Molecular Assessment of HPV in Patients with Head and Neck Tumors

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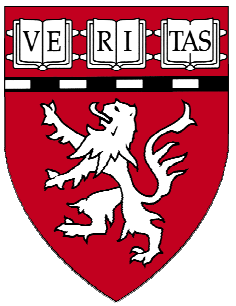
MASSACHUSETTS GENERAL HOSPITAL

PATHOLOGY

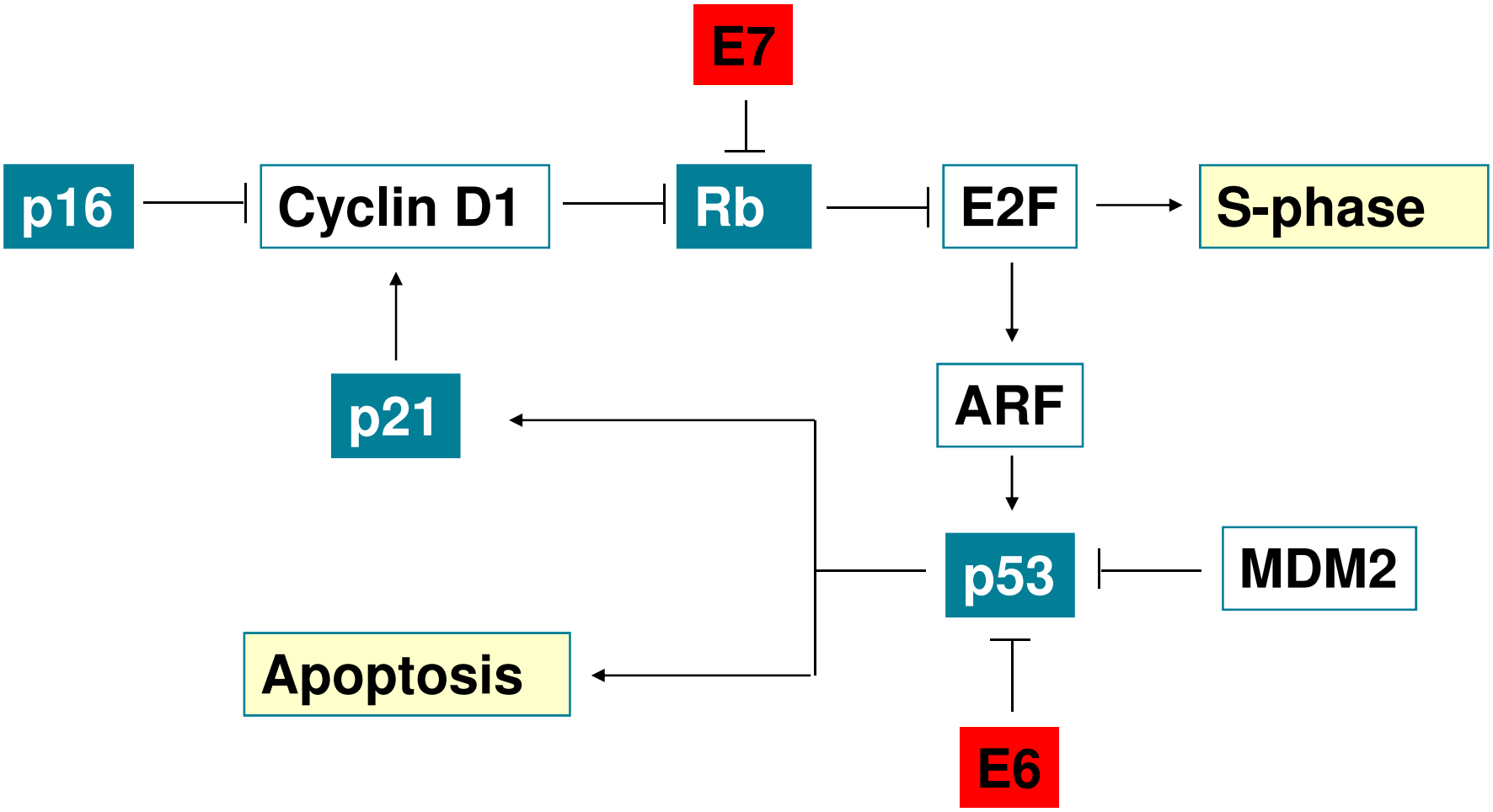
HARVARD MEDICAL SCHOOL



Agenda

- Detection of HPV in paraffin tissues
- HPV-related lesions of the head & neck
  - Oropharyngeal carcinomas
- Clinical value in HPV testing

Oncogenesis of HPV



HPV Detection Methods

- Polymerase chain reaction
- In situ hybridization
  - Multiplexed (High risk vs. low risk)
  - Type specific probes
- Other methods
  - Hybrid capture (cytology samples)
  - Other technologies
- p16 immunohistochemistry

PCR for HPV

- Types of assays
  - Qualitative PCR assays
  - Quantitative PCR assays
  - PCR with sub-typing assays
    - line probe assay

Normal Tissue with HPV

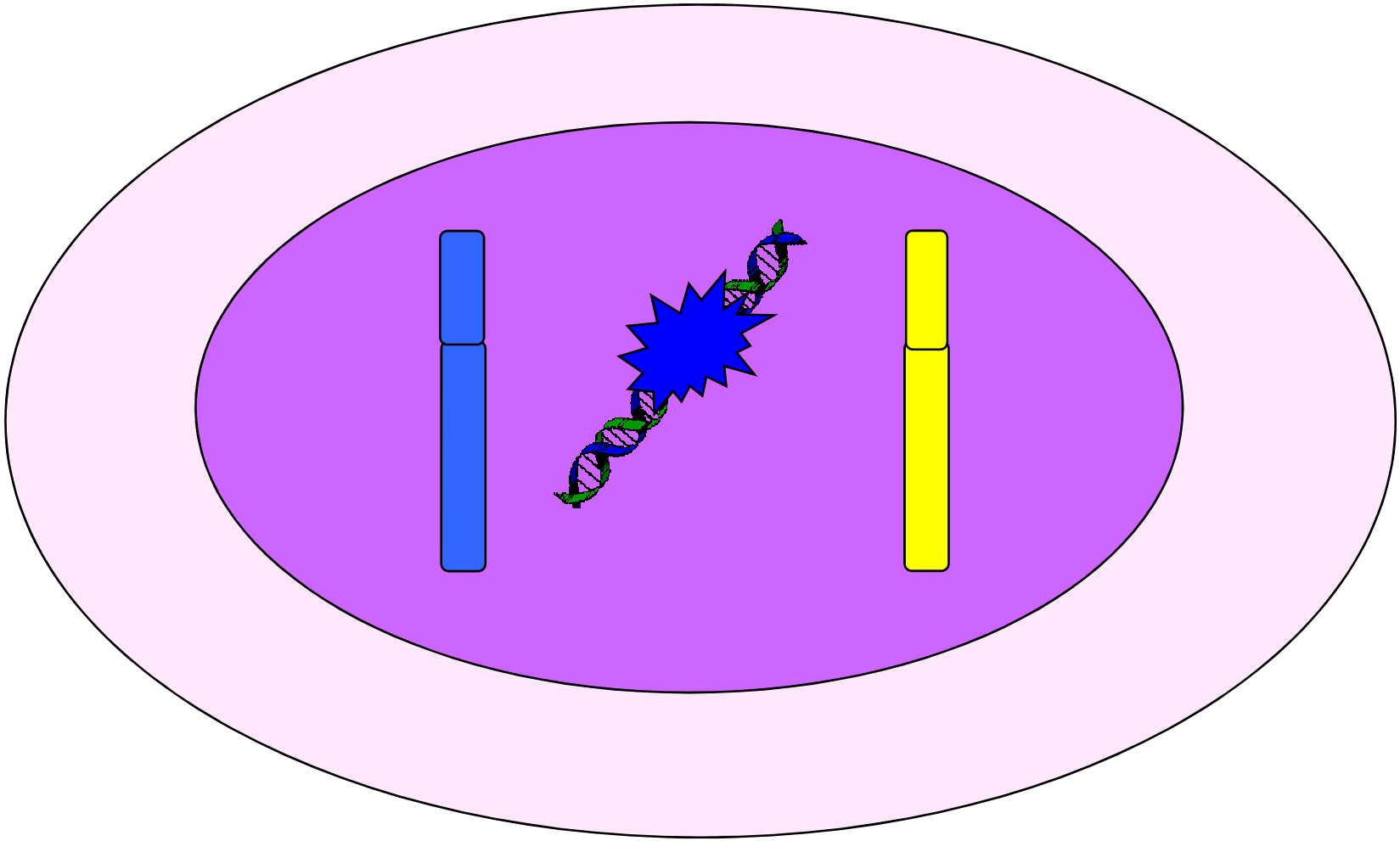
	HPV detection in normal	Population
Ernster JA (2009)	0%	Normal paraffin embedded tonsils
Kreimer AR (2010)	3.5%	Meta-analysis, oral tissue

