# Translational Research in Head & Neck Cancer


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# Outline

1. **HPV & OPC**
2. **PMH Data**
3. **Outstanding Research Questions**
4. **Conclusions**

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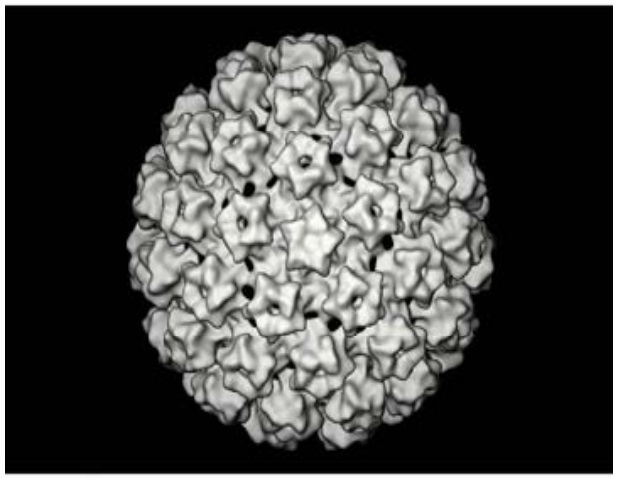
# HPV

- dsDNA virus; ~7.9 kb
- >300 sub-types identified
- HPV16 & 18 account for >98% HPV-positive HNSCC



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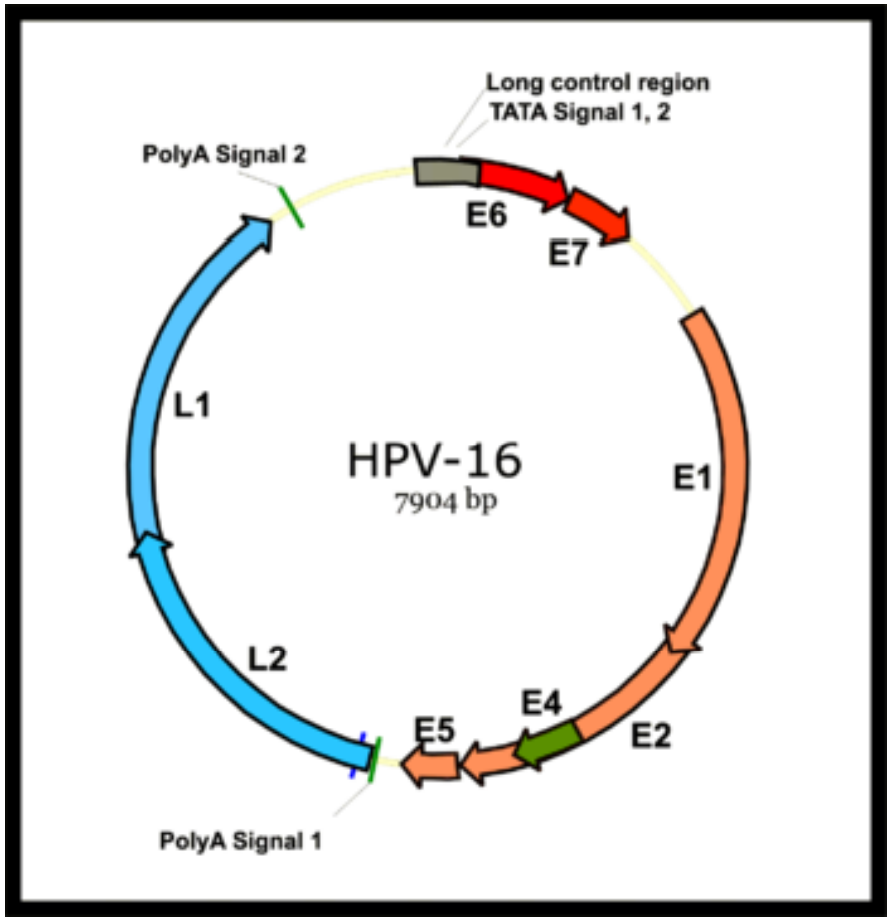
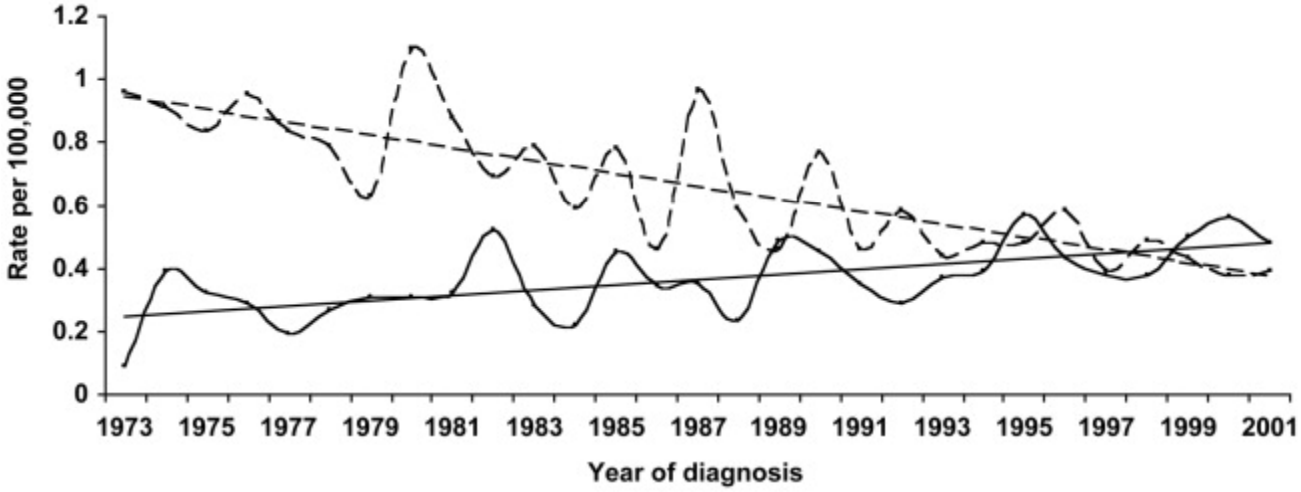
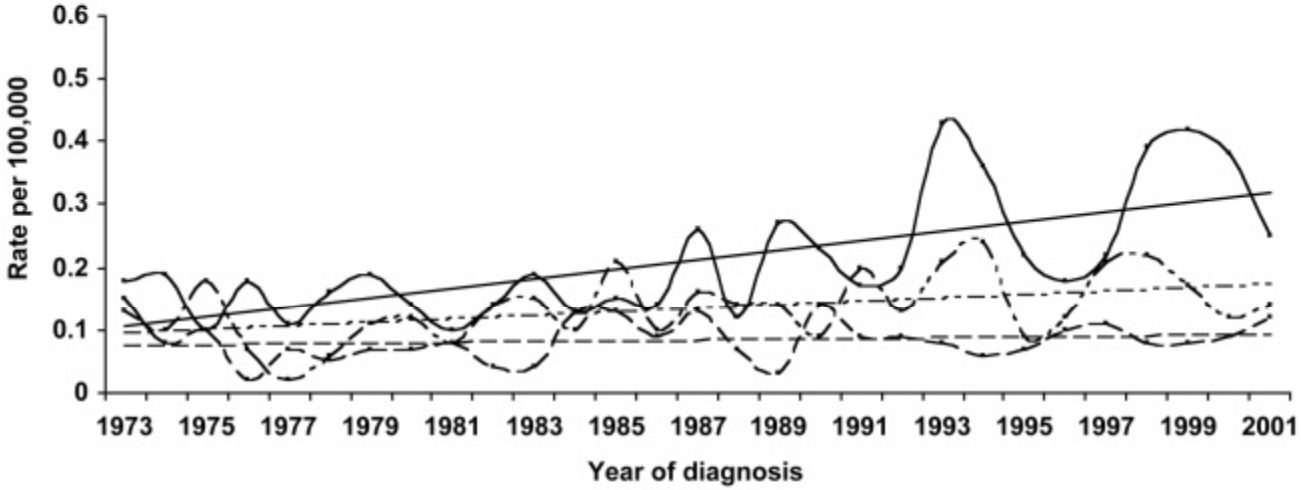



