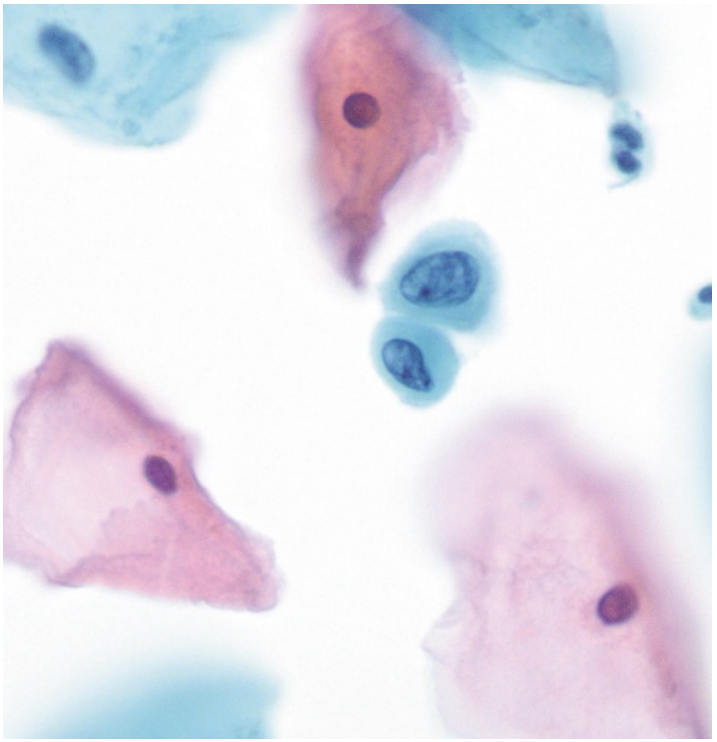
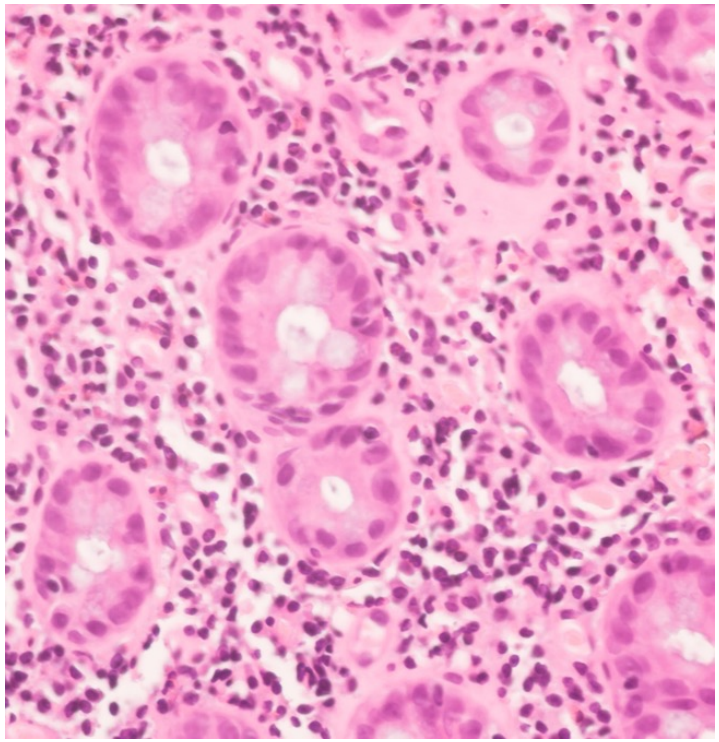
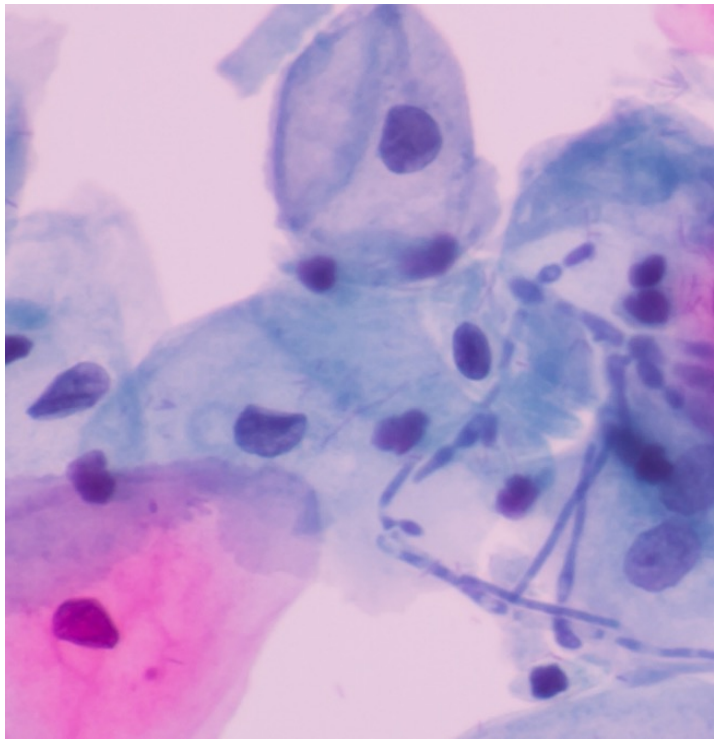