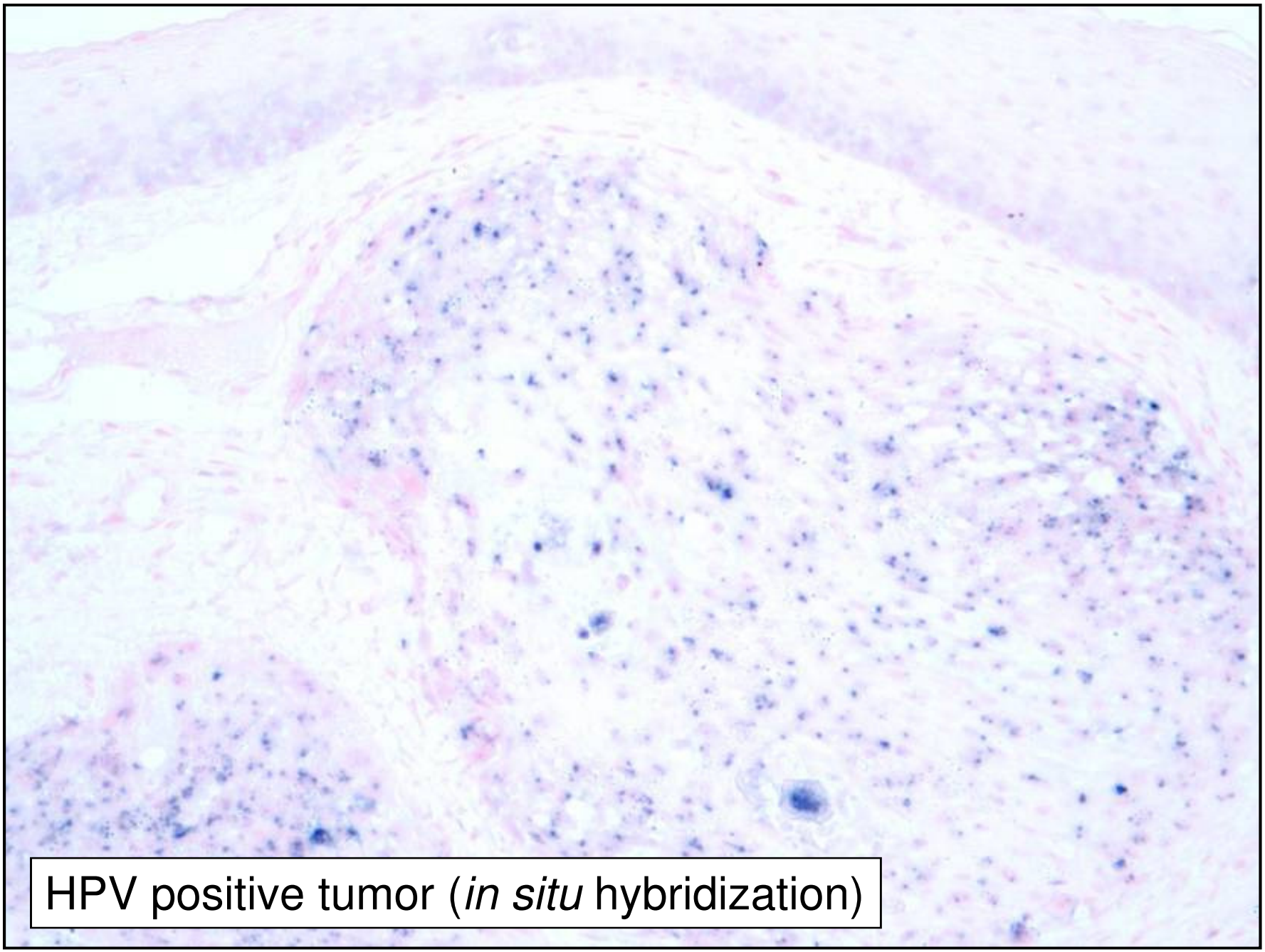
Ernster JA, Arch Oto Head Neck Surg, 135:554, 2009
Kreimer AR, Sex Transm Dis, 2010

HPV Detection Methods

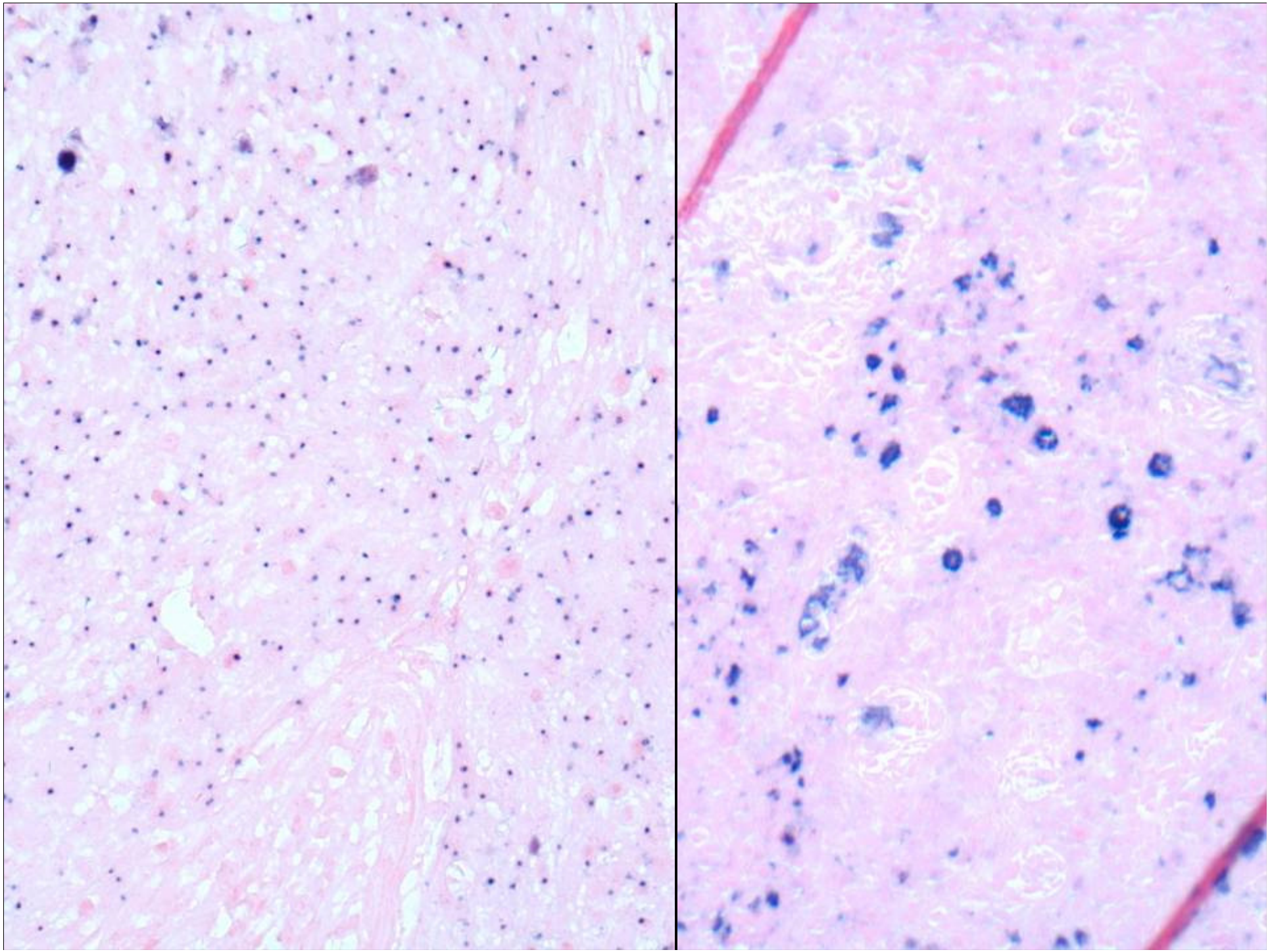
- In situ hybridization
  - Multiplexed (High risk vs. low risk)
  - Sub-type specific probes

HPV In Situ Hybridization





HPV positive tumor (*in situ* hybridization)