- **E6 – inactivates p53**
- **E7 – destabilizes Rb & increase p16**
- **Net result: failure to die; uncontrolled proliferation**



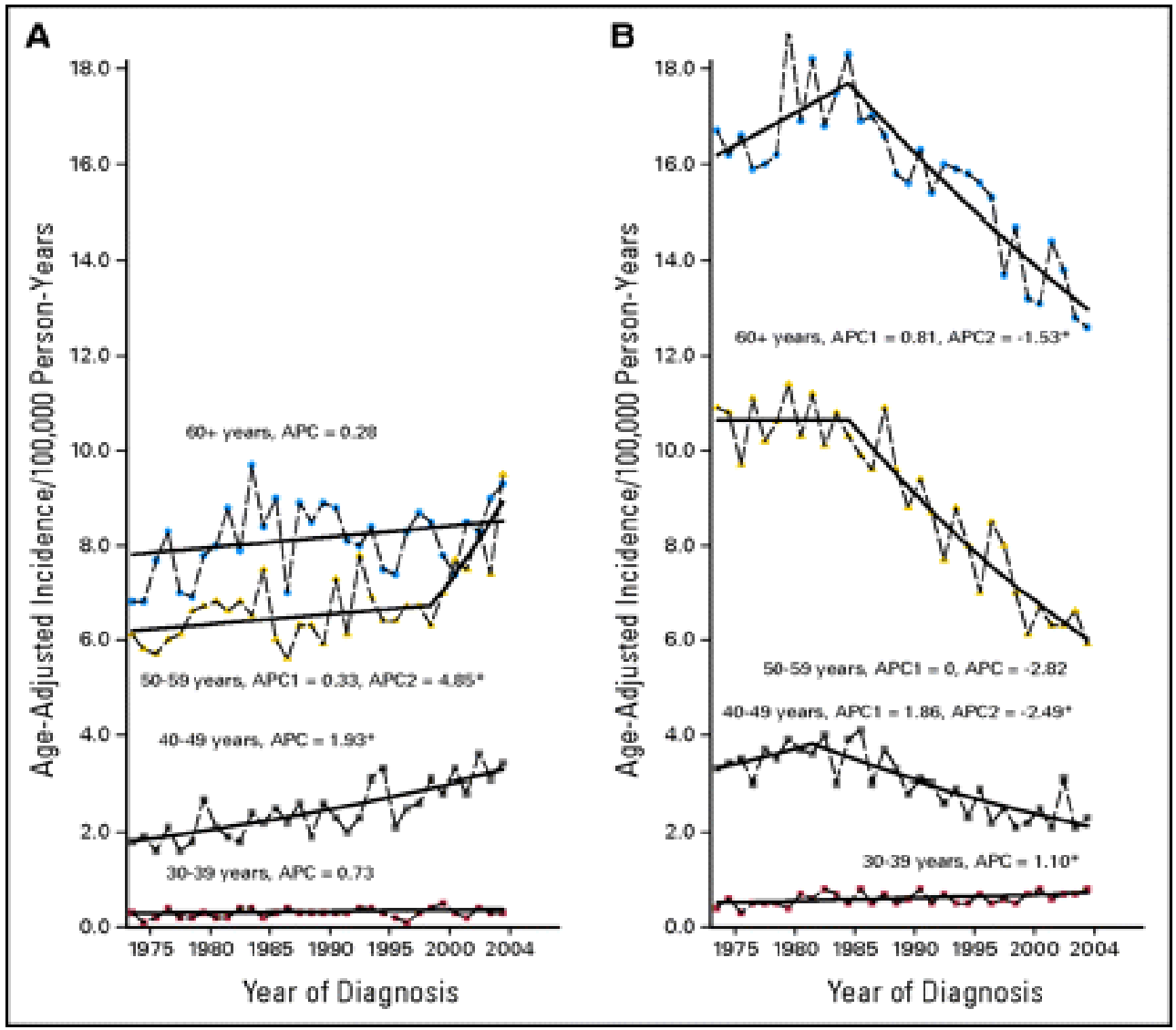

**A** —— Oral tongue: PC = +465; APC = +2.1; $P < 0.001$
– – –Other oral cavity sites: PC = -59; APC = -3.13; $P < 0.001$




——Tonsil: PC = +41; APC = +3.9; $P < 0.001$
– - - –Base of tongue: PC = +8.6; APC = +1.73; $P = 0.04$
**B** – – – Other pharynx: PC = -21; APC = 0.24; $P = 0.7$

