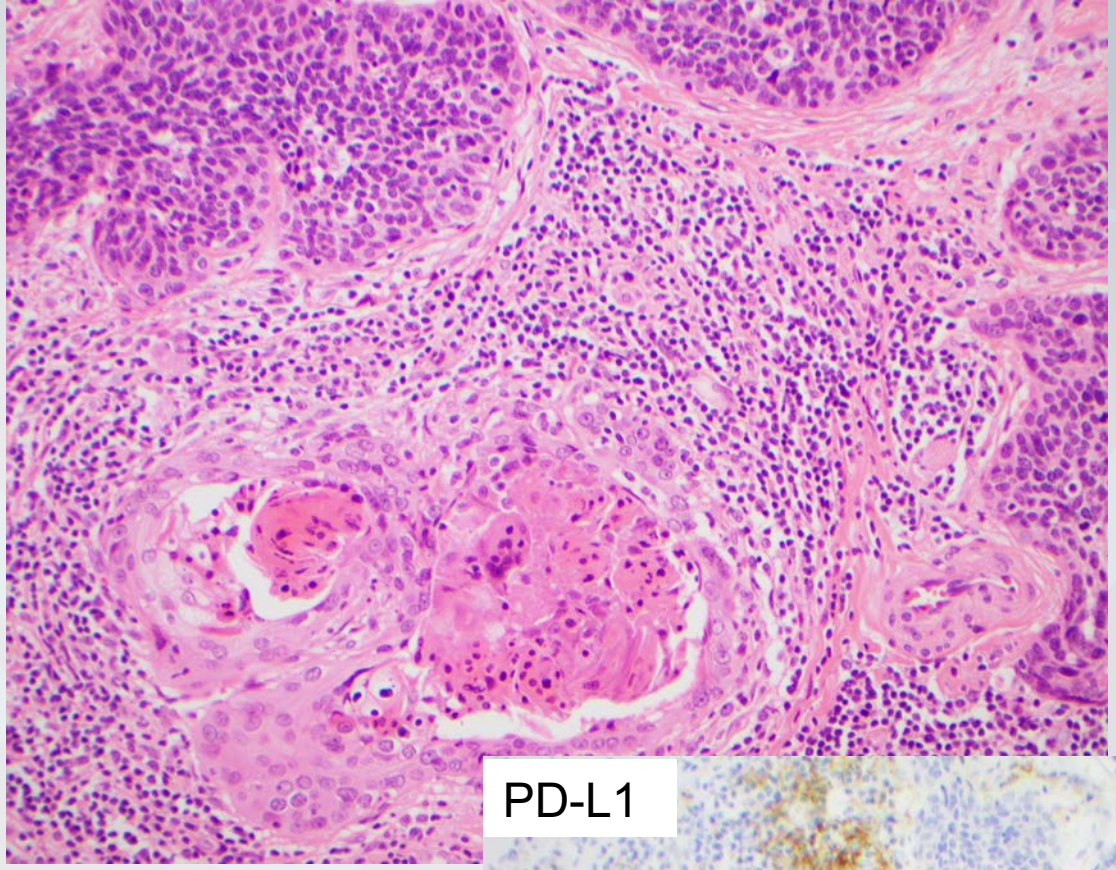
Disclosure of Relevant Financial Relationships

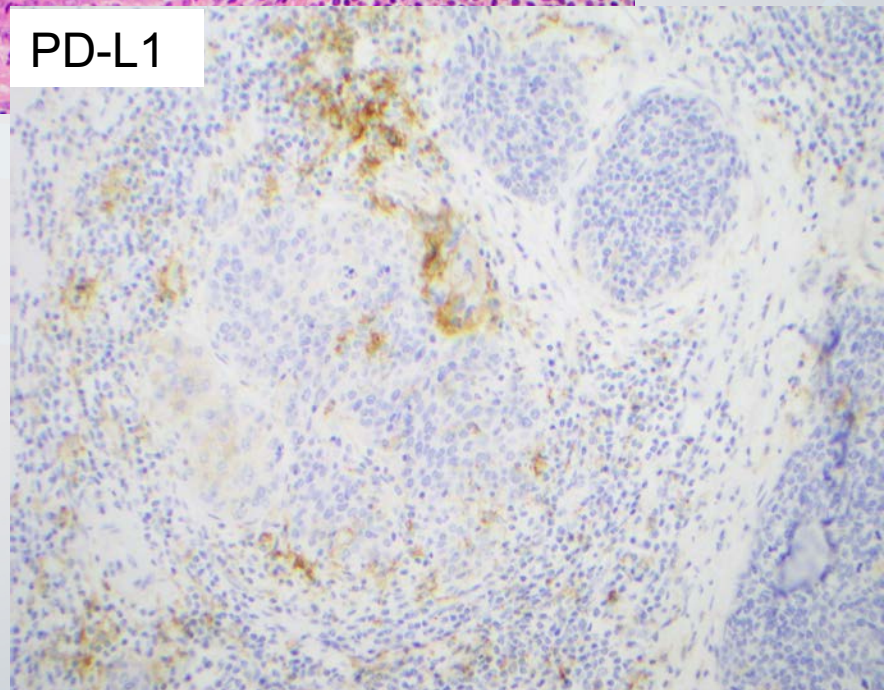
The faculty, committee members, and staff who are in position to control the content of this activity are required to disclose to USCAP and to learners any relevant financial relationship(s) of the individual or spouse/partner that have occurred within the last 12 months with any commercial interest(s) whose products or services are related to the CME content. USCAP has reviewed all disclosures and resolved or managed all identified conflicts of interest, as applicable.

Anne M. Mills reported no relevant financial relationships.

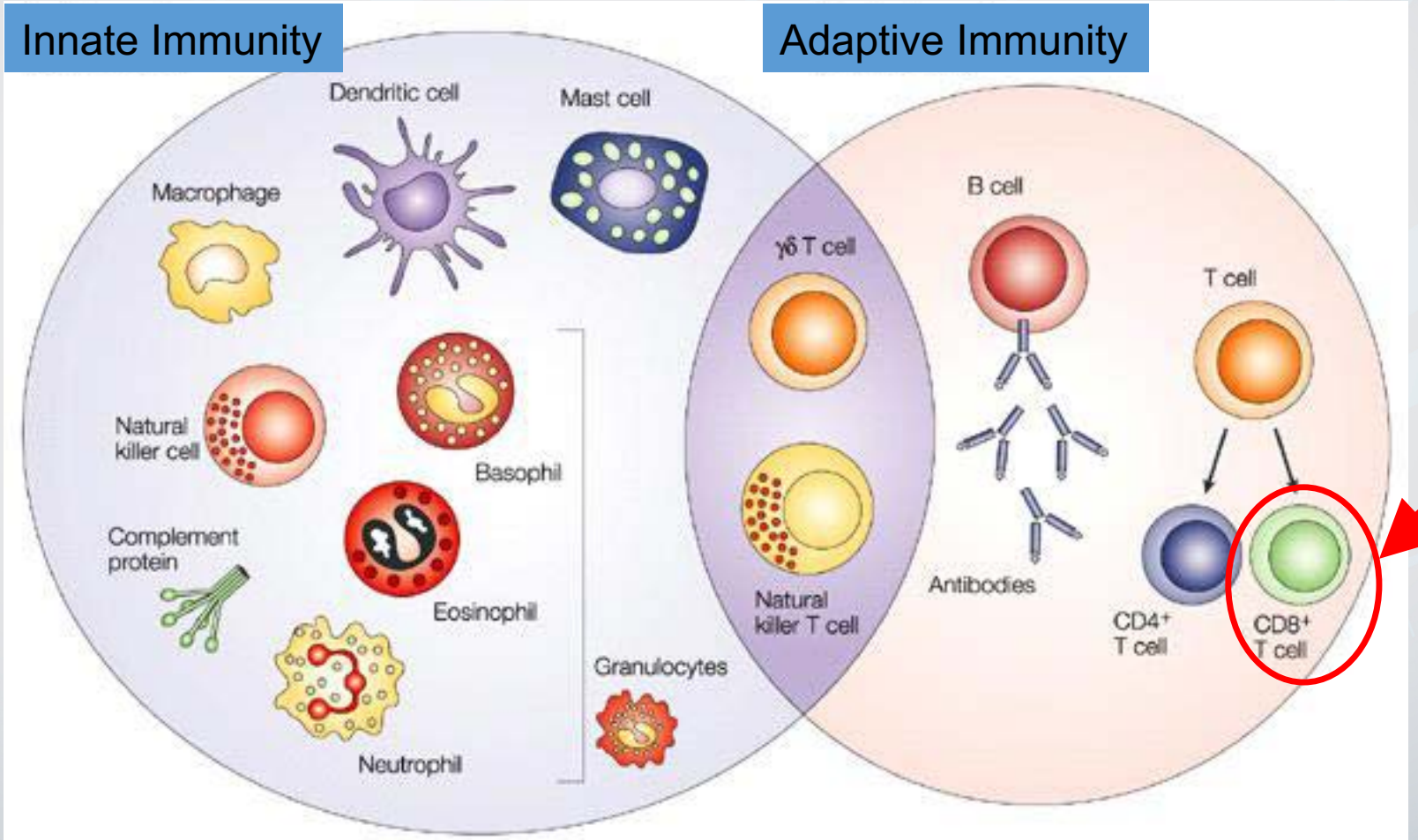
PLEASE TURN OFF YOUR CELL PHONES

- A 35-year-old woman presents with recurrent cervical squamous cell carcinoma following chemotherapy and radiation.
- Your colleagues in the Gynecologic Oncology group would like to be able to offer pembrolizumab in this patient.
- Here's her PD-L1 stain.
- Does she qualify for therapy?