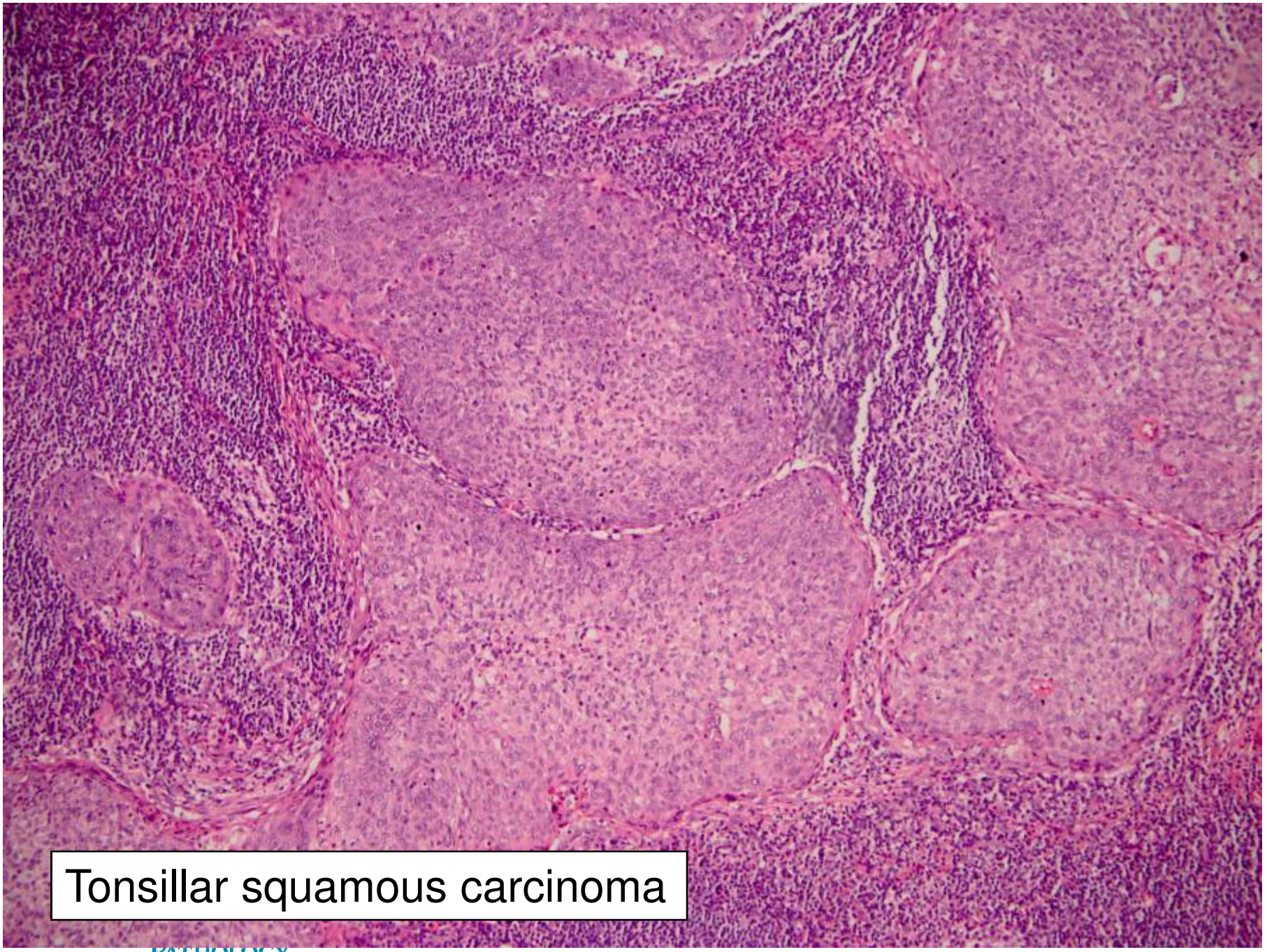


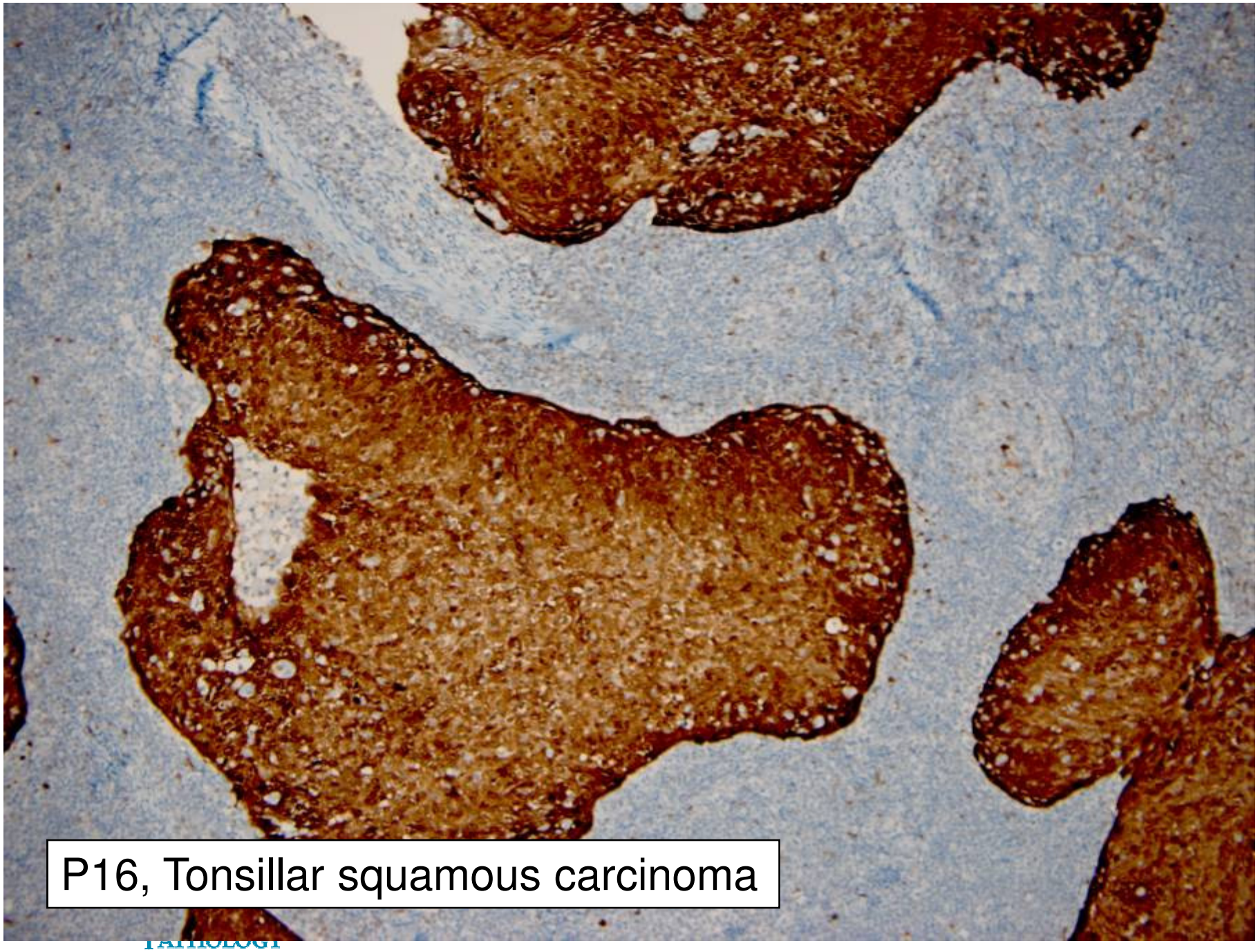
Surrogate Marker: p16

	HPV (+) *(types 16/18)*	HPV (-)
p16 (+)	100%	21%
p16 (-)	0%	79%

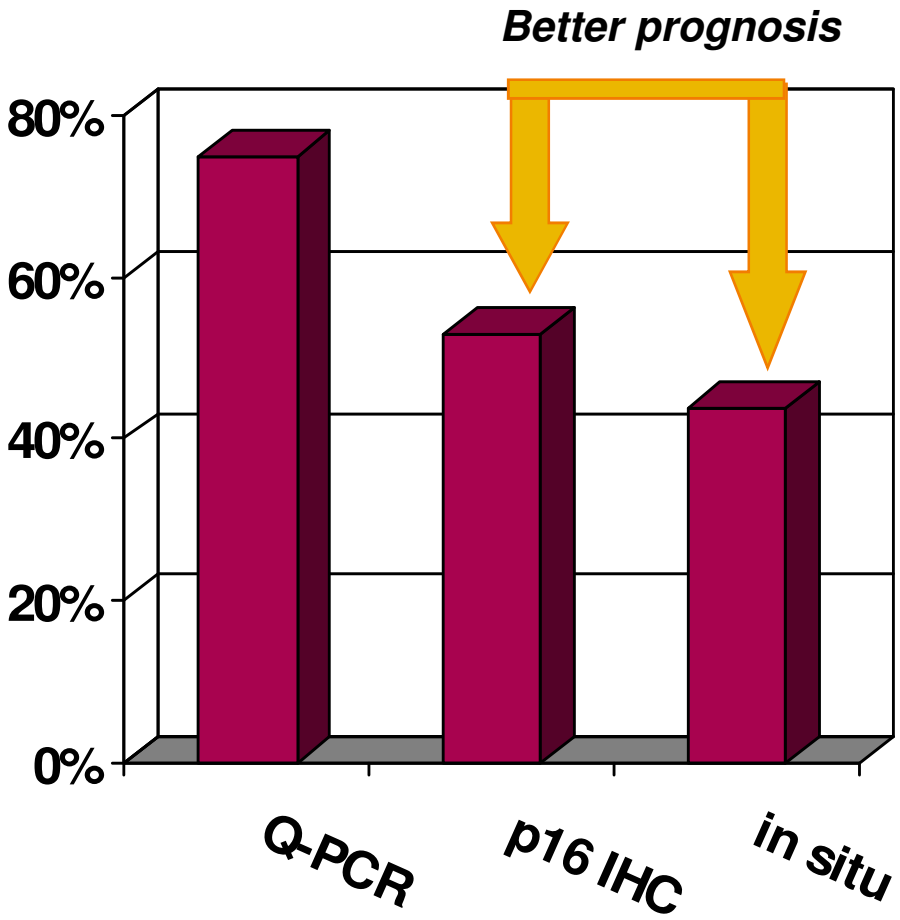
- ***Other Sub-types?***
- ***P16 over-expression***

See also: Abstract 1236, JE Lewis


Tonsillar squamous carcinoma

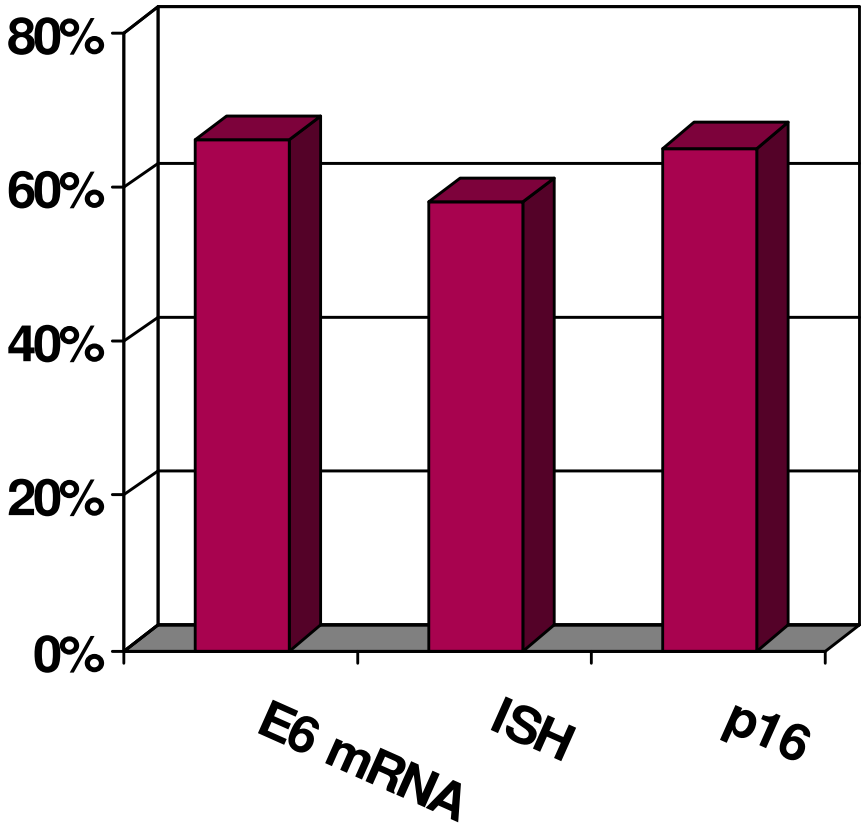

P16, Tonsillar squamous carcinoma

Virus Detection: HPV



Kuo KT, Mod Path, 21:376, 2008

Virus Detection: HPV



Shi W, J Clin Oncol, 27:6213, 2009

Agenda

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- HPV-related lesions of the head & neck
  - Oropharyngeal carcinomas
- Clinical value in HPV testing

Human Papillomavirus

- Relationship between SCC and HPV
  - Increasing incidence of HPV-related tumors
    - Based on SEER data, annual percentage increase is ~3%
  - ~25% of all HNSCC
    - Oropharynx: >50% of tumors are positive for HPV
    - Up to 95% are HPV-16