**Shiboski et al; *Cancer* 103:1843, 2005**

**HPV-Related** **HPV-Unrelated**




**Chaturvedi *et al*; *JCO* 26:612, 2008**

**Table 2. Associations of Oropharyngeal Cancer with Sexual Behaviors.***

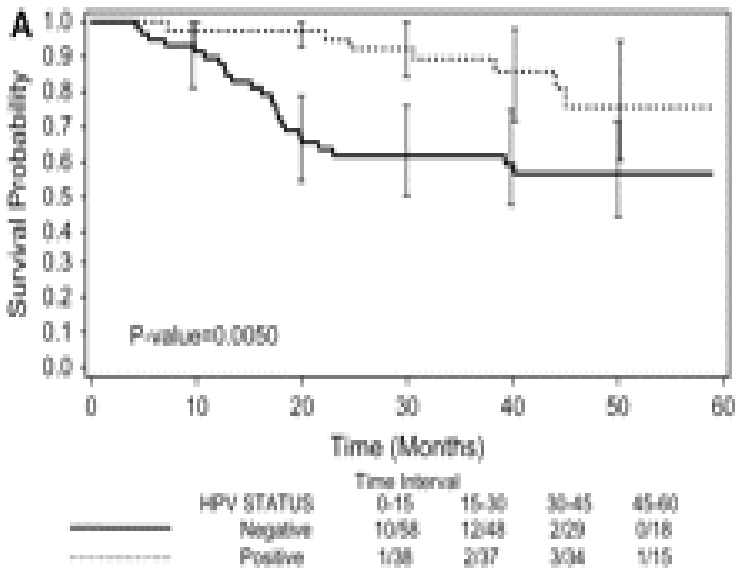
| Sexual Behavior | Patients with Oropharyngeal Cancer (N=100) | Control Patients (N=200) | Adjusted Odds Ratio (95% CI)† | |
|---|---|---|---|---|
| | | | All Patients | HPV-16+ Patients‡ |
| | *number (percent)* | | | |
| Lifetime no. of vaginal-sex partners | | | | |
| 0–5 | 31 (31) | 108 (54) | 1.0 | 1.0 |
| 6–25 | 41 (41) | 63 (32) | 2.2 (1.2–4.0) | 2.7 (1.4–5.5) |
| ≥26 | 28 (28) | 29 (14) | 3.1 (1.5–6.5)§ | 4.2 (1.8–9.4)¶ |
| Lifetime no. of oral-sex partners | | | | |
| 0 | 12 (12) | 38 (19) | 1.0 | 1.0 |
| 1–5 | 46 (46) | 110 (55) | 1.9 (0.8–4.5) | 3.8 (1.0–14.0) |
| ≥6 | 42 (42) | 52 (26) | 3.4 (1.3–8.8)‖ | 8.6 (2.2–34.0)** |
| Anal sex | | | | |
| No | 55 (55) | 129 (64) | 1.0 | 1.0 |
| Yes | 45 (45) | 71 (36) | 1.3 (0.8–2.2) | 1.6 (0.9–2.8) |
| Casual-sex partner†† | | | | |
| No | 42 (42) | 120 (60) | 1.0 | 1.0 |
| Yes | 58 (58) | 80 (40) | 1.7 (1.0–3.0) | 2.4 (1.2–4.7) |
| Age at first intercourse | | | | |
| 18 yr or older | 30 (30) | 87 (44) | 1.0 | 1.0 |
| 17 yr or younger | 70 (70) | 113 (56) | 1.3 (0.7–2.3) | 2.1 (1.1–3.6) |
| Condom use | | | | |
| Usually or always | 28 (28) | 90 (45) | 1.0 | 1.0 |
| Never or rarely | 72 (72) | 110 (55) | 2.2 (1.2–3.8) | 2.1 (1.1–4.0) |
| Sex with same-sex partner | | | | |
| No | 92 (92) | 186 (93) | 1.0 | 1.0 |
| Yes | 8 (8) | 14 (7) | 1.0 (0.4–2.6) | 1.1 (0.3–3.3) |
| Sexual partner with history of HPV-associated cancer‡‡ | | | | |
| No | 86 (86) | 190 (95) | 1.0 | 1.0 |
| Yes | 3 (3) | 2 (1) | 3.0 (0.5–20.5) | 3.9 (0.6–25.8) |
| Unsure | 11 (11) | 8 (4) | 2.3 (0.8–6.5) | 2.8 (0.9–8.5) |

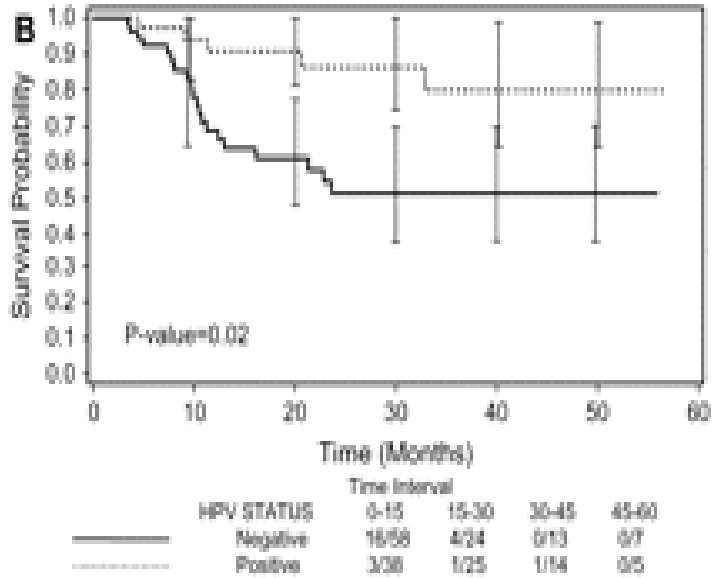
* The study was designed to have statistical power of 80% to detect an odds ratio of 2 or more for associations between sexual behavior and oropharyngeal cancer on the basis of the prevalence of sexual behaviors in case and control patients reported by Schwartz et al.,[9] a two-tailed $\alpha$ level of 0.05, and a 2:1 ratio of control patients to case patients. CI denotes confidence interval.
† Odds ratios were adjusted for age, sex, tobacco use, alcohol use, dentition and toothbrushing, and presence or absence of a family history of head and neck cancer. To evaluate trends in odds, ordinal variables were modeled as single, continuous, independent variables.
‡ This analysis was restricted to the 72 case patients with HPV-16-positive tumors (determined with the use of in situ hybridization; see Table 3), plus all 200 control patients.
§ P for trend=0.002.
¶ P for trend=0.001.
‖ P for trend=0.009.
** P for trend <0.001.
†† A casual-sex partner was defined as a partner in a "one-night stand" or a partner who was a stranger.
‡‡ Cancers considered to be associated with HPV included cervical, vulvar, vaginal, anal, penile, and head and neck cancers.

**D'Souza *et al*; *NEJM* 356:1944, 2007**

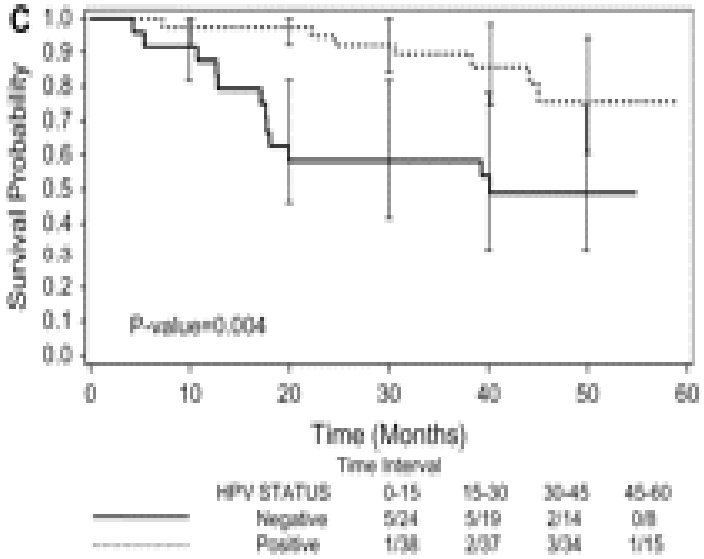
**OS** **DFS**

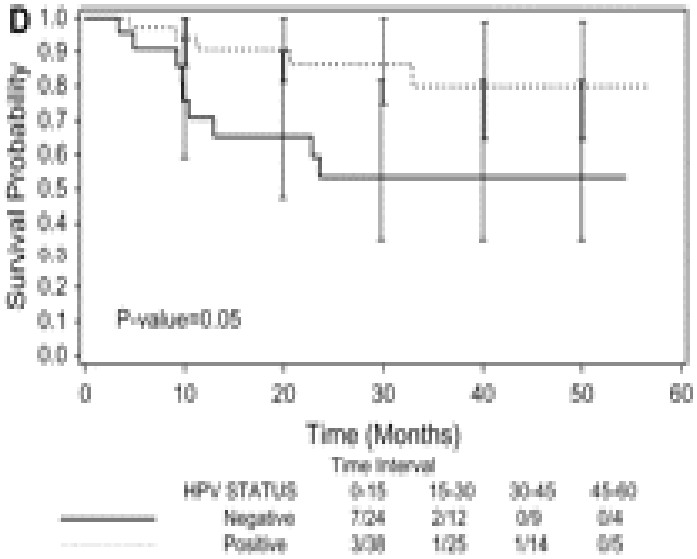
**All Patients**




**OPC**




**Fakhry *et al*; *JNCI* 100:261, 2008**

# Hypothesis

**HPV+ve OPCs are associated with increased p16 protein expression, and have an improved outcome, compared to HPV-ve OPC.**