Moderne Diagnostik: HPV vs PAP Screening

Dr. Hans Georg Mustafa

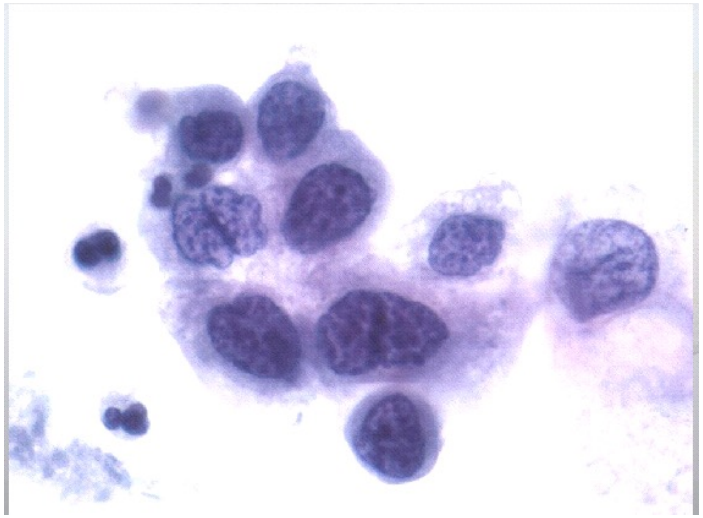
medilab

Interessenskonflikte

keine

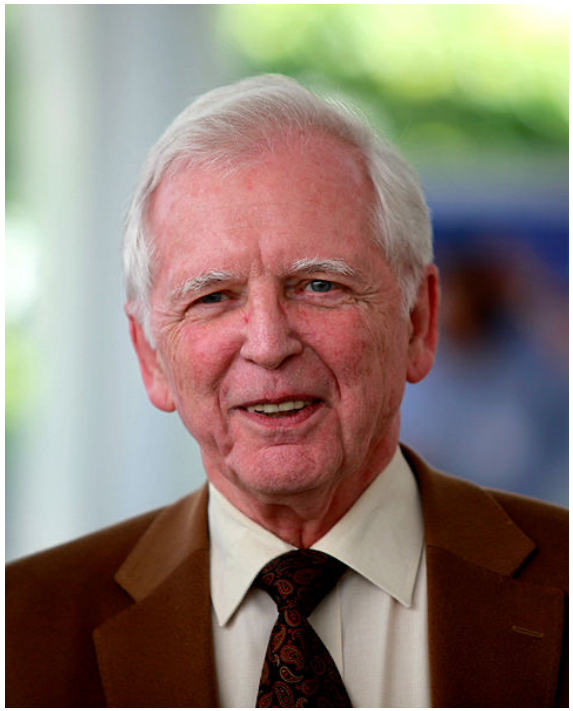
1928 griech. Arzt **Papanicolaou** entwickelt eine spezielle Färbung, um Krebszellen im Abstrich vom Gebärmutterhals zu erkennen.

1960er Pap Screening wird in vielen Ländern eingeführt



George Nicolas Papanicolaou

Prof. Harald zur Hausen
Mitte der 70 Jahre Publikation der Hypothese: HPV – Viren und Gebärmutterhalskrebs stehen in einem Zusammenhang
Anfang der 80 Jahre Isolierung von HPV 16 und 18 aus dem Gewebe von Gebärmutterhalskrebs.