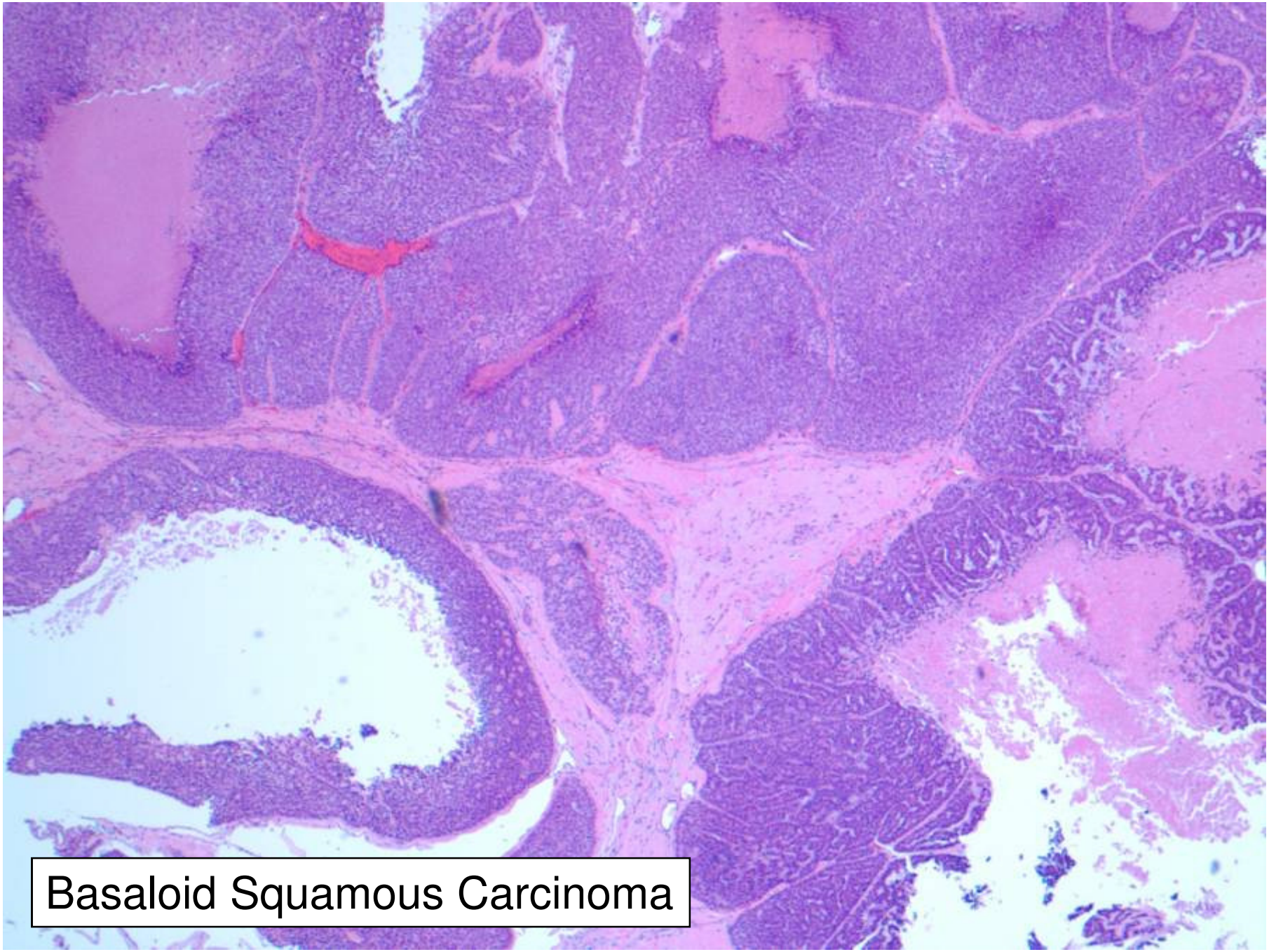
Ryerson AB, Cancer 113:2901, 2008
Syrjanen S, J Clin Virol, 32:S59, 2005

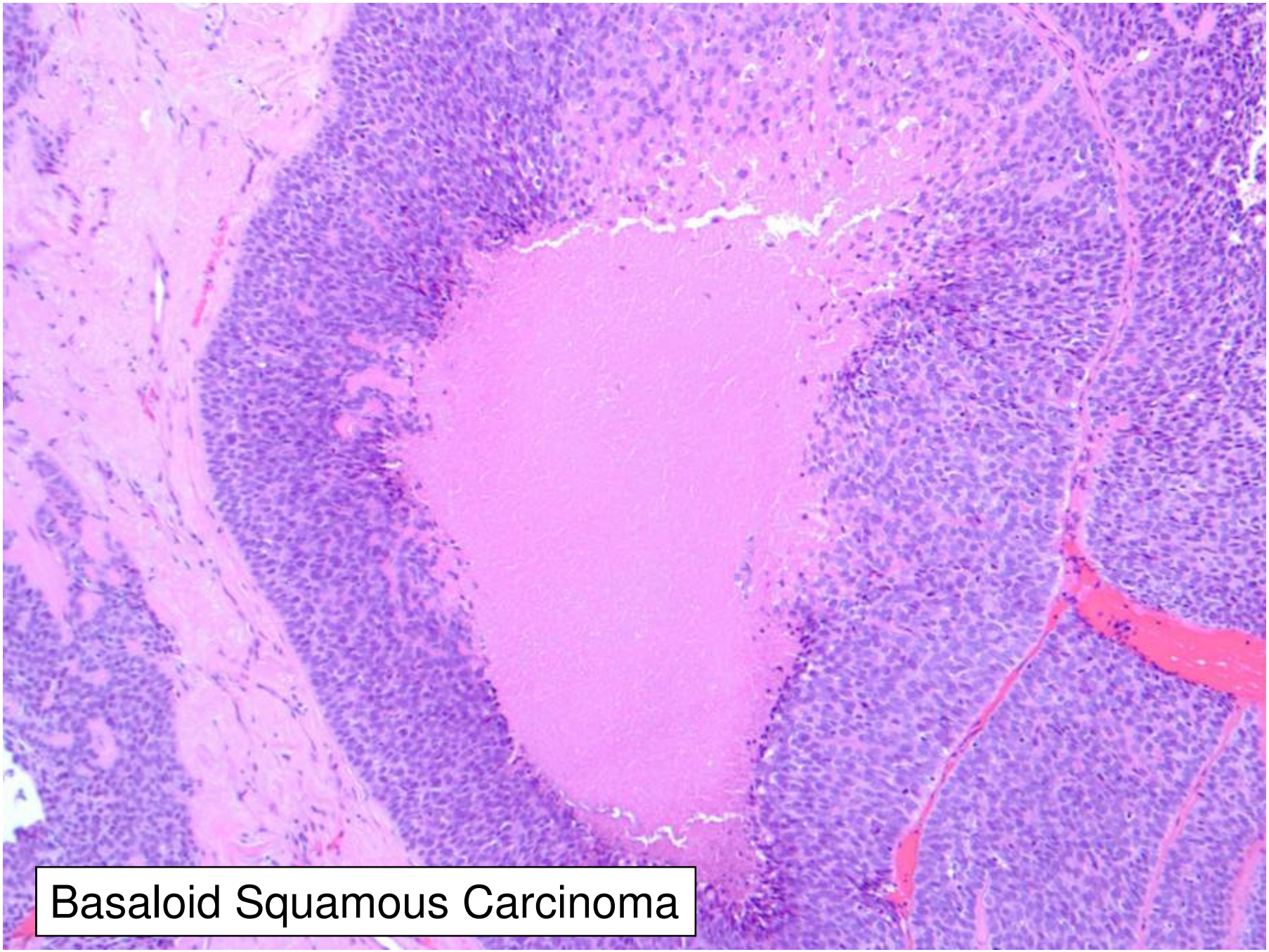
Oropharyngeal Squamous Carcinoma

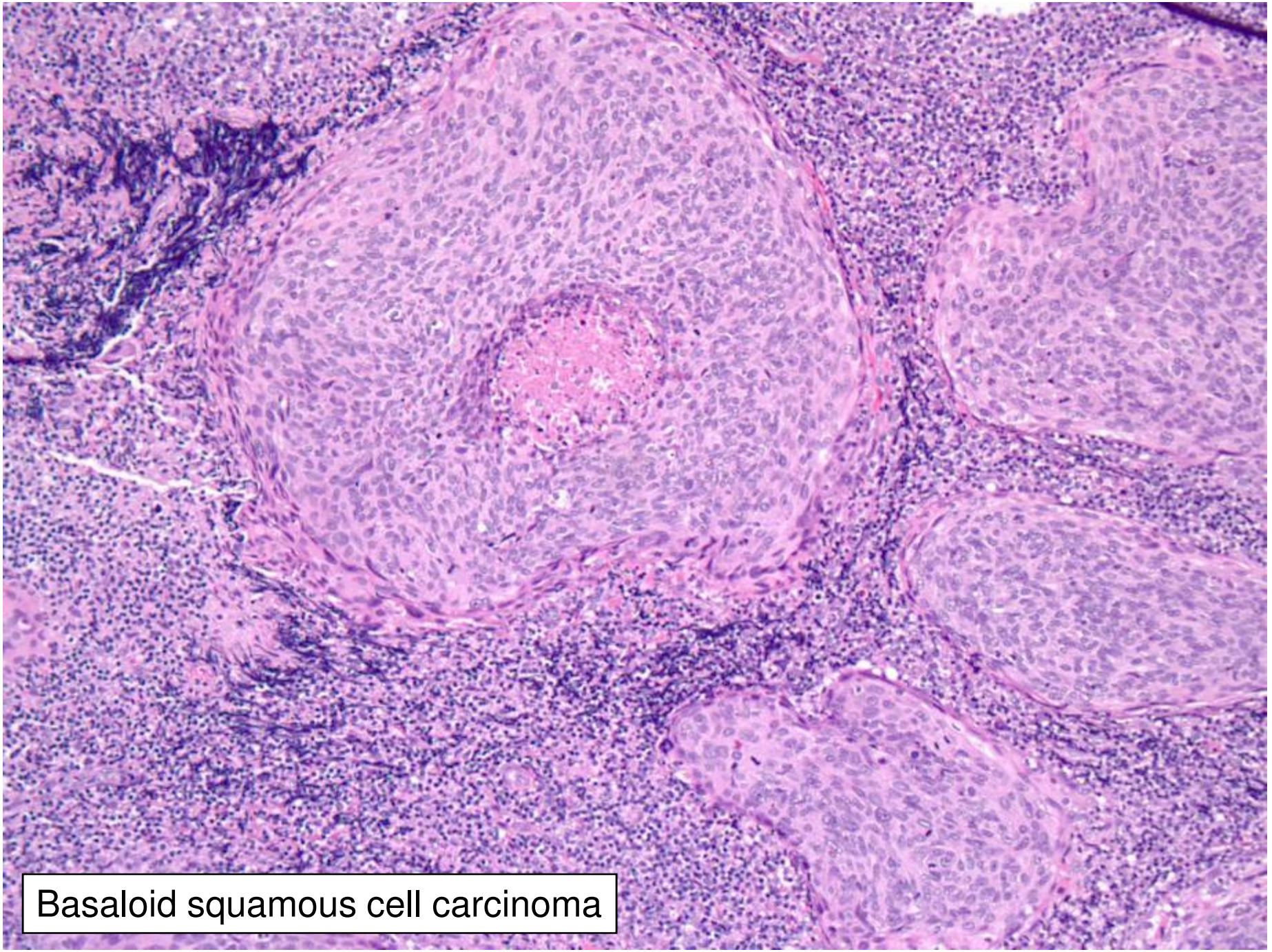
- Basaloid squamous cell carcinoma
- Non-keratinizing squamous carcinoma
- Lymphoepithelial carcinoma