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# Methods & Materials

- **In July 2003, a prospective clinical database was established (Anthology of Outcomes)**
- **Real-time clinical data: demographics, stage, treatment, and outcome**
- **3200 patients currently registered**


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# Methods & Materials

- **2003 – 2006, 112 FFPE biopsies of OPC patients treated at PMH**
- **Histology was reviewed by Dr. P-O**
- **Expression of p53, EGFR and p16 were determined by IHC**
- **HPV16 E6 mRNA determined using qRT-PCR**


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# Demographics

**Age**

| | |
|---|---|
| **Median** | **57** |
| **Range** | **27- 93** |

| **Gender** | **Frequency** | **Percent** |
|---|---|---|
| **Male** | **83** | **74%** |
| **Female** | **29** | **26%** |

**Smoking/Drinking History:**

| **Non-smoker/Non-Drinker** | **Frequency** | **Percent** |
|---|---|---|
| | **27** | **24%** |

# T&N Category

| T-category | Frequency | Percent |
|---|---|---|
| Tx | 4 | 3% |
| T1 | 13 | 12% |
| T2 | 51 | 46% |
| T3 | 23 | 20% |
| T4 | 21 | 19% |

| N-category | Frequency | Percent |
|---|---|---|
| N0 | 21 | 19% |
| N1 | 16 | 14% |
| N2 | 70 | 63% |
| N3 | 5 | 4% |

| Stage category | Frequency | Percent |
|---|---|---|
| I | 4 | 4% |
| II | 11 | 10% |
| III | 16 | 14% |
| IV | 81 | 72% |


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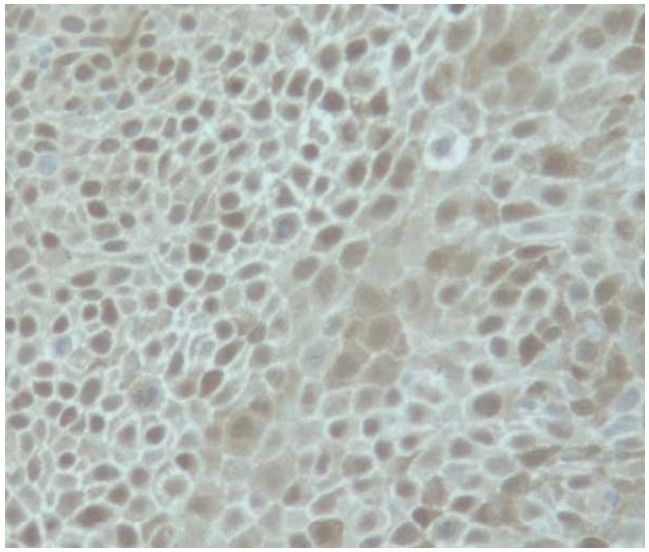
# Treatment Approach

| **Treatment** | Frequency | Percent |
|---|---|---|
| CRT (70 Gy/35#/7weeks) | 44 | 39% |
| RT alone | 68 | 61% |
| 70Gy/35#/7weeks RT alone | 13 | (19%) |
| HARDWINS (64Gy/40#/4weeks) | 17 | (25%) |
| 60 Gy/25#/5weeks | 25 | (37%) |
| 66 Gy/30#/6weeks | 9 | (13%) |
| Other | 4 | (6%) |

| **RT Technique** | Frequency | Percent |
|---|---|---|
| IMRT | 34 | 30% |
| Non-IMRT | 78 | 70% |

# p16 Immuno-expression

**p16 : score 3**



**classification**

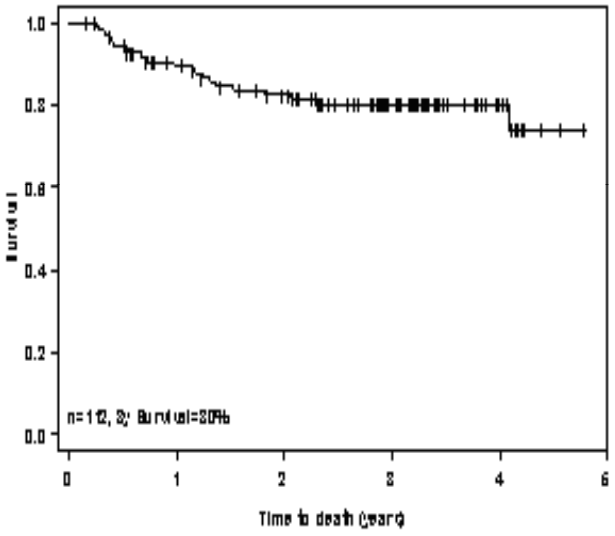
**more than 25% of tumor cells**

**Score 0 : no staining or non-specific staining**
**Score 1 : weak intensity and incomplete staining**
**Score 2 : moderate intensity and complete staining**
**Score 3 : strong intensity and complete staining**

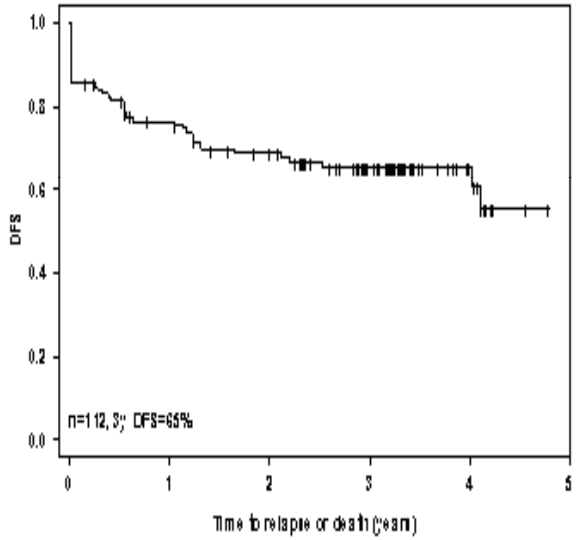
**definition**

**p16-Positive : Score 2 & 3**
**64/112 (57%)**

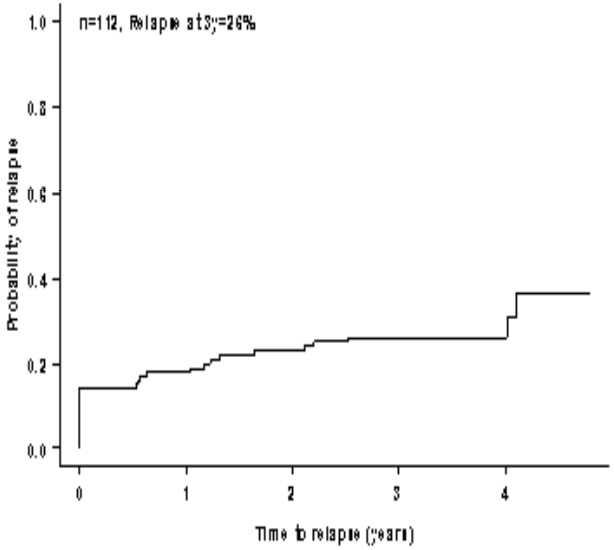
# 3-year OS, DFS & Probability of Relapse