2008 Nobelpreis

Onkogene DNS-Viren

Der einzige mit Sicherheit durch ein DNS-Virus verursachte Tumor beim Menschen ist die *Verucca vulgaris* (Papovavirus). *Epstein-Barr Virus* (EBV) wird für das *Burkitt-Lymphom* und *nasopharyngeale Karzinome* verantwortlich gemacht. Beim *Gebärmutterhalskarzinom* finden sich neuerdings zunehmend Hinweise auf eine virale Genese: *HSV-2* (Herpes simplex Virus 2) und *HPV* (human papilloma virus, besonders Typ 16 und 18). Die letzteren verursachen *flache Kondylome* mit typischen zytologischen Veränderungen (Koilozytose), die als Präkanzerosen gelten.

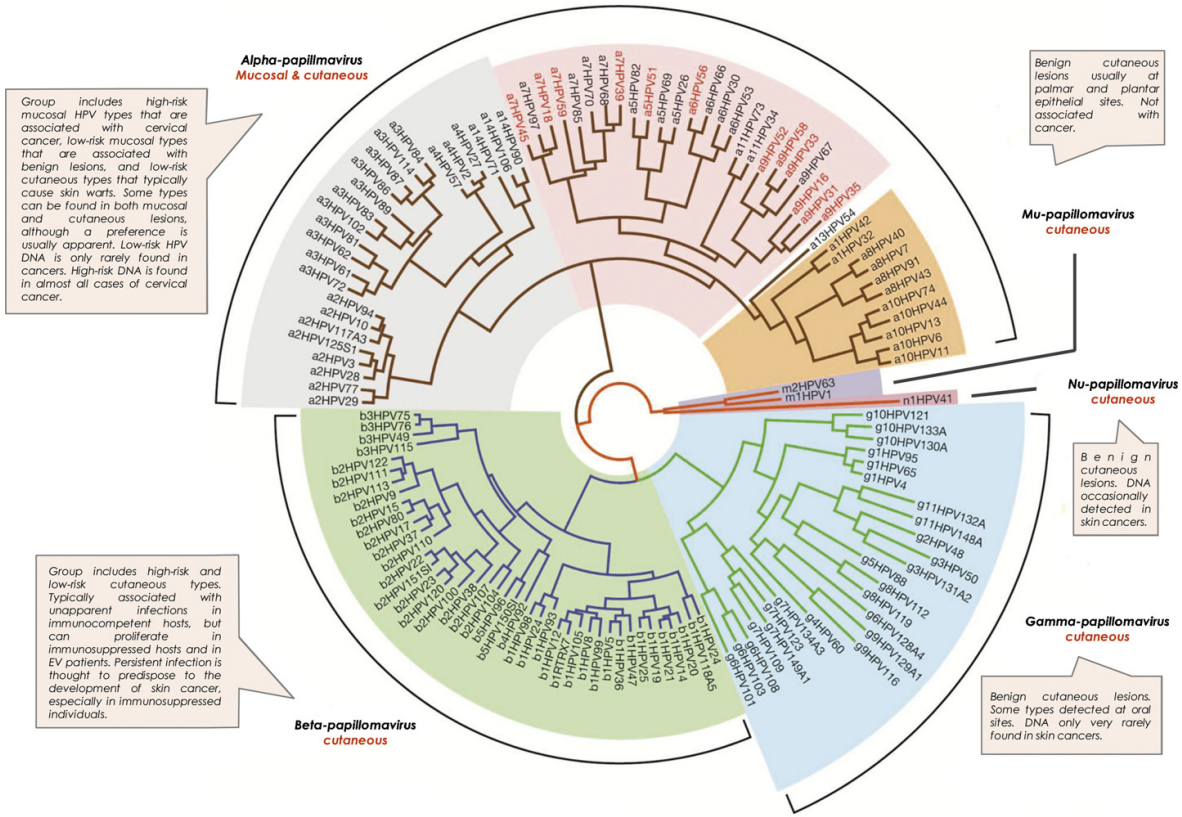


Figure 1 Evolutionary Relationship between Human Papillomaviruses

i) UNINFECTED EPITHELIUM

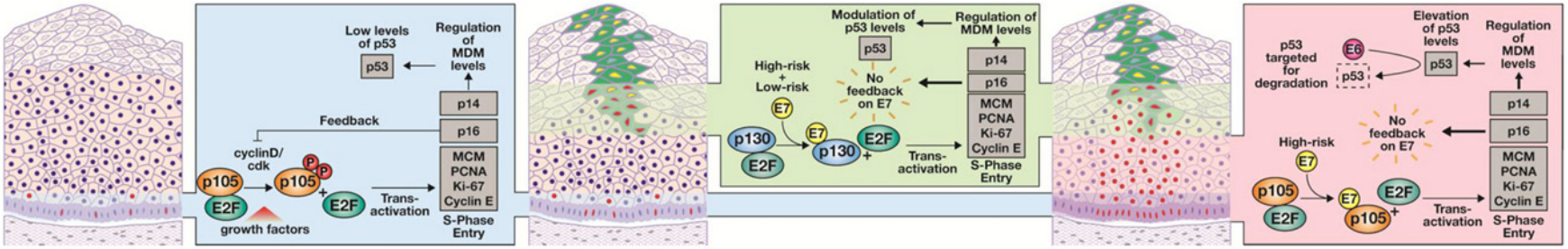
Cell cycle entry and cell proliferation only in the basal and parabasal cell layers stimulated by growth factors (blue box). No cell cycle entry in the superficial cell layers.

ii) LOW-RISK HPV INFECTION

E6/E7 expression stimulates cell cycle entry (but not cell proliferation) in the upper epithelial layers allowing genome amplification in low and also high-risk HPV infections (green box). Basal cell proliferation may still be stimulated by growth factors as shown for the uninfected epithelium (blue box).

iii) HIGH-RISK HPV INFECTION

In high-risk HPV infections, E6/E7 expression stimulates additional cell cycle entry and cell proliferation in the lower and middle epithelial layers leading to neoplasia (red box). E6/E7 also drive cell cycle entry in the upper epithelial layers to allow genome amplification as shown for low-risk HPV infections (green box).