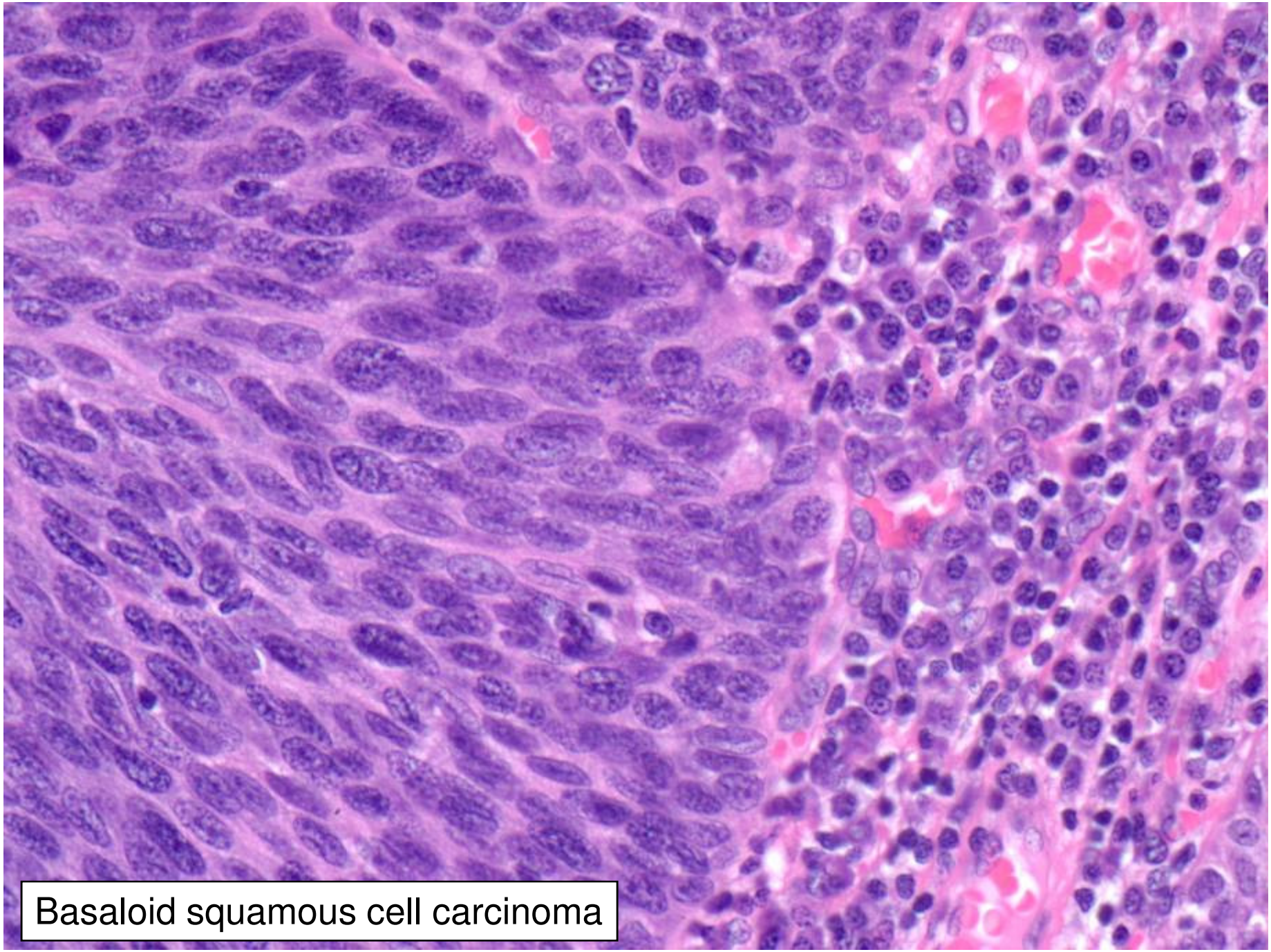
Basaloid Squamous Carcinoma

- Histology
  - Rounded nests and sheets of cells
  - Basaloid morphology
    - High N:C ratio
    - Mitoses and apoptosis
    - Peripheral palisading
  - Comedo-type necrosis


Basaloid Squamous Carcinoma

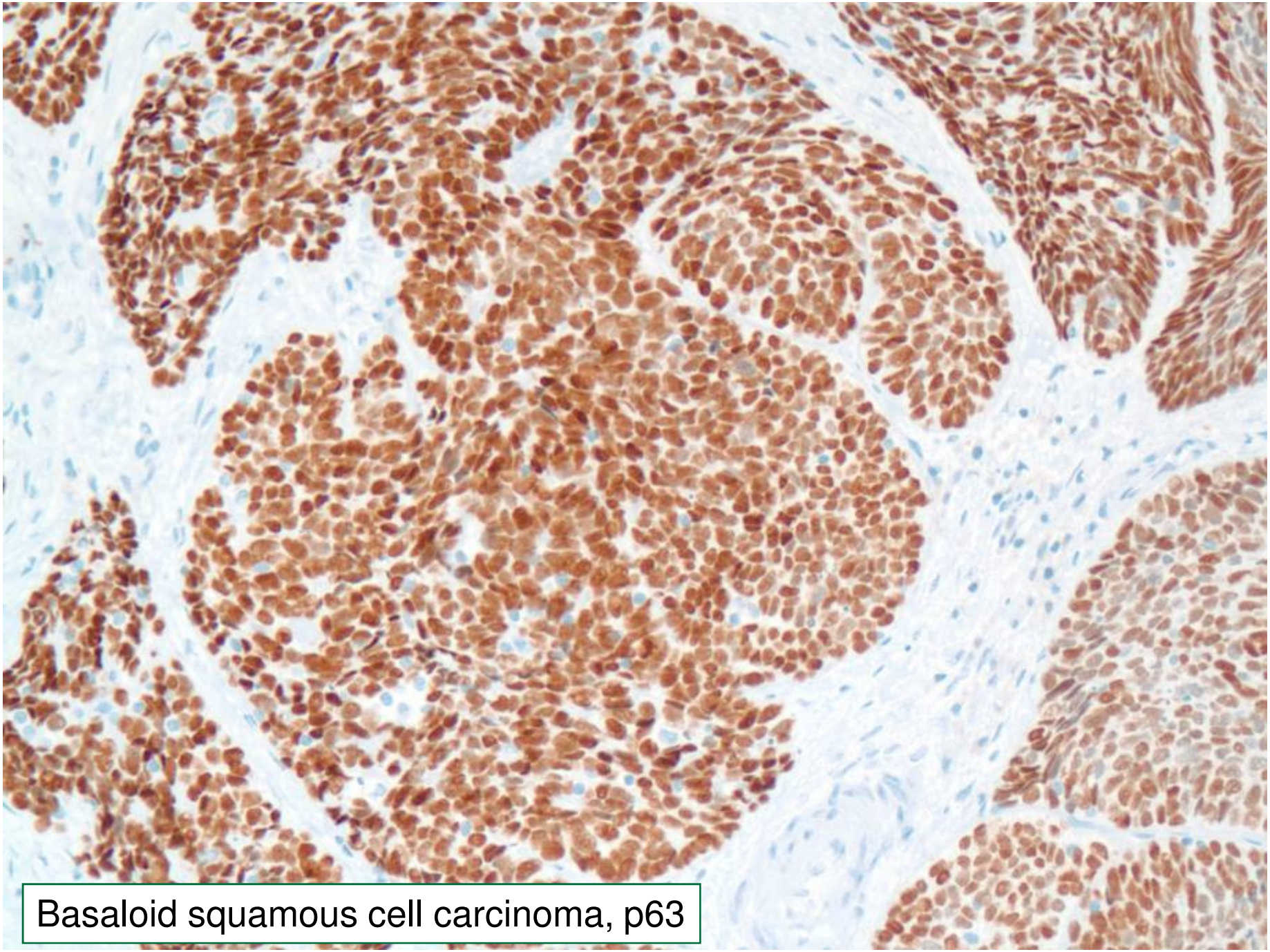

Basaloid Squamous Carcinoma


Basaloid squamous cell carcinoma


Basaloid squamous cell carcinoma

Basaloid Squamous Cell Carcinoma

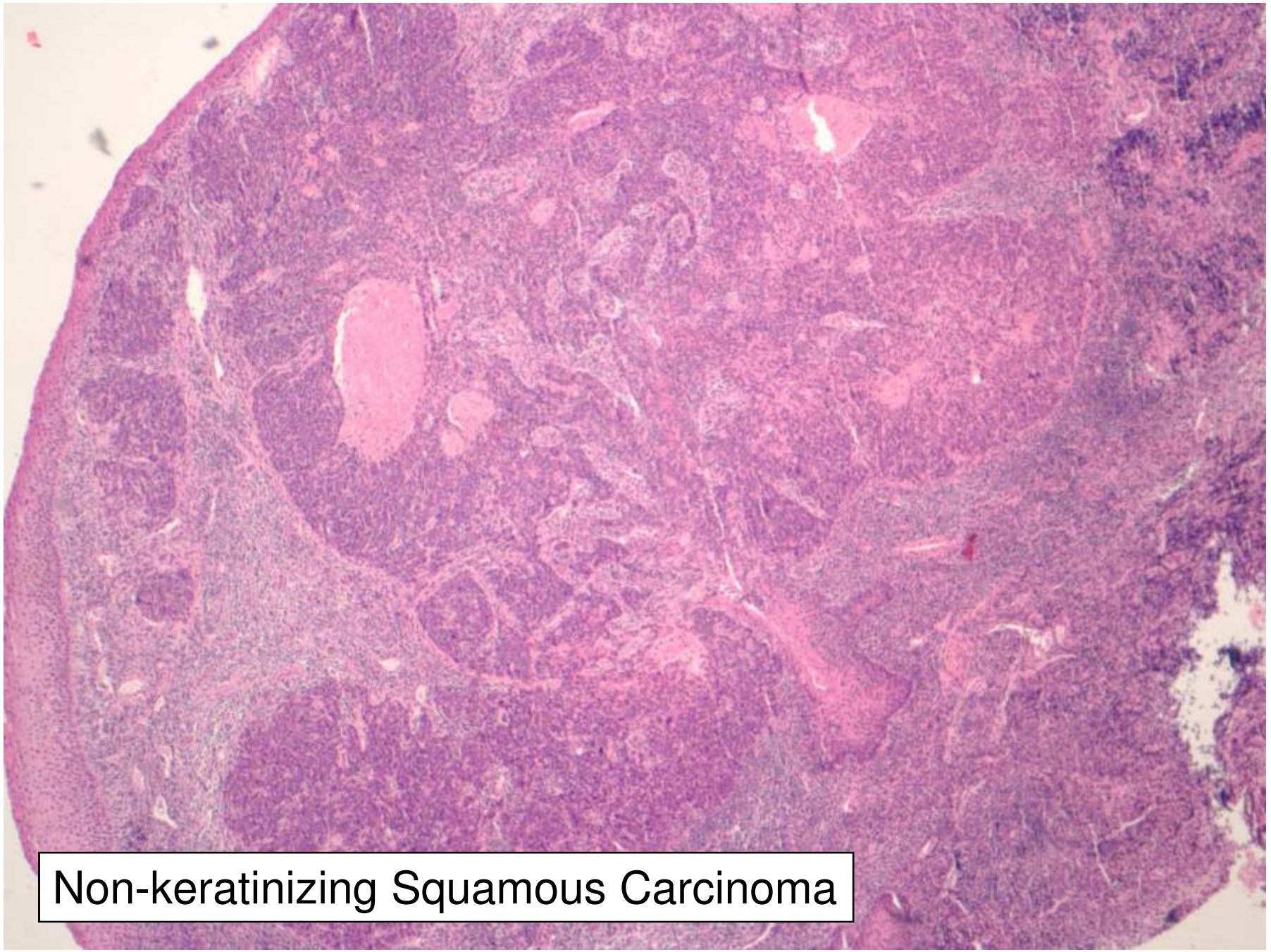
- IHC
  - Cytokeratins
  - Negative for myoepithelial markers
  - p63 positive