**Overall survival**
**3 year: 80%**



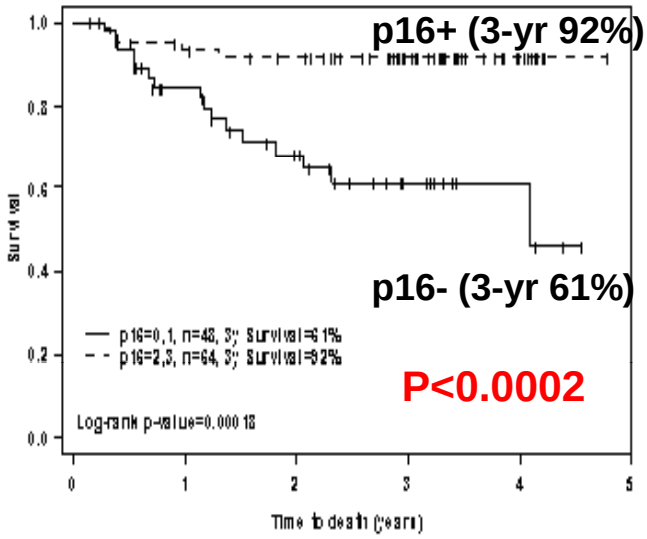

**Disease-free survival**
**3 year: 65%**



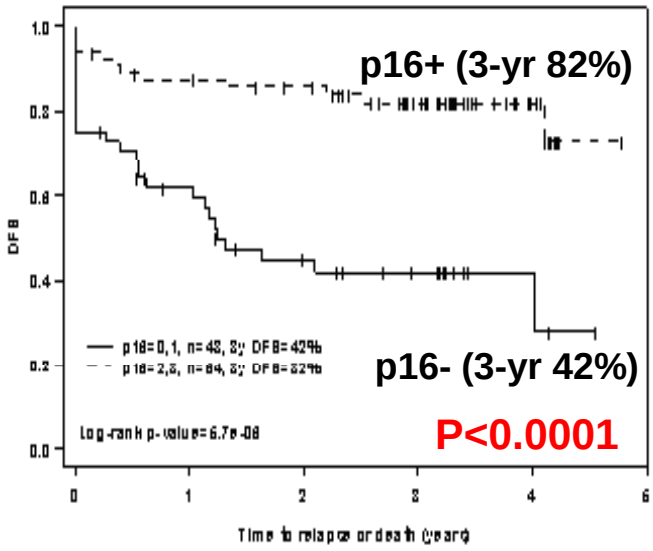

**Probability of relapse**
**3 year: 26%**

# OS, DFS, and Relapse with p16

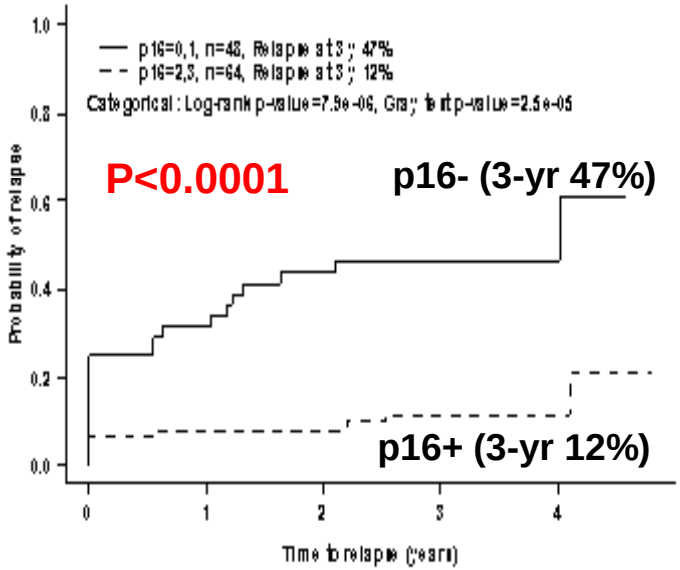
**OS**




**DFS**




**Probability of Relapse**

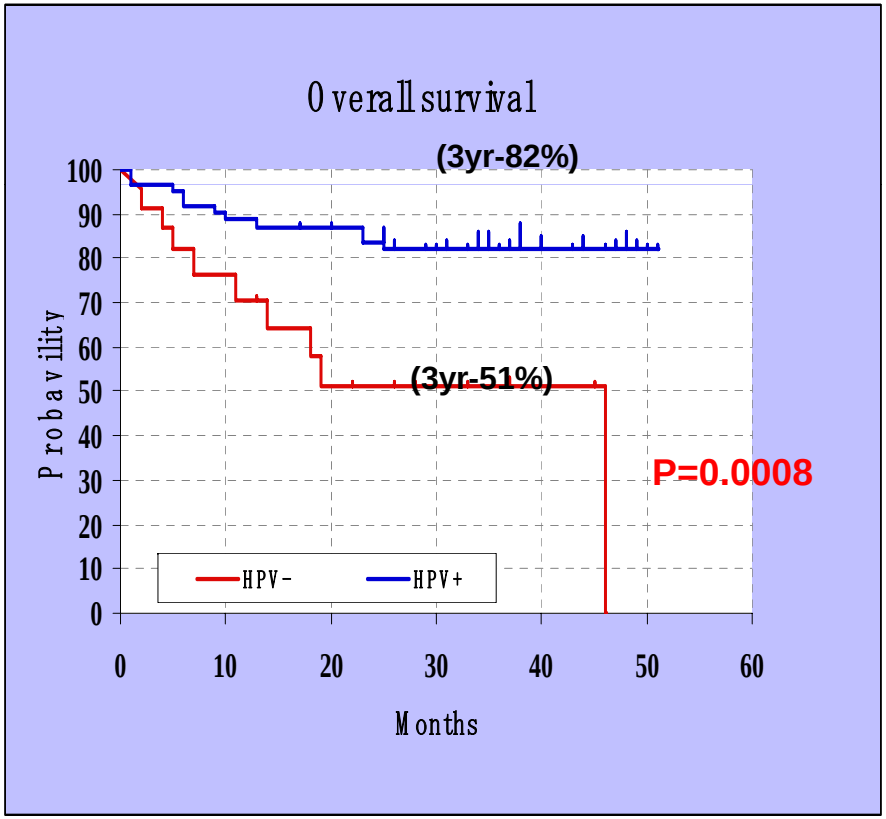


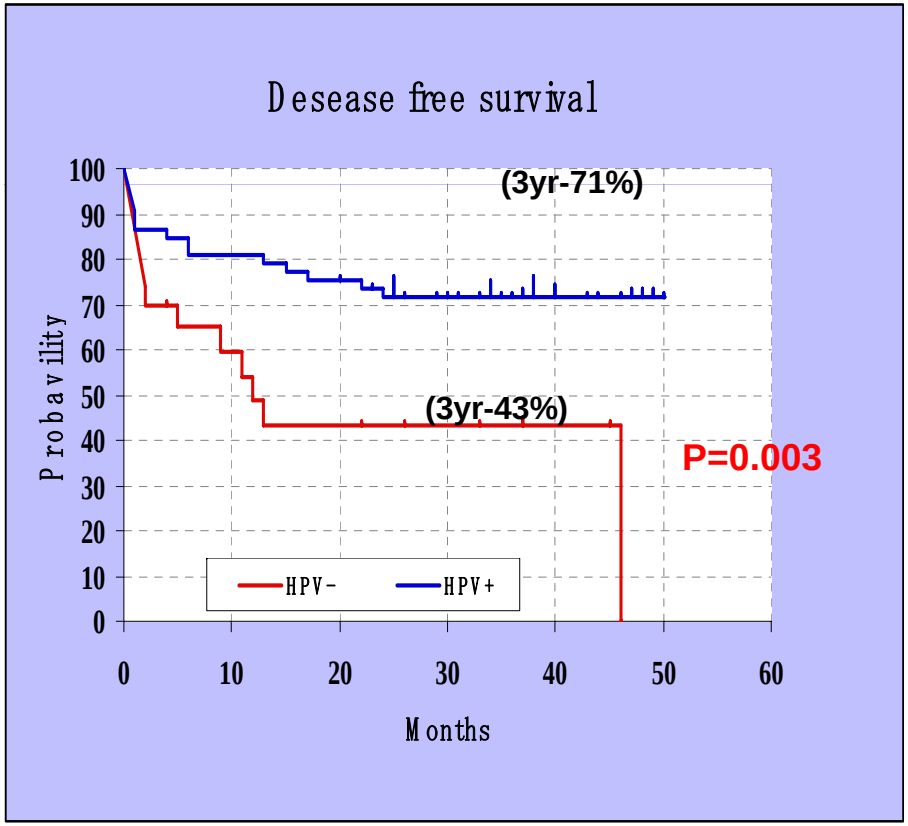
# p16 Expression Correlates with HPV16 E6 (86 cases)

| | HPV-negative (n=23) | HPV-positive (n=63) | |
|---|---|---|---|
| p16-negative | 20 (23%) | 14 (16%) | 34 |
| p16-positive | 3 ( 3%) | 49 (57%) | 52 |