The Two Arms of the Immune System



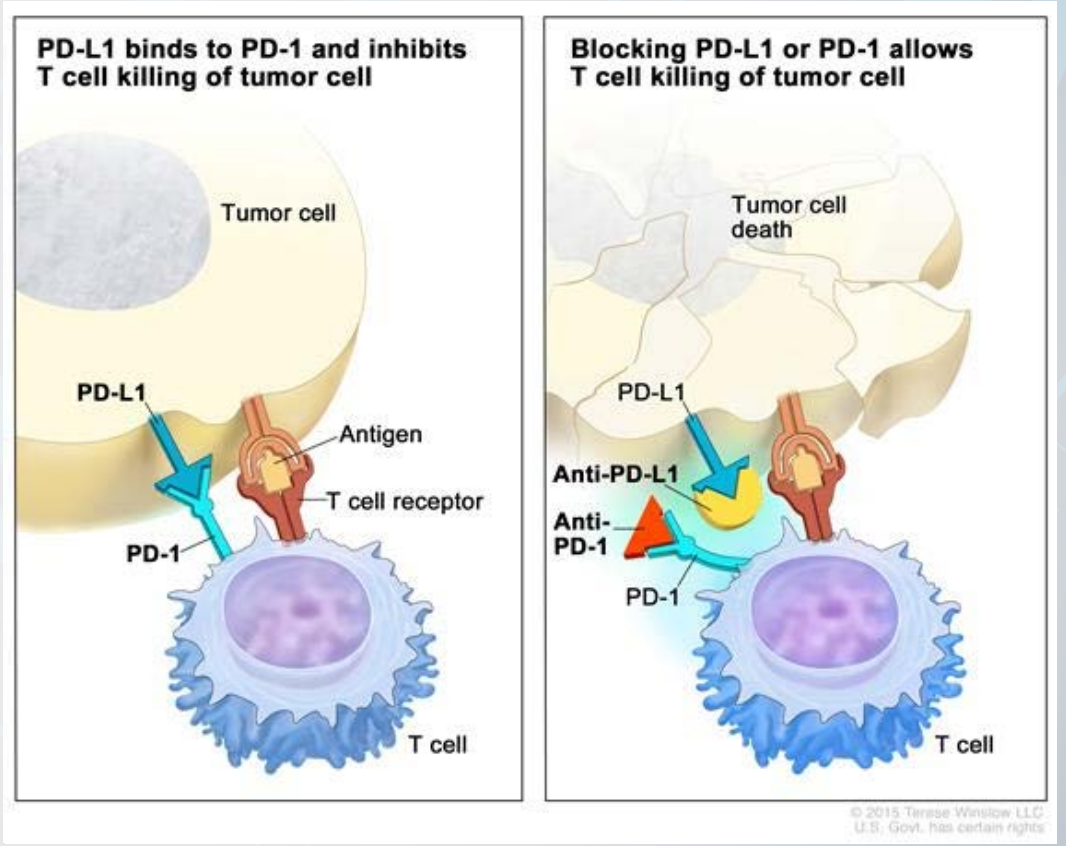
Nature Reviews Cancer, 2004; 4, 11-22.

Immunotherapy 101

- Immune checkpoints such as PD-1 put the brakes on the adaptive immune response to prevent perpetual activation following infection etc.
  - The PD-1/PD-L1 interaction promotes **immune tolerance.**

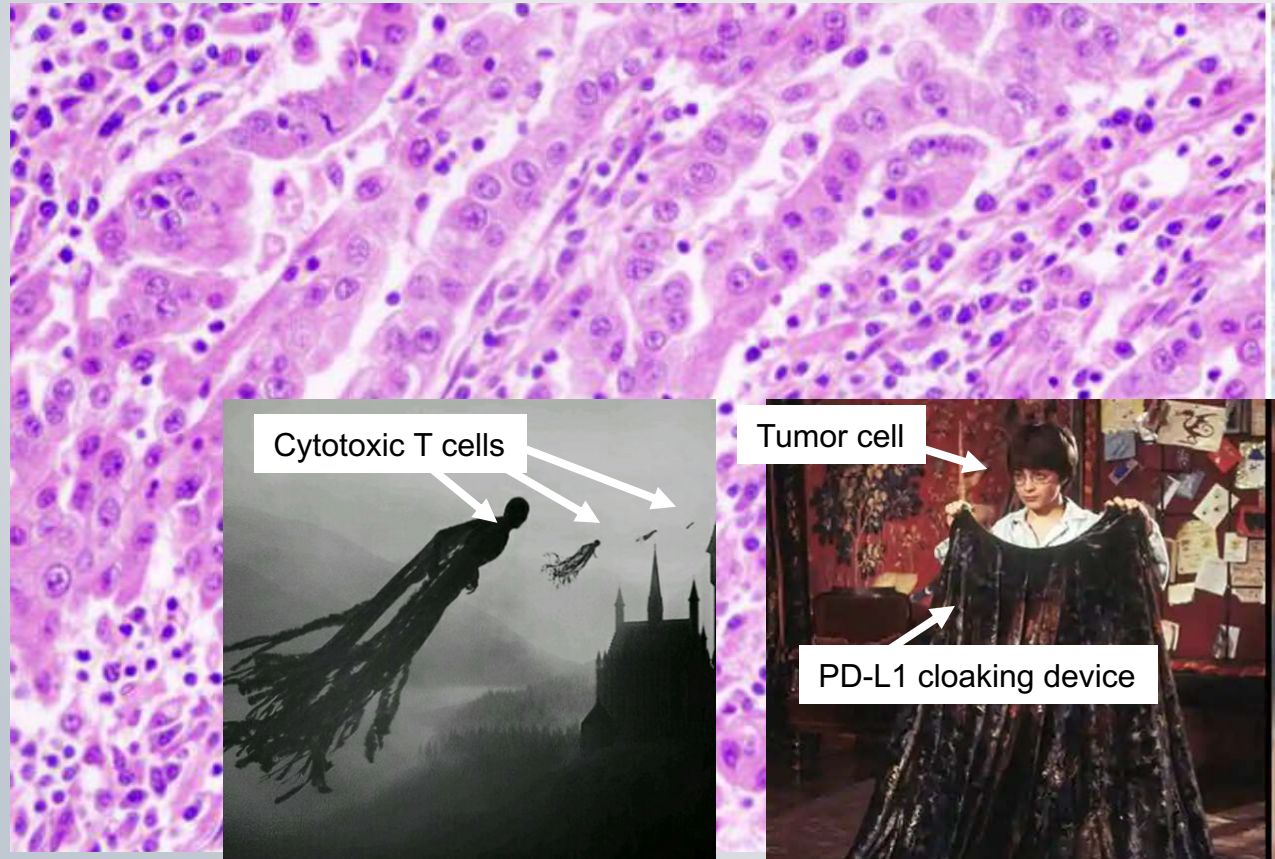
- Checkpoint ligands such as PD-L1 can be co-opted by tumor cells as a “cloaking device” to evade immune attack.

- Blocking these inhibitory checkpoints (or their ligands) “takes off the cloak” and allows cytotoxic T cells to recognize and attack tumor.



https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-checkpoint-inhibitor

Programed cell death ligand-1 (PD-L1): *A Cancer Cloaking Device*



The Oncologist
The official journal of the Society for Translational Oncology

FDA Approval Summary: Pembrolizumab for Treatment of Metastatic Non-Small Cell Lung Cancer: First-Line Therapy and Beyond

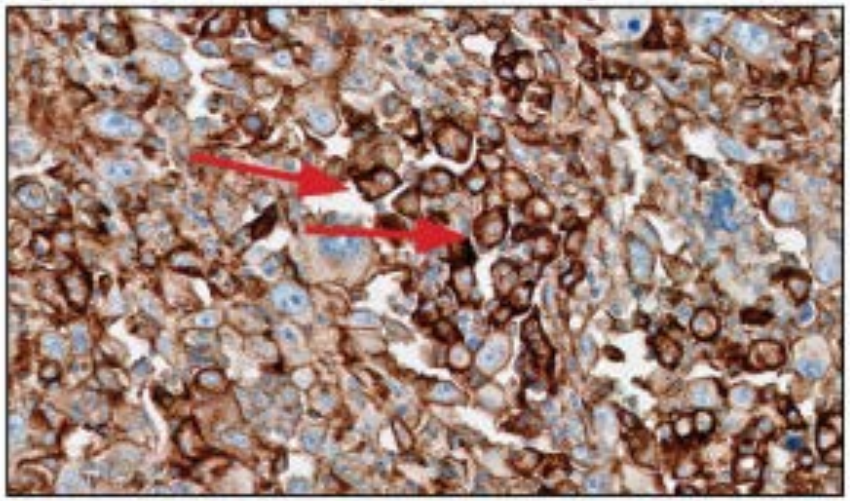
Abstract

On October 24, 2016, the U.S. Food and Drug Administration (FDA) approved pembrolizumab (Keytruda; Merck & Co., Inc., https://www.merck.com) for treatment of patients with metastatic non-small cell lung cancer (mNSCLC) whose tumors express programmed death-ligand 1 (PD-L1) as determined by an FDA-approved test, as follows: (a) first-line treatment of patients with mNSCLC whose tumors have high PD-L1 expression (tumor proportion score [TPS] ≥50%), with no epidermal growth factor receptor (*EGFR*) or anaplastic lymphoma kinase (*ALK*) genomic tumor aberrations, and (b) treatment of patients with mNSCLC whose tumors express PD-L1 (TPS ≥1%), with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab.

TPS=Tumor Proportion Score

TPS=Percentage of viable tumor showing partial or complete membranous staining of any intensity.