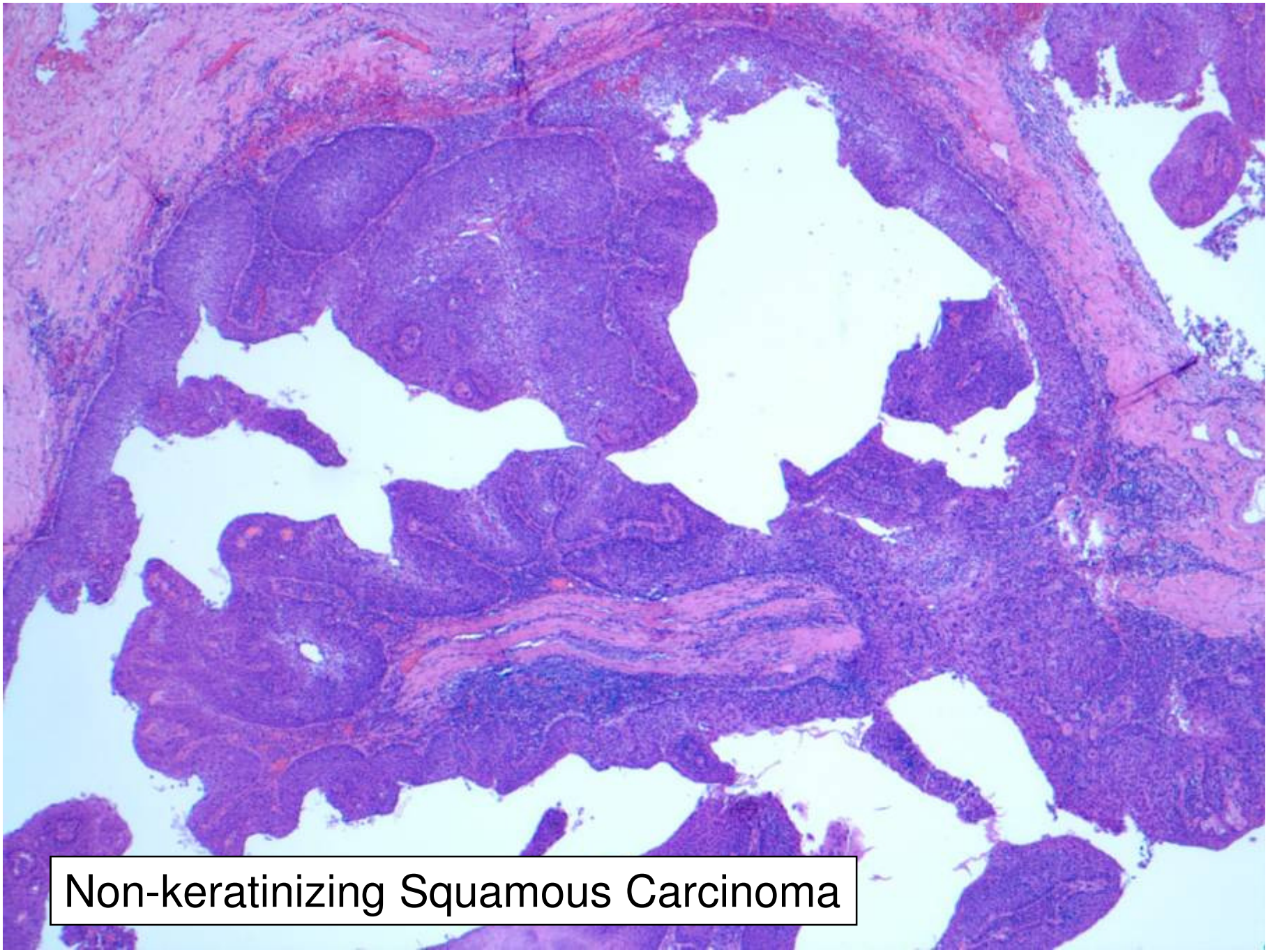


Basaloid squamous cell carcinoma, p63

Non-keratinizing Squamous Carcinoma

- Histology
  - Sheets of cells
  - Limited keratinization
  - Cytology
    - High N:C ratio
    - Pleomorphism
  - Tracks along tonsillar crypts


Non-keratinizing Squamous Carcinoma


Non-keratinizing Squamous Carcinoma

HPV and Tumor Characteristics

	HPV Positive	HPV Negative
Demographics	•5 years younger •Non-smokers/non-drinkers	•Typical ages •Tobacco and alcohol
Site	Tonsil & Tongue base	All locations
Histology	Poorly differentiated, non-keratinizing, basaloid	Keratinizing SCC
Genetics	•p53 inactivated by E6 •Rb inactivated by E7 •p16 over-expressed	•p53 inactivated by mutation •Rb inactivated by cyclin D1 amplification •Inactivation of p16

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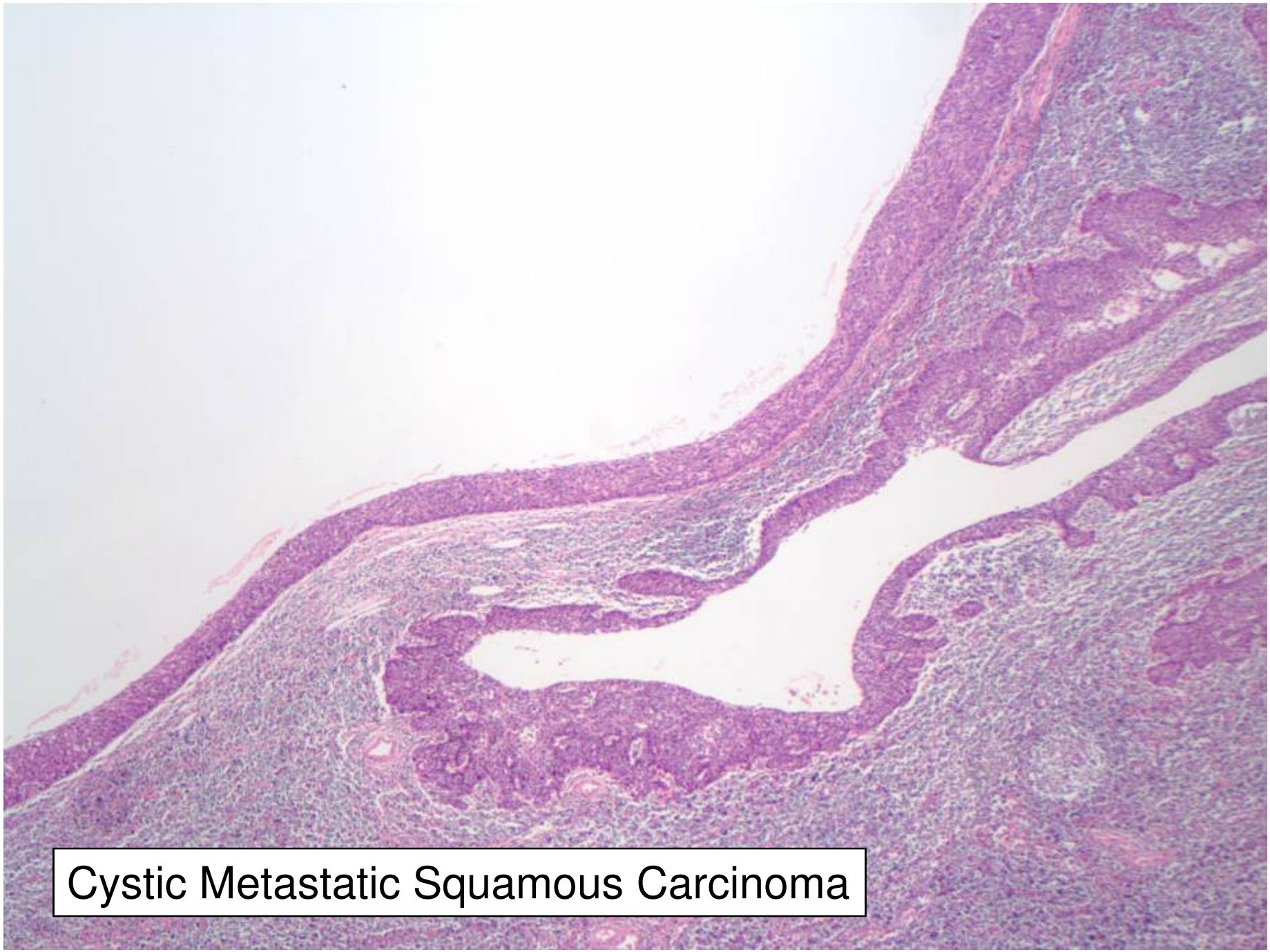
Why Test for HPV?