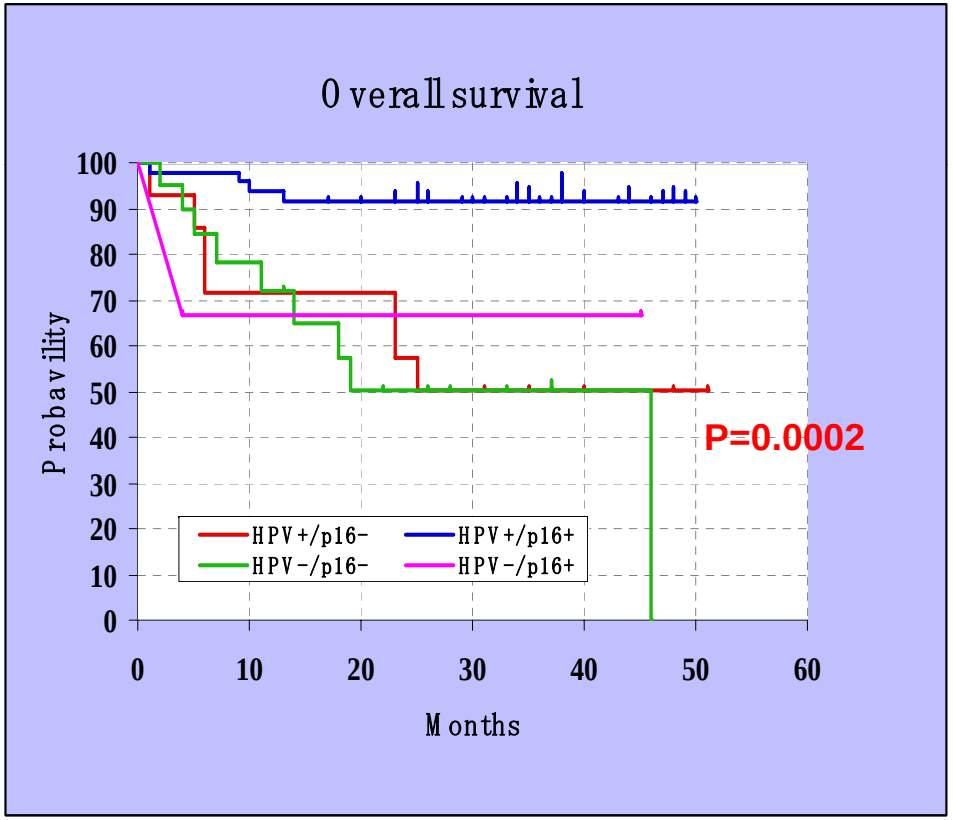
**P<0.0001**

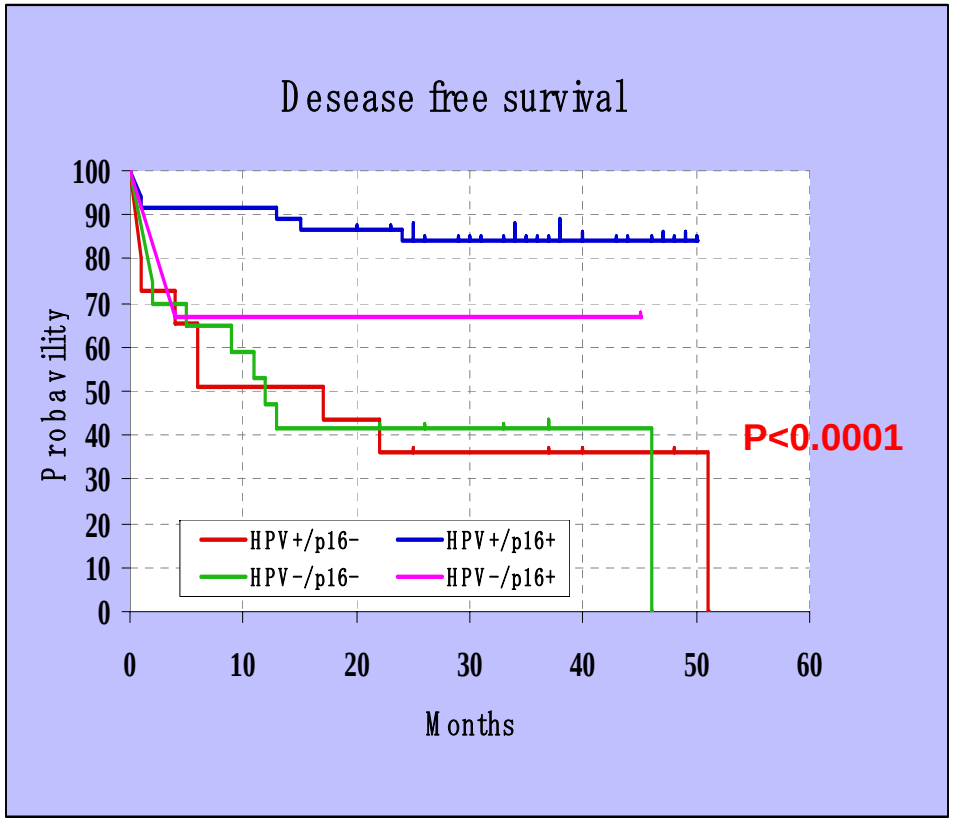
# OS and DFS in Relation to HPV16



# OS and DFS in Relation to HPV16 & p16 Expression



# Preliminary Conclusions

1. **Between 2003-2006, 57% of OPCs are p16 over-expressing.**

2. **In a sub-group, 73% of OPCs harbour HPV16 E6 mRNA.**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# Preliminary Conclusions