- Negative=TPS<1%
- Positive=TPS≥1%
- Need to provide “exact” percentage in addition to positive/negative as treatment threshold varies (most common cut-offs are 1% and 50%).



PD-L1 primary antibody exhibiting linear membrane staining distinct from cytoplasmic staining (arrows) (20× magnification).

FDA approves pembrolizumab for advanced cervical cancer with disease progression during or after chemotherapy

On June 12, 2018, the Food and Drug Administration approved pembrolizumab (Keytruda, Merck and Co. Inc.) for 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.

Pembrolizumab was investigated in 98 patients with recurrent or metastatic cervical cancer enrolled in a single cohort of KEYNOTE 158 (NCT02628067), a multicenter, non-randomized, open-label, multi-cohort trial. Patients were treated with pembrolizumab intravenously at a dose of 200 mg every 3 weeks until unacceptable toxicity or documented disease progression. Among the 98 patients, approval was based on 77 (79%) patients who had tumors that expressed PD-L1 with a CPS ≥1 and who had received at least one line of chemotherapy for metastatic disease. PD-L1 status was determined using the PD-L1 IHC 22C3 pharmDx Kit.

The major efficacy outcomes were objective response rate (ORR) according to RECIST 1.1 as assessed by blinded independent central review, and response duration. With a median follow-up time of 11.7 months, the ORR in 77 patients was 14.3% (95% CI: 7.4, 24.1), including 2.6% complete responses and 11.7% partial responses. The estimated median response duration based on 11 patients with a response by independent review was not reached (range 4.1, 18.6+ months); 91% had a response duration of greater than or equal to 6 months. No responses were observed in patients whose tumors did not have PD-L1 expression (CPS <1).

What about vulva?

Gynecol Obstet Invest. 2019;84(1):94-98. doi: 10.1159/000491090. Epub 2018 Jul 17.

Pembrolizumab in Recurrent Squamous Cell Carcinoma of the Vulva: Case Report and Review of the Literature.

Shields LBE[1], Gordinier ME[2].

Author information

Abstract

Advanced vulvar cancer is associated with a very poor prognosis. Surgical resection is the mainstay of treatment, with radiation indicated for areas at high risk for recurrence. When surgical and radiation options have been exhausted, the effectiveness of systemic chemotherapy is poor. No biologic or targeted agents have been approved for the management of advanced or recurrent vulvar cancer. Pembrolizumab, a humanized monoclonal antibody against programmed death 1 (PD-1), has been successfully used as a target of tumor immune therapy in small cell lung cancer and melanoma. We present the first case in the literature of a patient with recurrent vulvar cancer who was treated successfully with pembrolizumab. Caris next-generation testing revealed a PD-L1 and PD-1 mutation (PD-L1 positive, 2+, 100%). She attained a complete clinical remission after 2 cycles, and a CT scan after 6 cycles revealed a significant response by RECIST criteria. After completing 10 cycles, treatment was stopped due to complications of severe malnutrition related to narcotic abuse. A CT scan 10 weeks after the final treatment revealed no adenopathy. Pembrolizumab is a safe and effective chemotherapeutic agent to treat recurrent vulvar carcinoma.

If you are asked to perform PD-L1 IHC on a vulvar squamous cancer, report it as you would a cervical case.

- No existing FDA approval for any immunotherapeutic agent in vulvar squamous cell carcinoma.
- Case reports suggest that pembrolizumab can be safe and effective in these tumors, but large studies are lacking.

CPS=Combined Positive Score

$$\frac{\text{\#PD-L1 staining cells (tumor cells, lymphocytes, and macrophages)}}{\text{\# viable tumor cells}} \times 100$$

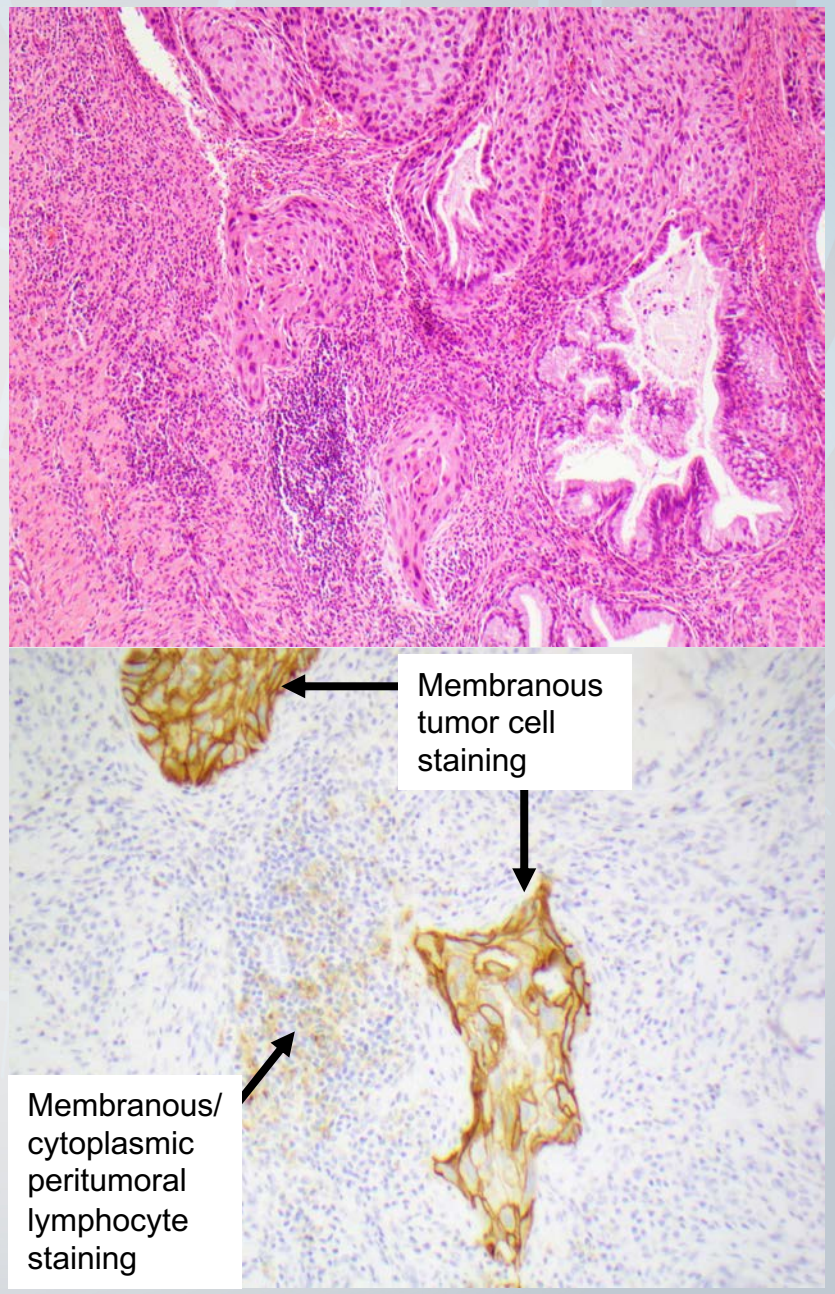
- Negative=CPS<1
- Positive=CPS≥1

Pet Peeves:

- **CPS is NOT a %.** The numerator and the denominator refer to different collections of cells!
- **It's "CPS" not "CPS Score."** The latter is redundant.

How to Assess the CPS:

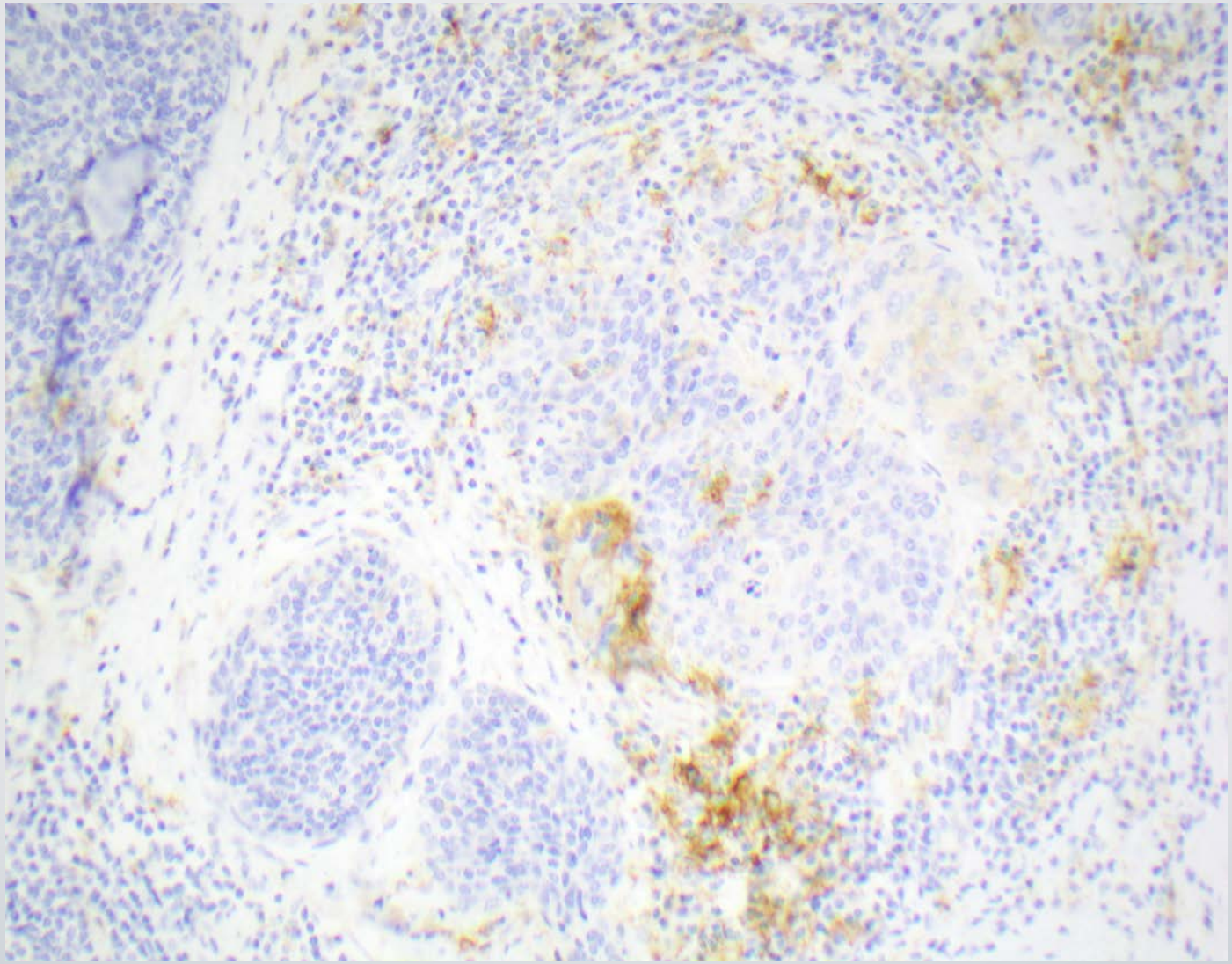
- Any CPS from 1-100 is positive.
  - 100 is the maximum allowable score.
- CPS is averaged across the entire tumor.
  - Don't just count the hot spots!
- CPS should be assessed at 20x to ensure that even focal positivity is captured.
- Tumor cell staining must be membranous.
- Immune cell staining may be membranous or cytoplasmic.
- PD-L1+ lymphocytes and macrophages must be associated with response to the tumor.
  - Location can be either intratumoral or peritumoral.
  - Lymphoid aggregates count, provided they are within or immediately adjacent to the tumor.