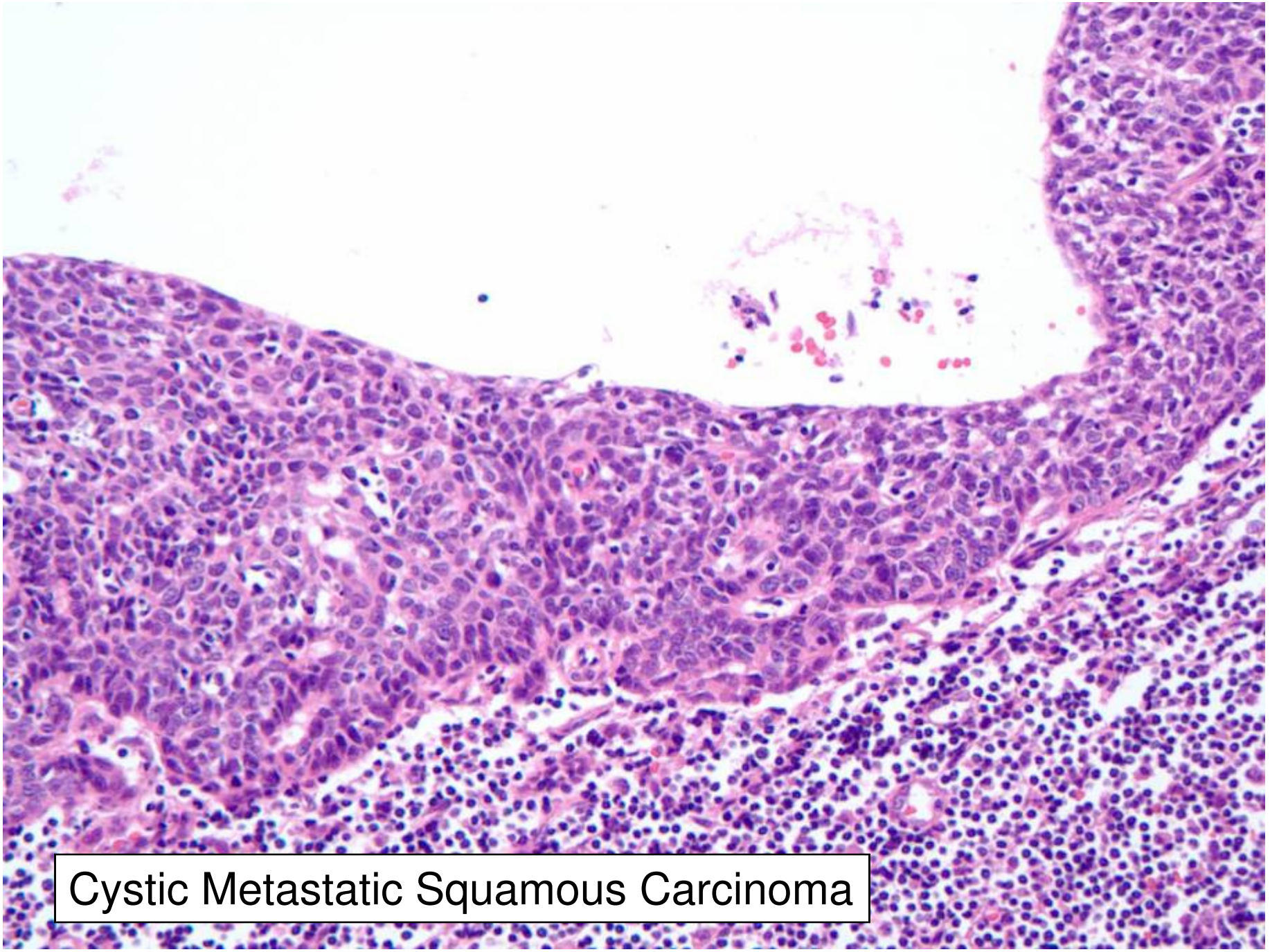
- Epidemiologic
- Diagnostic
- Prognostic
- Therapeutic

Cystic Metastasis and Unknown Primary

- Histology
  - Ribbon like epithelium
  - Thickness of tonsillar type epithelium
  - Can have endophytic or exophytic areas

- Cytology
  - Moderate N:C ratio
  - No maturation
  - Bland appearance


Cystic Metastatic Squamous Carcinoma


Cystic Metastatic Squamous Carcinoma

Unknown Primary

Site	*Percentage*
Tonsil/tongue base	63%
Nasopharynx	8%
Other	10%
None found	20%

HPV as a Diagnostic Tool

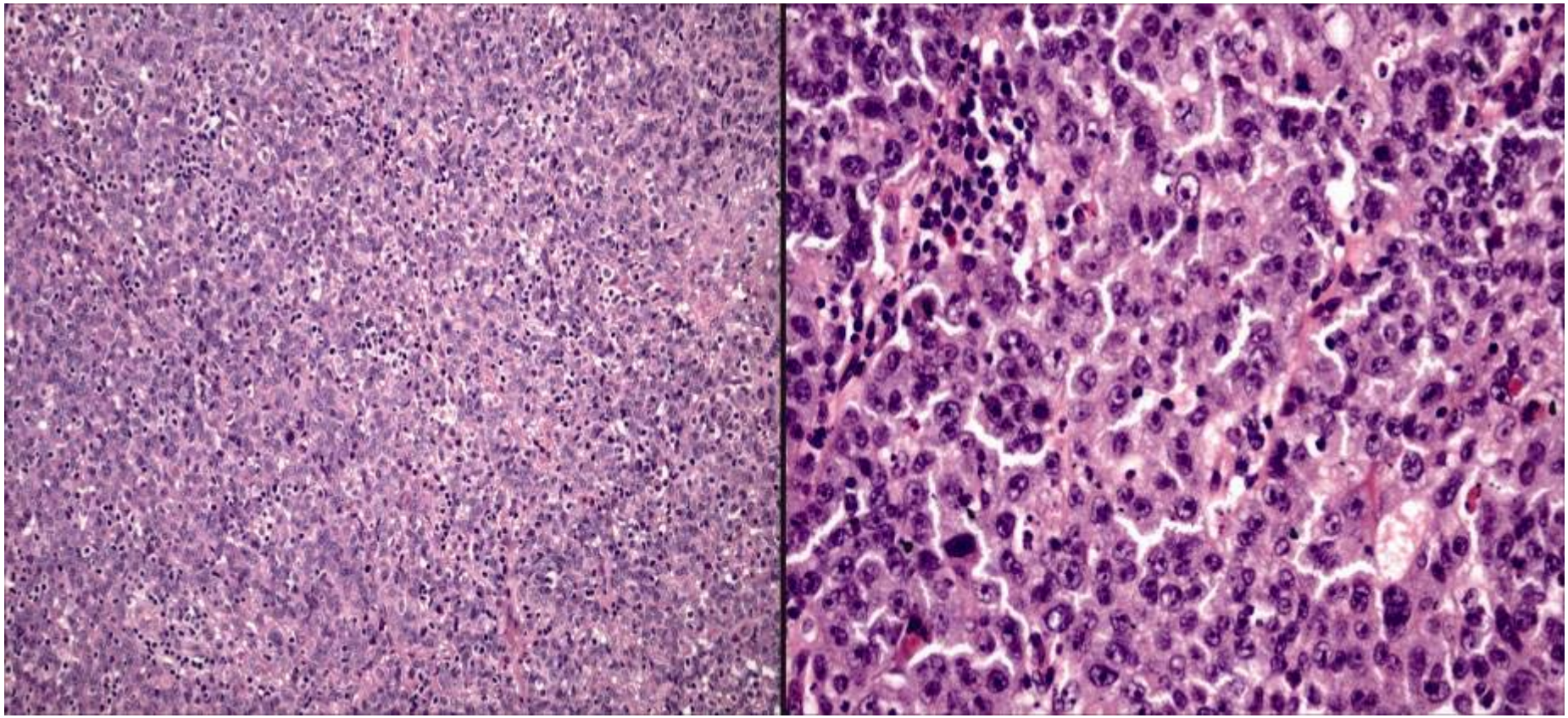
- HPV in cystic lymph node metastases
  - Between 50 and 80% will be positive when originating from an oropharyngeal site

Desai PC, Exp Mol Pathol, 87:94, 2009
Goldenberg D, Head Neck, 30:898, 2008

Oral HPV-related Lymphoepithelial Carcinoma

	P16	HPV 16 ISH	HPV PCR
Singhi (#1256)	22/22 (100%)	19/22 (86%)	N/A
Carpenter (#1221)	14/15 (93%)	8/14 (57%)	6/6 (100%) (ISH -)

Oral HPV-related Lymphoepithelial Carcinoma



Courtesy of Dr. Ed Stelow

Why Test for HPV?

- Epidemiologic
- Diagnostic
- Prognostic
- Therapeutic

Prognostic Value of HPV

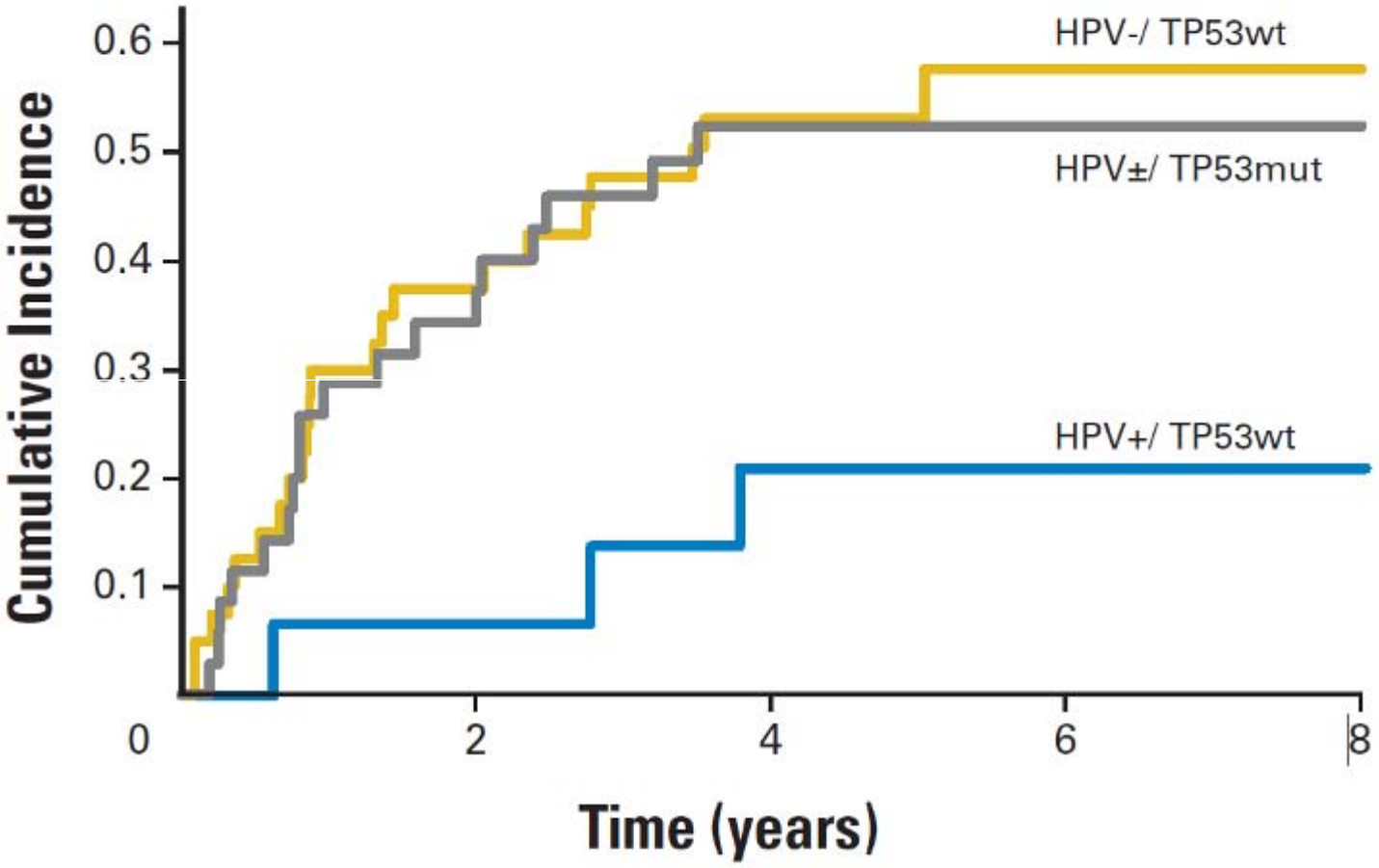
Table 3. Three- and 5-Year Estimates of Overall Survival, Crude Cumulative Incidence of Tumor Relapse, and Second Tumors Accordingto HPV/*TP53* Status