3. **P16 over-expression is significantly associated with presence of HPV16 E6.**

4. **Both p16 over-expressing, and HPV-positive OPCs are associated with improved OS, DFS, and reduced relapse rates.**


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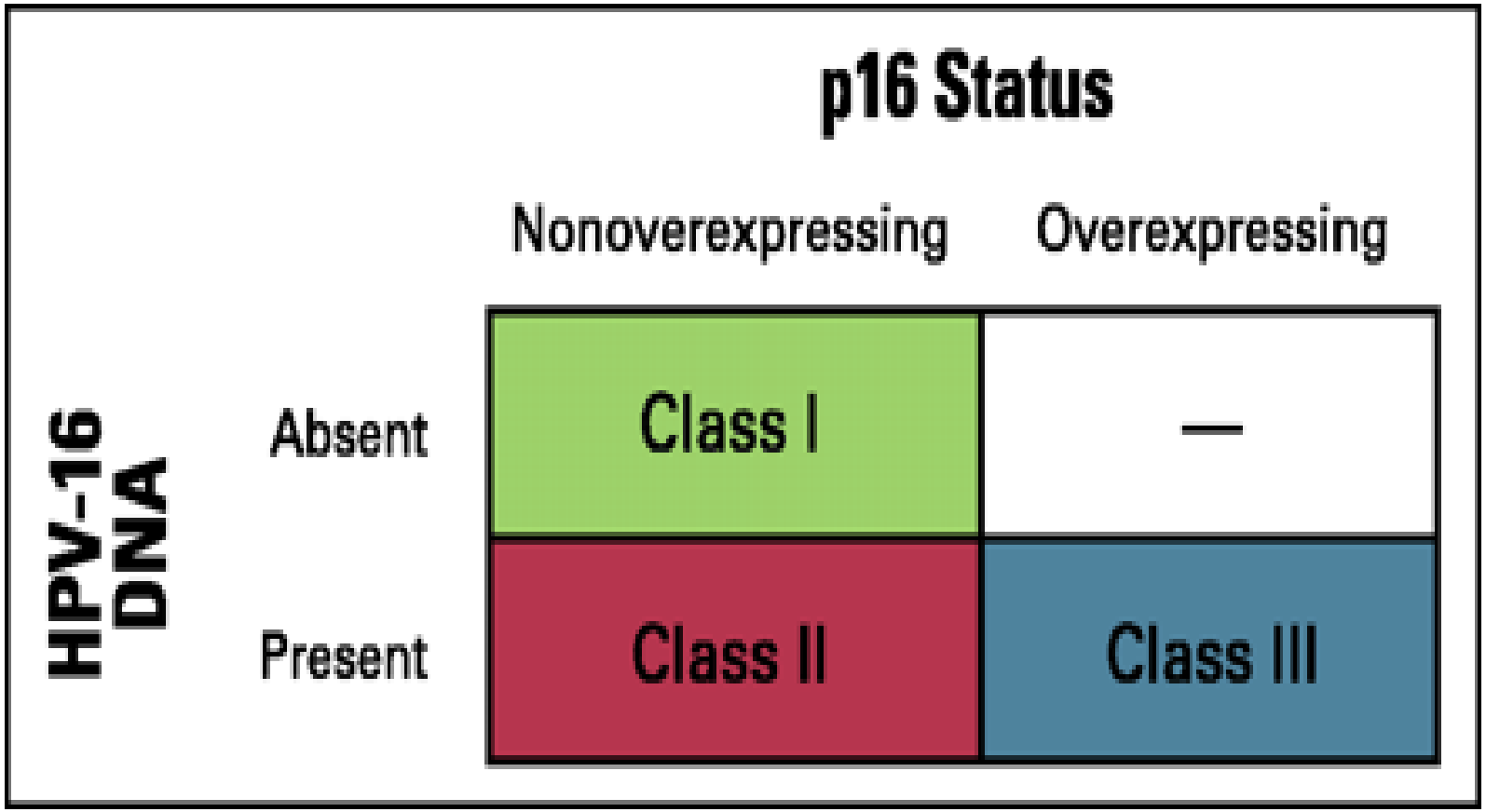
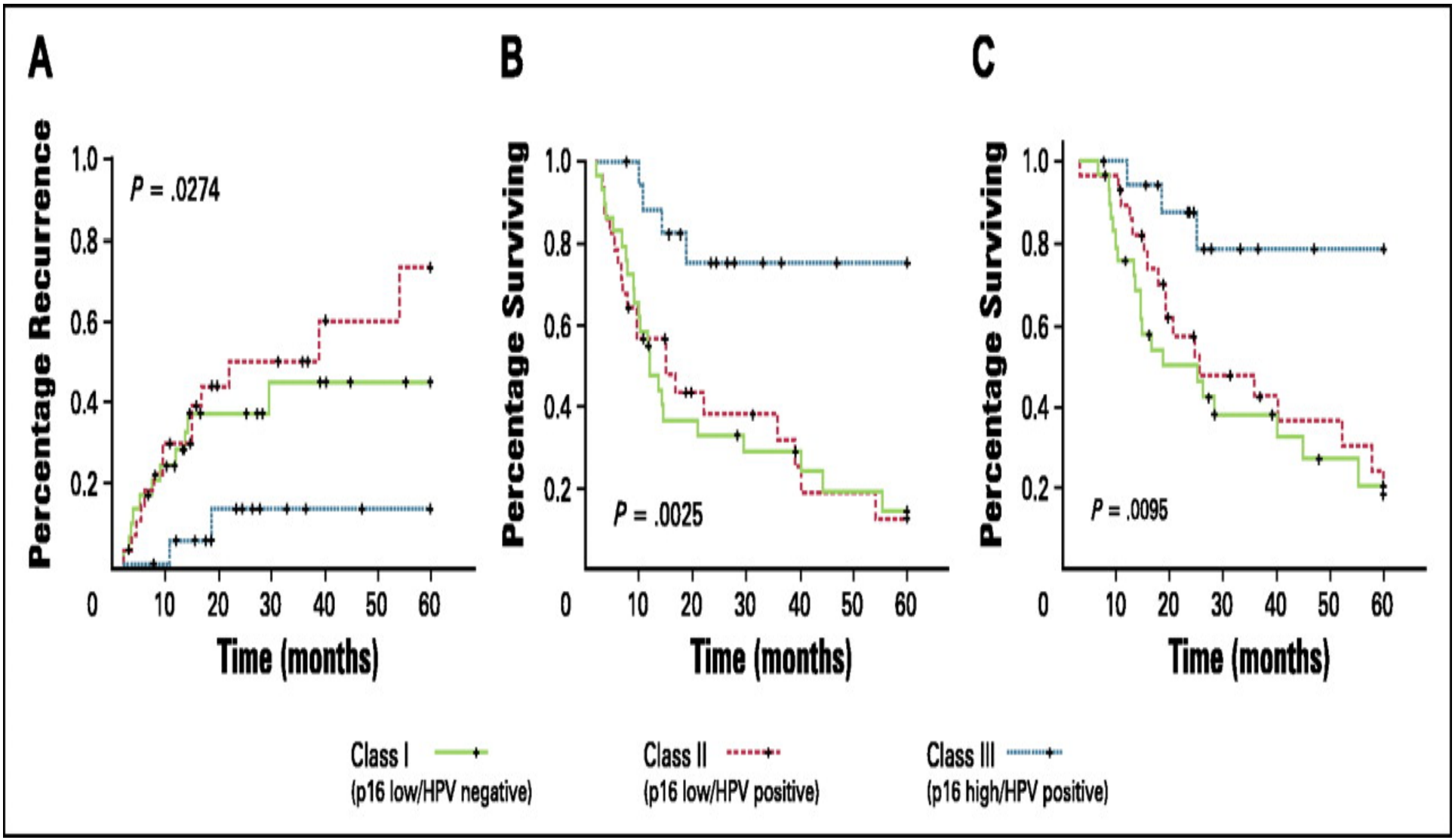
# What are the Mechanism(s)?