What doesn’t count:

- Cells in stroma distant from tumor **do not** count.
- In nodal metastases, immune cells in normal nodal tissue adjacent to the metastatic deposit **do not** count.
- Immune cells associated with dyplasia and normal structures **do not** count.
- Plasma cells often show weak positive staining, but **do not** count.

What if staining is focal or heterogeneous?



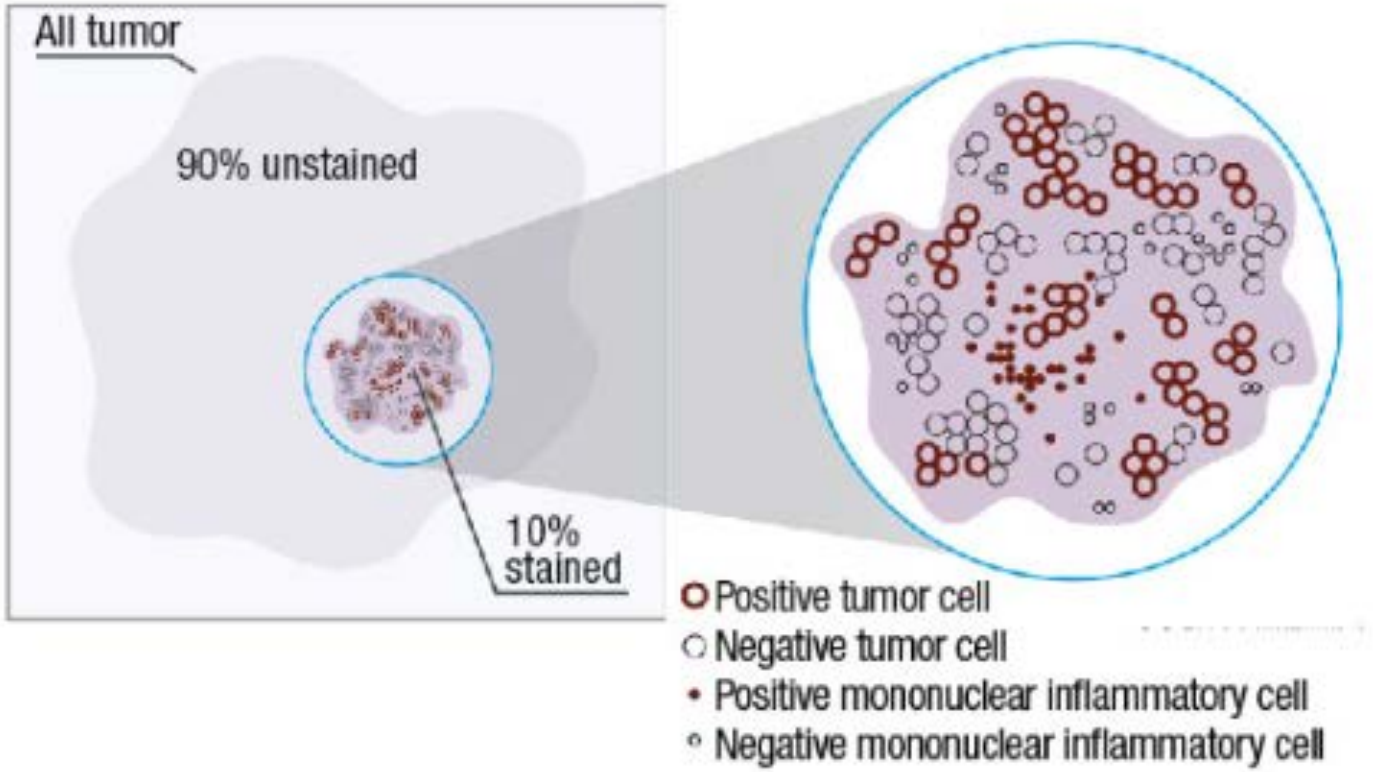
Focal staining

Calculate the CPS for the focus of positivity, then multiply by the proportion of positive tumor.

- Example 1: 10% of the tumor has a CPS=80,
  - 80 x 0.10=8, overall **CPS=8 (≥1, positive)**

- Example 2: 10% of the tumor has a CPS=5
  - 5 x 0.10=0.5, overall **CPS=0.5 (<1,negative)**

Focal staining:



In the stained area, 50 of 100 tumor cells are PD-L1 positive, and there are 34 PD-L1-positive mononuclear inflammatory cells (MIC).

Combined positive score:

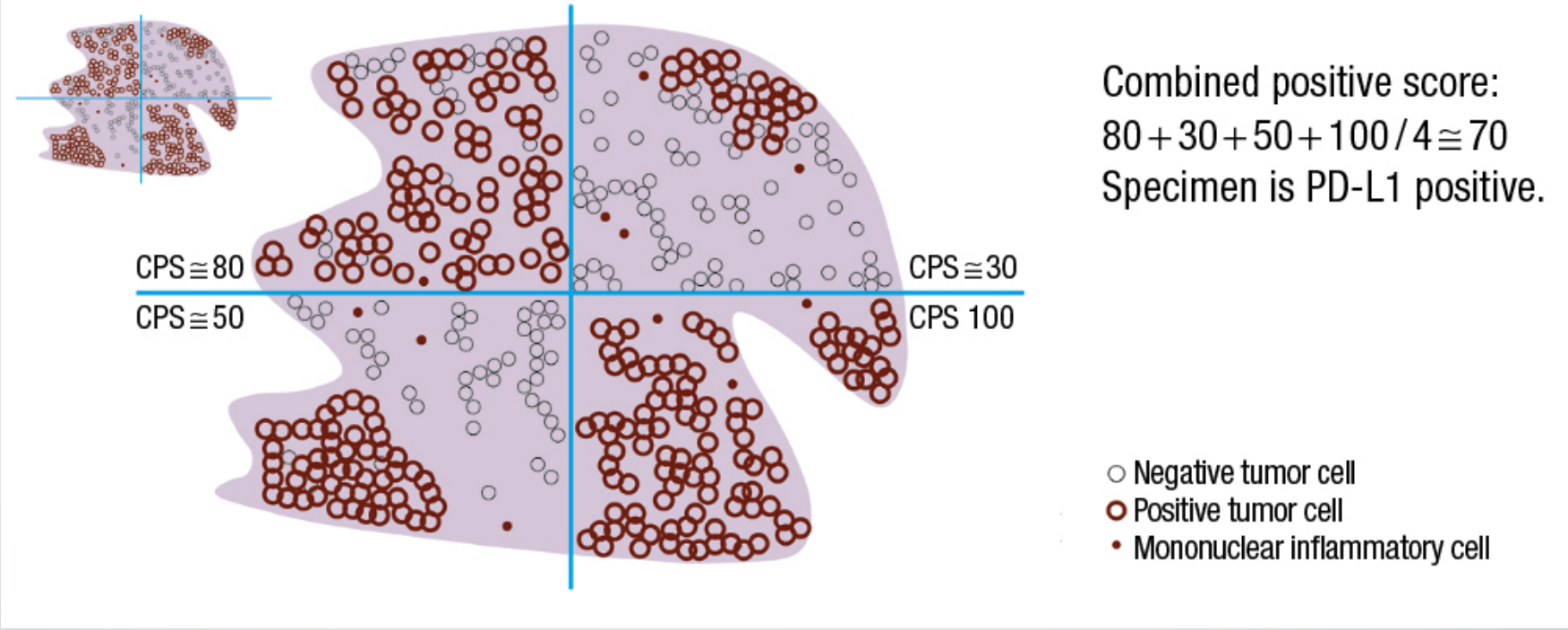
$$\frac{84 \text{ positive cells}}{100 \text{ tumor cells}} \times 100 \cong 80$$

10% of 80 = 8
Specimen is PD-L1 positive.