Outcome	HPV Positive/ TP53 wt	HPV Positive or Negative/ *TP53* or Mutated HPV Negative/*TP53* wt	Total No. of Patients
No. of patients	15	75	90
% Survival, years			
3	93	60	64
5	79	46	50
% Cumulative incidence of tumor relapse, years			
3	14	47	42
5	21	53	48
% Cumulative incidence of second tumors, years			
3	0	3	2
5	0	12	10

Abbreviations: HPV, human papillomavirus; wt, wild type.

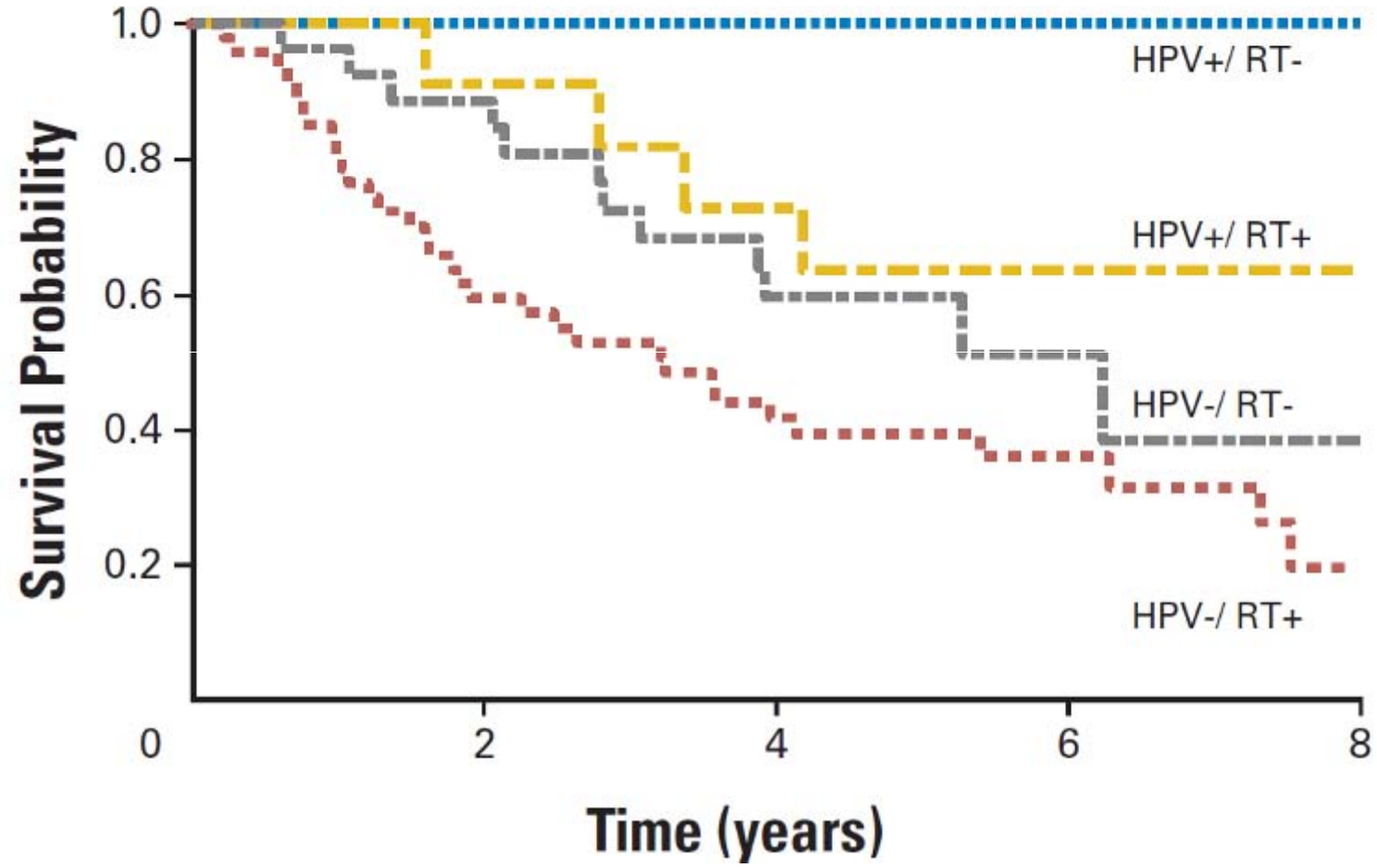
Licitra, J Clin Onc, 24:5630, 2006.

Cumulative Incidence Relapse



Licitra, J Clin Onc, 24:5630, 2006.

Survival Rates



Licitra, J Clin Onc, 24:5630, 2006.

HPV: Unanswered Questions

- What tumors should be tested?
- What test should be done?
- What value should be reported?
  - Qualitative: Positive vs. negative
  - Quantitative: Copy number
  - Sub-typing: High vs. low or exact subtype

Summary

- Detection of HPV in paraffin tissues
- HPV-related lesions of the head & neck
  - Oropharyngeal carcinomas
- Clinical value in HPV testing

Molecular Assessment of HPV in Patients with Head and Neck Tumors

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HPV Viral Oncogenesis in Head and Neck Tumors

There are a number of viruses that are known to be associated with tumorigenesis. The most common are Epstein-Barr Virus (EBV) and human Papillomavirus (HPV). Of course, HPV is particularly well known for its association with carcinomas of the uterine cervix. In fact, HPV testing of cervical smear specimens has become standard of care for the management of certain subsets and age groups of women.

Recent evidence has suggested that a subset of head and neck squamous cell carcinomas is also associated with HPV. These tumors are commonly in a younger population and often afflict patients who do not have traditional risk factors (non-smokers and non-drinkers) [1]. These tend to occur in the orpharynx, particularly in the tonsillar and tongue base regions [2, 3]. The tumors often have a characteristic morphology, including either a nonkeratinizing appearance or basaloid squamous cell carcinoma features [4]. These tumors, as with non-HPV associated tumors in these locations, can present with bulky lymph node metastases, and sometimes a primary tumor is not discovered despite very careful clinical workups. Detection of HPV virus DNA can be useful in directing the clinical to the oropharynx in search of a primary. Most HPV positive tumors of squamous origin are from these locations.

The most common subtype of HPV in oropharyngeal carcinomas is HPV 16 [5]. HPV 18 and HPV 33 can also be seen. HPV related oropharyngeal squamous cell carcinomas have a different prognosis [6] They have a decreased rate of second primary tumors, less local recurrence, and better survival rates [6]. They may also have different types of responses to chemotherapy and radiation therapy [7, 8].

Detecting HPV can be done with several different assays, including PCR based assays, in situ hybridization, and immunohistochemistry [9]. The

advantages of ISH are that the virus can be localized to the tumor and that it is not overly sensitive [10]. IHC is not very reliable, if the target is HPV. There is a surrogate marker that can be used to suggest HPV, and that is p16 by IHC. This correlates fairly well with the presence of HPV. Because p16 is also a tumor suppressor gene, alterations in expression will not always be associated with HPV. Tumors associated with HPV have a better prognosis and also have different response rates to traditional chemo and radiation therapy. Furthermore, there may be novel anti-viral therapies used in some HPV positive lesions, though perhaps not invasive carcinomas

The reasons that HPV testing may be implemented in a clinical setting include diagnostic implications (i.e., helping to suggest site of origin for a neck metastasis with an unknown primary), therapeutic and prognostic reasons, since HPV-related tumors have a different behavior and prognosis.

References

1. Andrews, E., W.T. Seaman, and J. Webster-Cyriaque, *Oropharyngeal carcinoma in non-smokers and non-drinkers: a role for HPV.* Oral Oncol, 2009. **45**(6): p. 486-91.
2. Termine, N., et al., *HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988-2007).* Ann Oncol, 2008. **19**(10): p. 1681-90.
3. Psyrri, A., L. Prezas, and B. Burtness, *Oropharyngeal cancer.* Clin Adv Hematol Oncol, 2008. **6**(8): p. 604-12.
4. El-Mofty, S.K. and S. Patil, *Human papillomavirus (HPV)-related oropharyngeal nonkeratinizing squamous cell carcinoma: characterization of a distinct phenotype.* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2006. **101**(3): p. 339-45.
5. *Prevalence of oncogenic human papillomavirus 16 and 18 in the palatine tonsils of the general adult population.* Arch Otolaryngol Head Neck Surg., 2009. **135**(6): p. 554-7.
6. *HPV and prognosis for patients with oropharynx cancer.* Eur J Cancer., 2009. **45**(Suppl 1): p. 383-5.
7. Syrjanen, S., *Human papillomavirus (HPV) in head and neck cancer.* J Clin Virol, 2005. **32 Suppl 1**: p. S59-66.
8. *Oropharyngeal squamous cell carcinoma treated with radiotherapy or radiochemotherapy: prognostic role of TP53 and HPV status.* Int J Radiat Oncol Biol Phys., 2009. **75**(4): p. 1053-9. Epub 2009 Jul 4.
9. Koyama, K., K. Uobe, and A. Tanaka, *Highly sensitive detection of HPV-DNA in paraffin sections of human oral carcinomas.* J Oral Pathol Med, 2007. **36**(1): p. 18-24.
10. Hopman, A.H., et al., *HPV in situ hybridization: Impact of different protocols on the detection of inetegrated HPV.* Int J Cancer, 2005.