**Weinberger et al; *JCO* 24:736, 2006**

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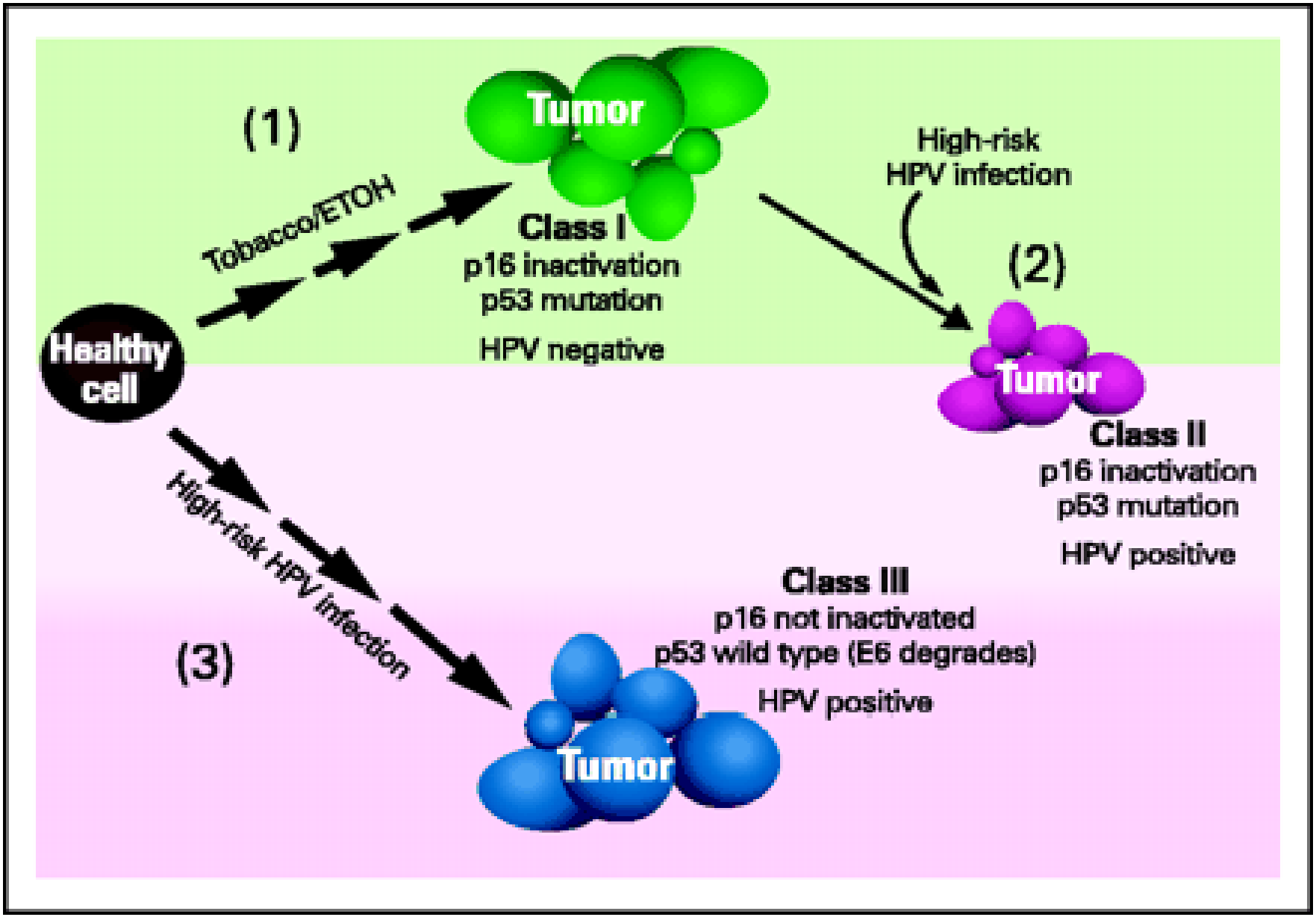


**Weinberger et al; *JCO* 24:736, 2006**

# Outstanding Questions

1. **Why do HPV-positive OPC fare better than HPV-negative disease?**
   a) **DNA repair defects b/o HPV genes**
   b) **Immunologic response**
   c) **Micro-RNA profiling**

2. **What host genetic factors lead to HPV-positive OPC?**
   a) **SNP profiles**


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# Outstanding Questions

3. **What is the most reliable & expedient method to diagnose HPV-positive OPC?**
   a) **HPV16 DNA ISH**
   b) **p16 IHC**

4. **Should HPV-positive OPC be treated differently?**
   a) **RT only**
   b) **Both RT and CT**


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# Outstanding Questions

5. **Is there a role for HPV vaccines for young male teenagers?**
   a) **Evidence supporting this possible approach**
   b) **Reduction in population burden**


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# Conclusion

1. **HPV status and p16 expression are amongst the most significant predictors for OPC.**

2. **An evolving entity, with complex biology, and challenges in clinical management, including health policy implications.**


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# PMH HNC Clinicians

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