http://www.captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

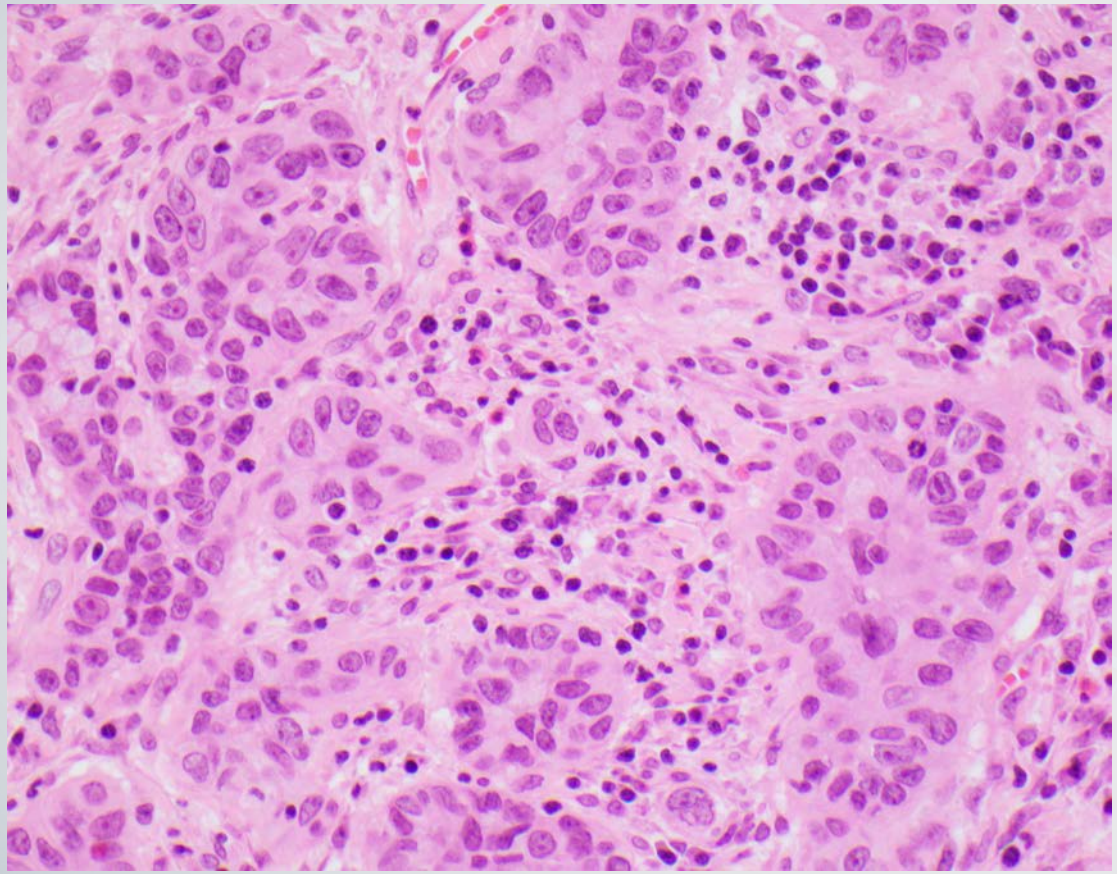
Heterogeneous staining:

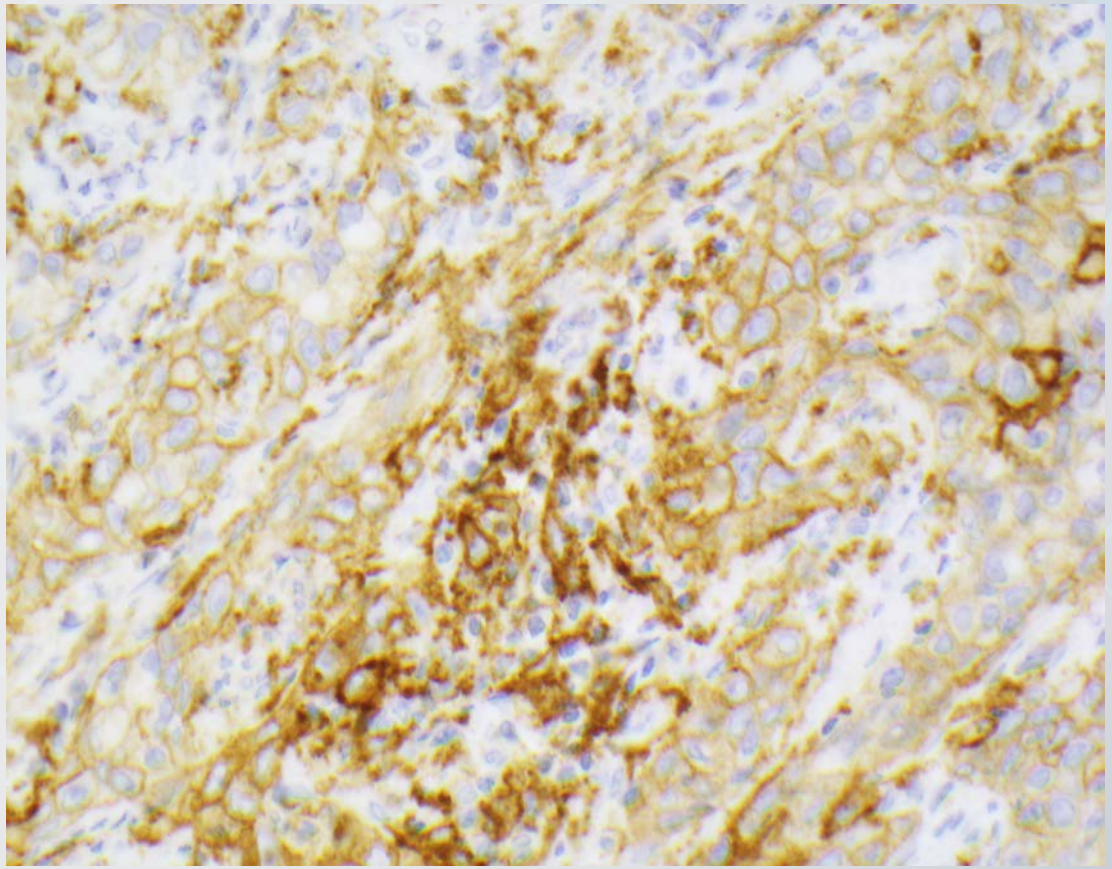
Use the “divide and conquer” technique: Divide the tumor into four equal quadrants, calculate the CPS by sector, and divide by 4.



http://www.captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

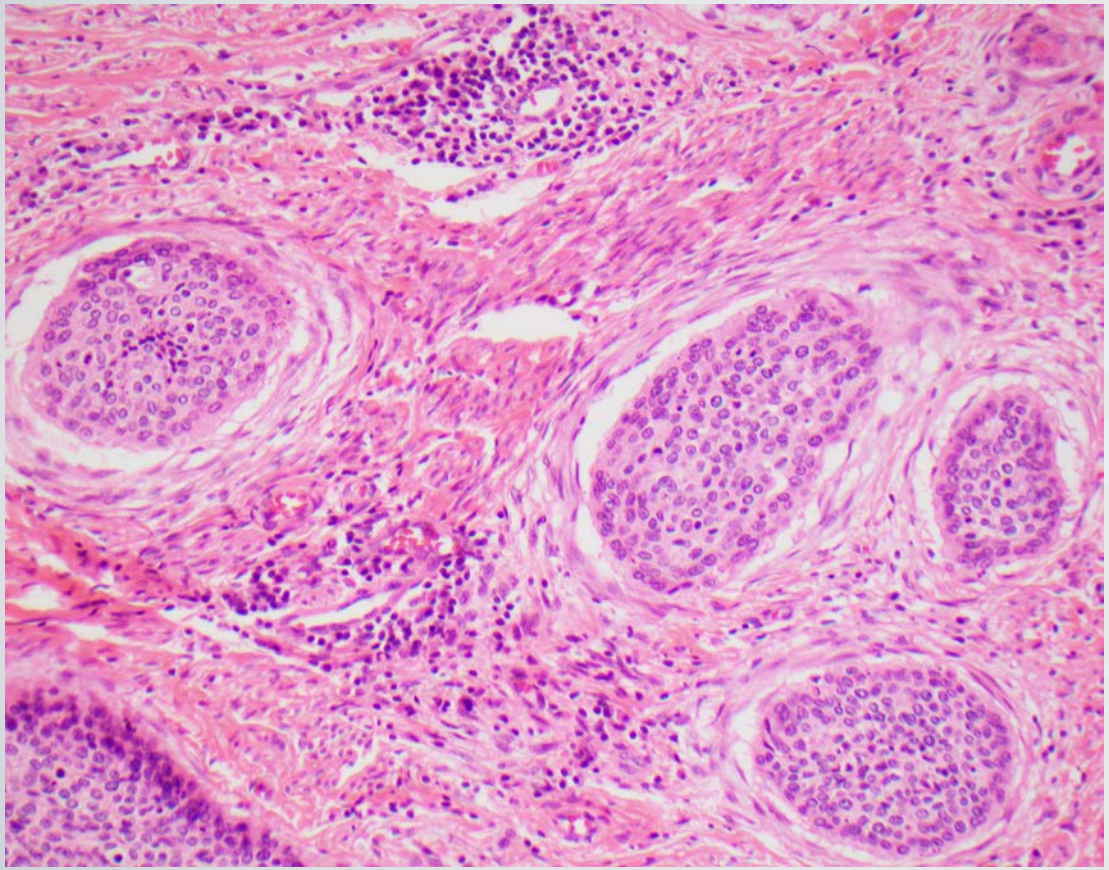
Example 1:

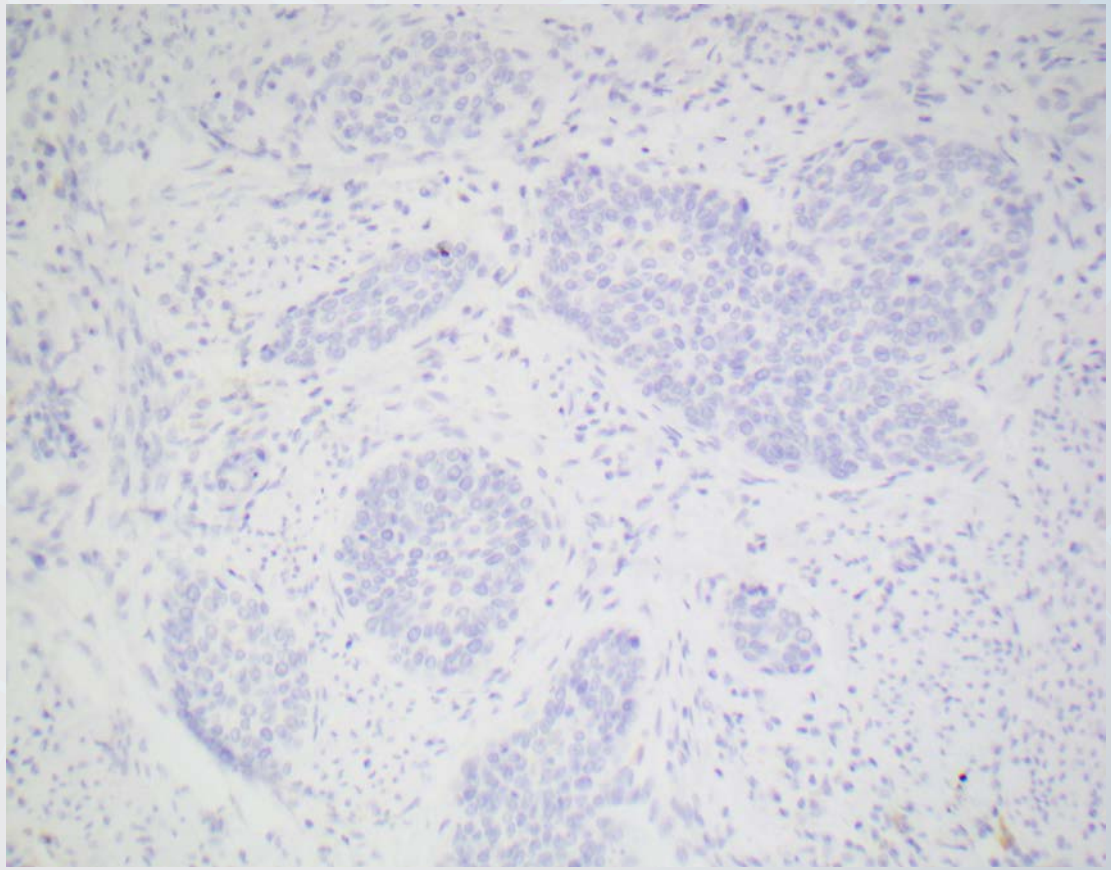




- Easy! Positive, CPS=100.
- With 100% of tumor cells staining, we can easily get to the maximum CPS of 100 without even bothering to count inflammatory cells.
- The entire tumor looked like this, so there was no need to average cross fields.

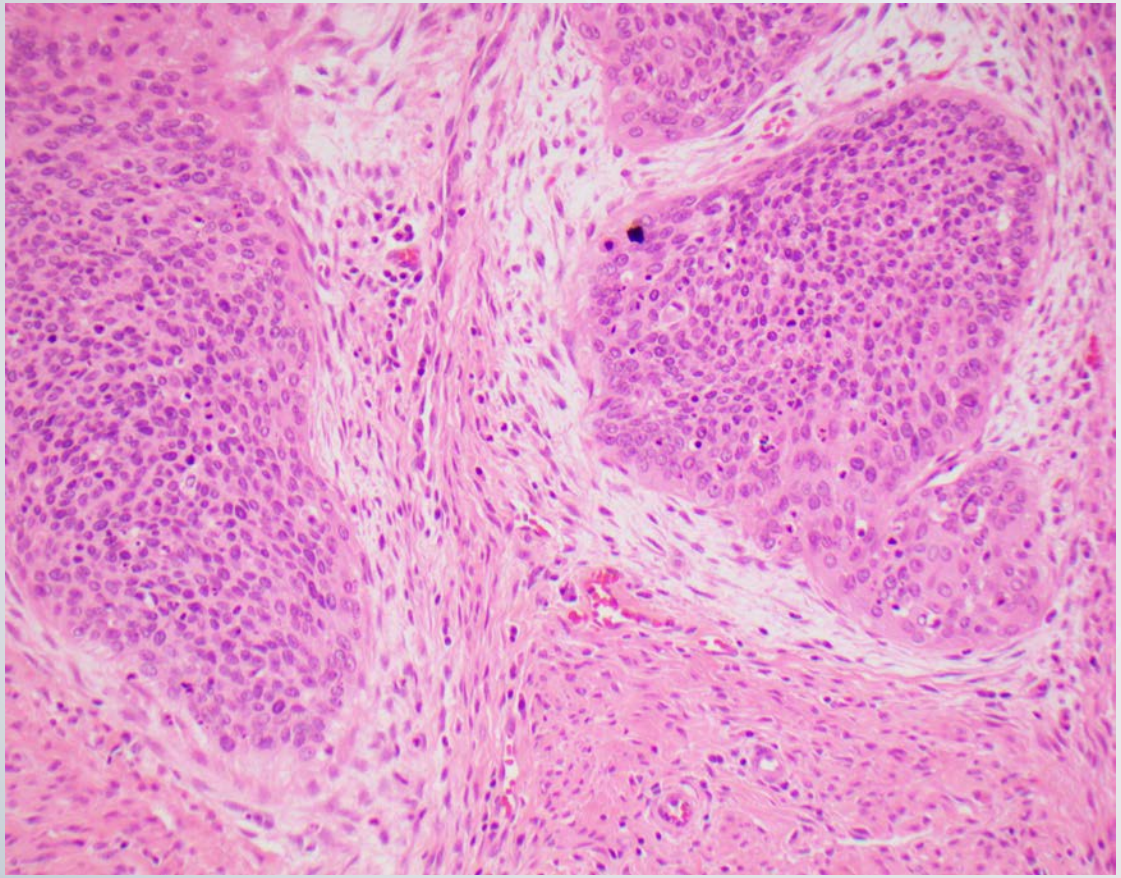
Example 2:

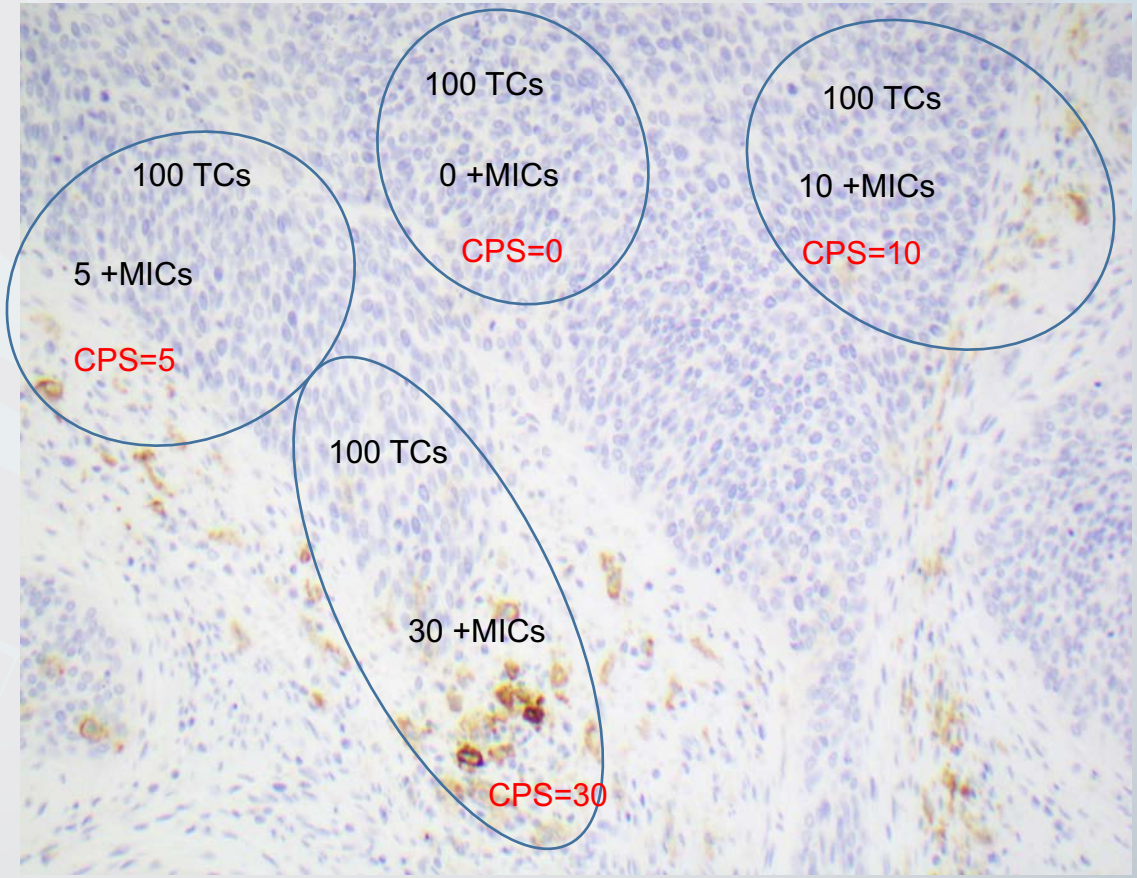




- Easy! Negative (CPS<1).
- What if another field of this case, representing 5% of the total tumor, had 10 PD-L1-positive macrophages? 10 x 0.05=0.5, CPS is still negative (<1).
- What if that field had 25 PD-L1-positive lymphocytes? 25 x 0.05=1.25, CPS is now **positive (≥1).**

Example 3: MIC

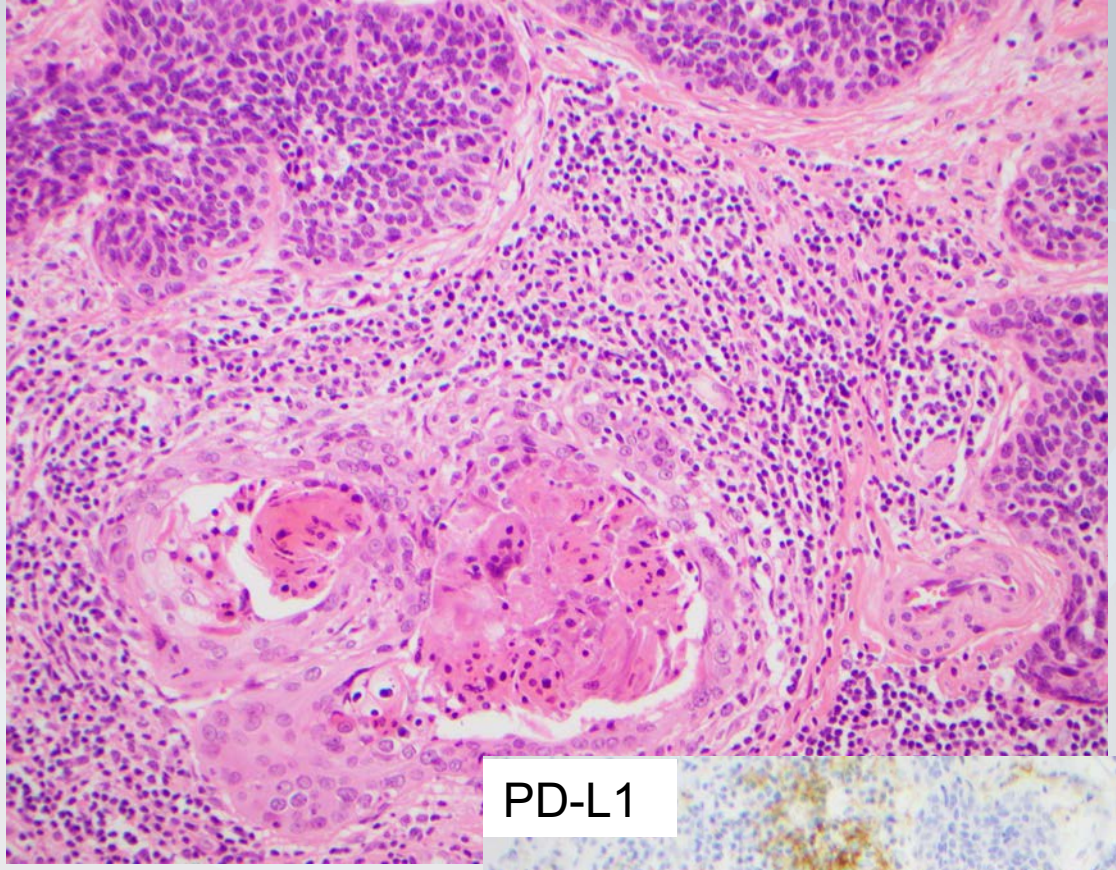


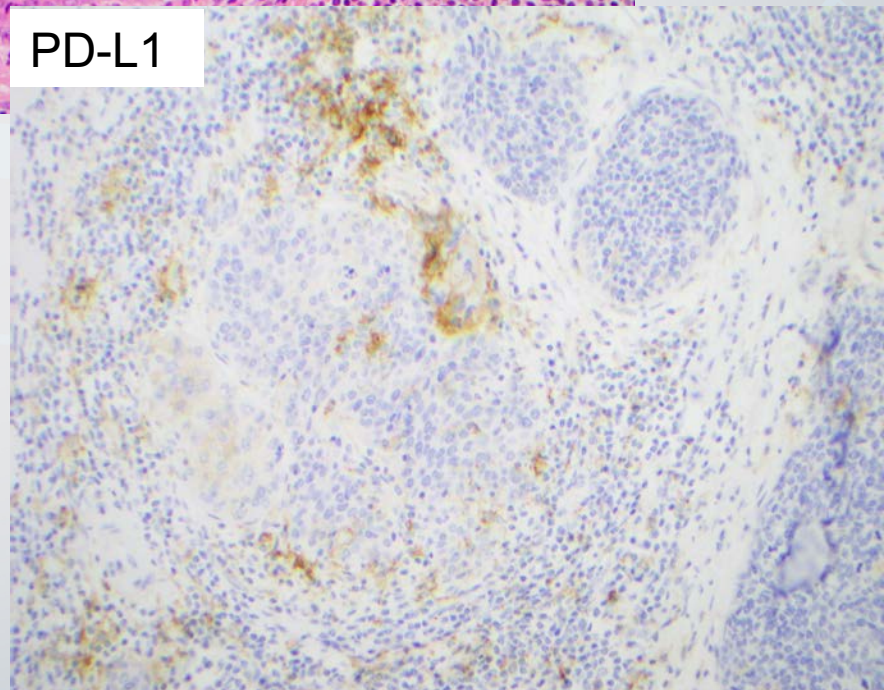


- The tumor cells are entirely negative, but scattered tumor-associated mononuclear inflammatory cells (MICs) are positive. Patchy staining like this was seen throughout the tumor.
- Averaging across four relatively representative fields, we get a CPS=11 (0+5+10+30=45, 45/4=11)

- Returning to our original patient…

- Does she qualify for pembrolizumab therapy, assuming that the pictured field is fairly representative of her entire tumor section?





YES!

How do I report?

PD-L1 CPS Quick text:

PD-L1 protein expression is quantified in cervical squamous cell carcinoma by using the Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS ≥ 1.

The CPS in this case is {negative (<1); positive (≥1)}.

How do I report? True Confessions

TRUE CONFESSION #1: I do not give an exact CPS in my clinical reports.

- No one complains.
- All they care about is positive (≥1) or negative (<1).

- *That said*, if the staining is impressive I will often add something descriptive such as:

  "*The CPS in this case is positive (≥1), with 80% of tumor cells and abundant associated inflammatory cells demonstrating expression*" or "*with a score >50*" etc.

- Similarly, if it's barely positive I may say:

  "*The CPS in this case is positive (≥1), with occasional tumor-associated lymphocytes demonstrating PD-L1 positivity.*"

How do I report? True Confessions

TRUE CONFESSION #2: We do not use the FDA-Approved Dako 22C3 antibody.

We validated the SP263 against the 223C and have excellent concordance.

- Our clinicians are fine with this.
- Patients have had no issues with drug access or insurance coverage.

We place this disclaimer in our reports:

Immunohistochemistry is performed for PD-L1 using the SP263 antibody on the Ventana BenchMark ULTRA. This assay has been internally validated against 223C antibody (Dako Link) at a cut-off of 50% staining (97% concordance).

Critical Caveats!

- Even in the setting of PD-L1 positivity, pembrolizumab response rates are LOW.
  - <3% of patients show complete response
  - <12% show partial response
- The CPS was designed to maximize sensitivity for responders, but may have been at cost of specificity.
- Are higher PD-L1 expression levels associated with better response rates?
  - Unclear.
- Could variables other than PD-L1 impact response?
  - YES.

FDA approves pembrolizumab for advanced cervical cancer with disease progression during or after chemotherapy

SHARE | TWEET | LINKEDIN | PIN IT | EMAIL | PRINT

On June 12, 2018, the Food and Drug Administration approved pembrolizumab (Keytruda, Merck and Co. Inc.) for 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.

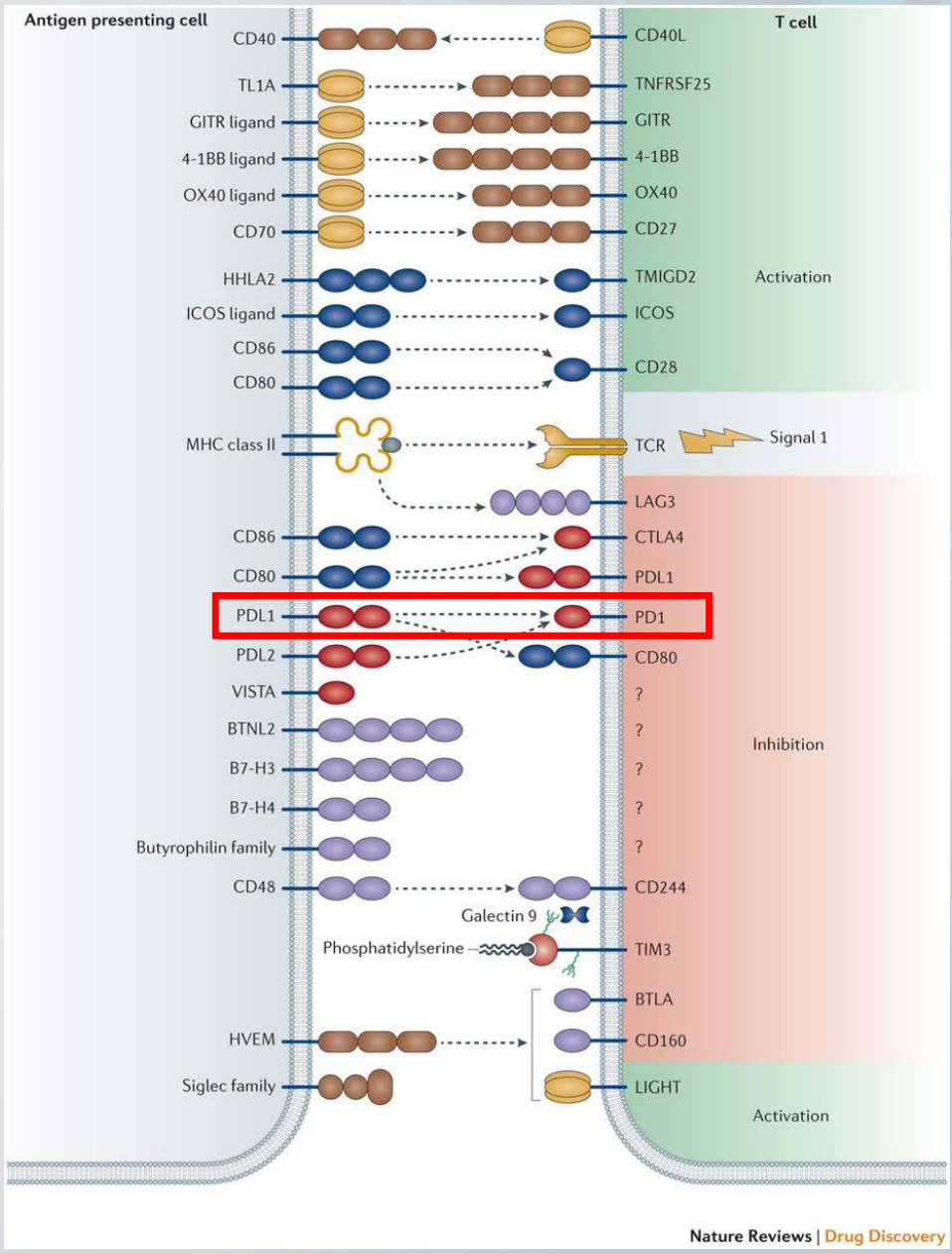
Pembrolizumab was investigated in 98 patients with recurrent or metastatic cervical cancer enrolled in a single cohort of KEYNOTE 158 (NCT02628067), a multicenter, non-randomized, open-label, multi-cohort trial. Patients were treated with pembrolizumab intravenously at a dose of 200 mg every 3 weeks until unacceptable toxicity or documented disease progression. Among the 98 patients, approval was based on 77 (79%) patients who had tumors that expressed PD-L1 with a CPS ≥1 and who had received at least one line of chemotherapy for metastatic disease. PD-L1 status was determined using the PD-L1 IHC 22C3 pharmDx Kit.

The major efficacy outcomes were objective response rate (ORR) according to RECIST 1.1 as assessed by blinded independent central review, and response duration. With a median follow-up time of 11.7 months, the ORR in 77 patients was 14.3% (95% CI: 7.4, 24.1), including 2.6% complete responses and 11.7% partial responses. The estimated median response duration based on 11 patients with a response by independent review was not reached (range 4.1, 18.6+ months); 91% had a response duration of greater than or equal to 6 months. No responses were observed in patients whose tumors did not have PD-L1 expression (CPS <1).

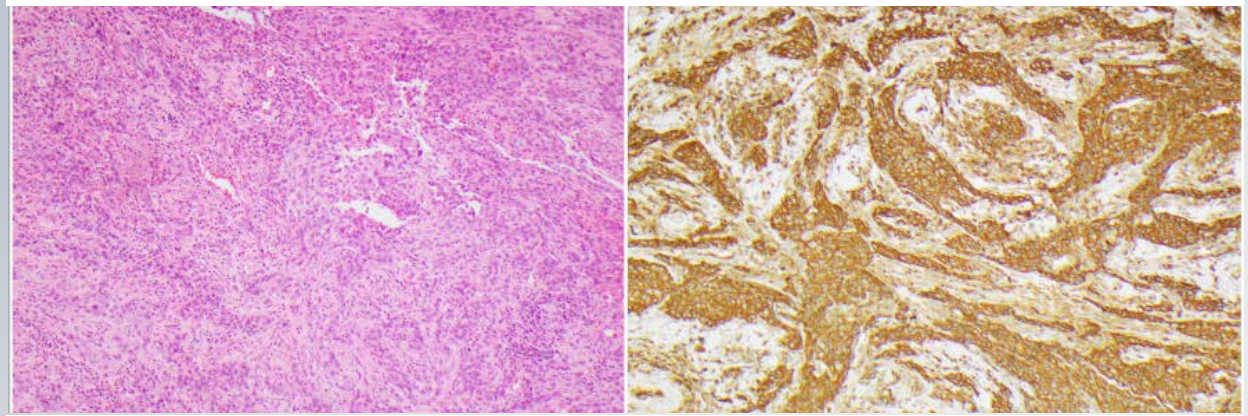
- The PD-1/PD-L1 axis is one of many immunoinhibitory checkpoints that tumors can enlist to evade the host immune system.
- CTLA-4 is the original checkpoint inhibitor target!
- Other immunosuppressive targets with drugs in clinical trials:
  - LAG-3, TIM-3, VISTA…
- Drugs are also being developed to target immuno-ACTIVATING checkpoints, such as OX40
- Tumors may also produce immunosuppressive enzymes, like IDO, which can be targeted.
- But there's an even better way to hide…

Combination cancer immunotherapy and new immunomodulatory targets

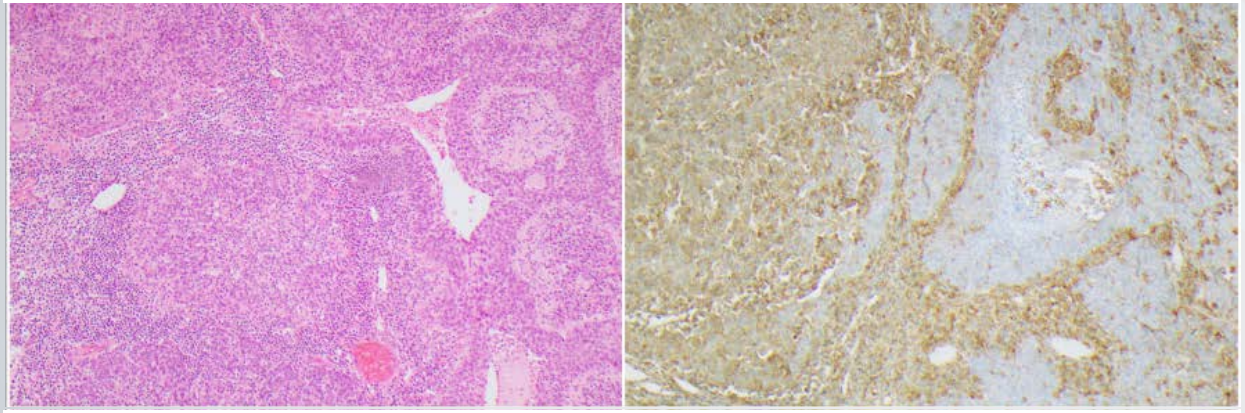
Kathleen M. Mahoney, Paul D. Rennert & Gordon J. Freeman



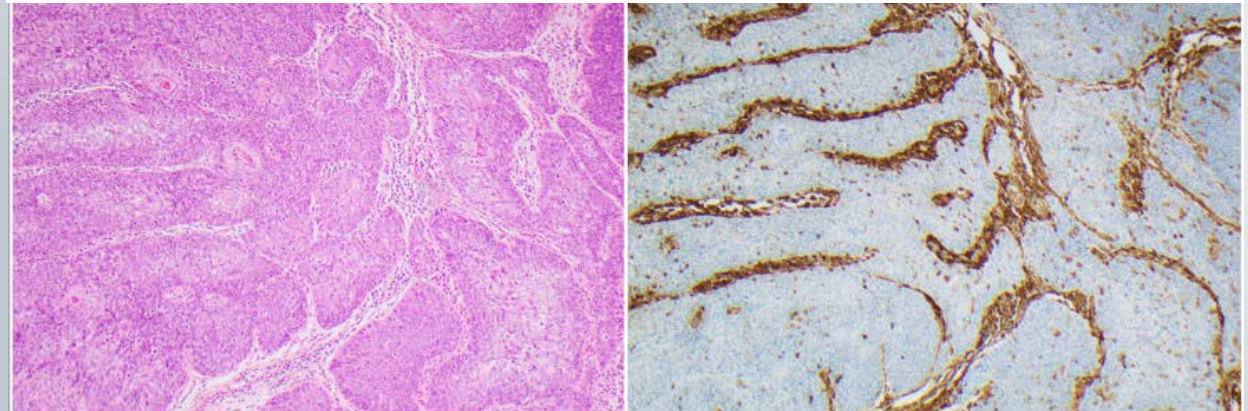
Intact MHC Class I



Clonal loss of MHC Class I



Diffuse loss of MHC Class I



Loss of MHC Class I Expression in HPV-Associated Cervical and Vulvar Neoplasia:

A Potential Mechanism of Resistance to Checkpoint Inhibition

Megan E Dibbern, MD[1]; Timothy Bullock, PhD[1]; Linda Duska, MD[2]; Mark H Stoler, MD[1]; Anne M Mills, MD[1]

Abstract #: 2560

Platform Presentation:
Tuesday Afternoon Gyn Session
1:00 PM - 2:45 PM
LACC 502 A*

Presentation Time: 1:45 PM - 2:00 PM

THANK YOU!