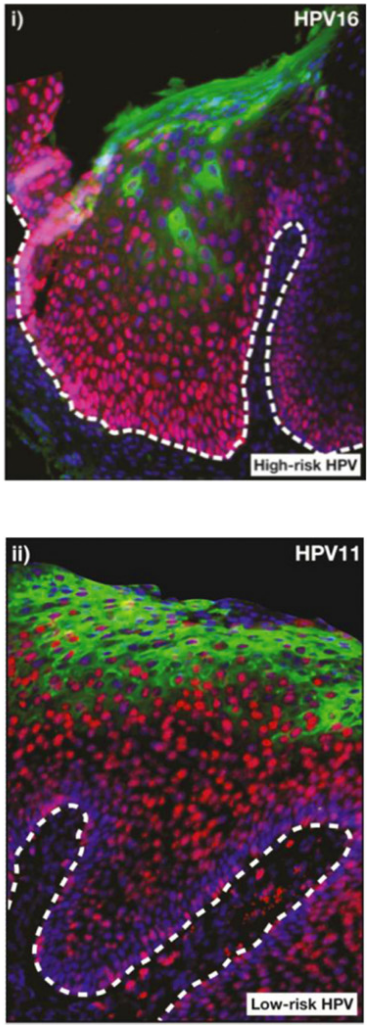


J. Doorbar et al.

A

	High-Risk Alpha	Low-Risk Alpha
E6	encodes E6* products	no E6* products
	binding and degradation of… •p53 •specific PDZ-domain proteins (e.g. Dlg, MAGI-1, Scribble)	weaker binding (no degradation) of… •p53 •no binding of PDZ-domain proteins
	interact with the E6AP ubiquitin ligase inhibition of p53 transactivation and acetylation	
	inhibition of apoptosis	unknown
	bypass of growth arrest following DNA damage	normal growth arrest following DNA damage
	inhibition of keratinocyte differentiation	unknown
	inhibition of interferon response	weaker inhibition of interferon response
	activation of signaling pathways… •Akt •Wnt •Notch •mTORC1	unknown
	telomerase activation	no activation
	c-myc activation	no activation
E7	binding and degradation of… •pRb •p107 •p130 binding (no degradation) of… •E2F1 •Cullin2 •HDAC	weaker binding (no degradation) of… •pRb •p107 •E2F1 binding of… •p130
	binding of regulatory proteins including E2F6, p600, HAT, PP2A induction of cell cycle entry and DNA synthesis role in genome amplification	
	induction of genome instability	no stimulation of instability
	suppression of STAT-1 function	no suppression
	immortalization and transformation functions	no such functions
	activation of signaling pathways… •Akt	unknown

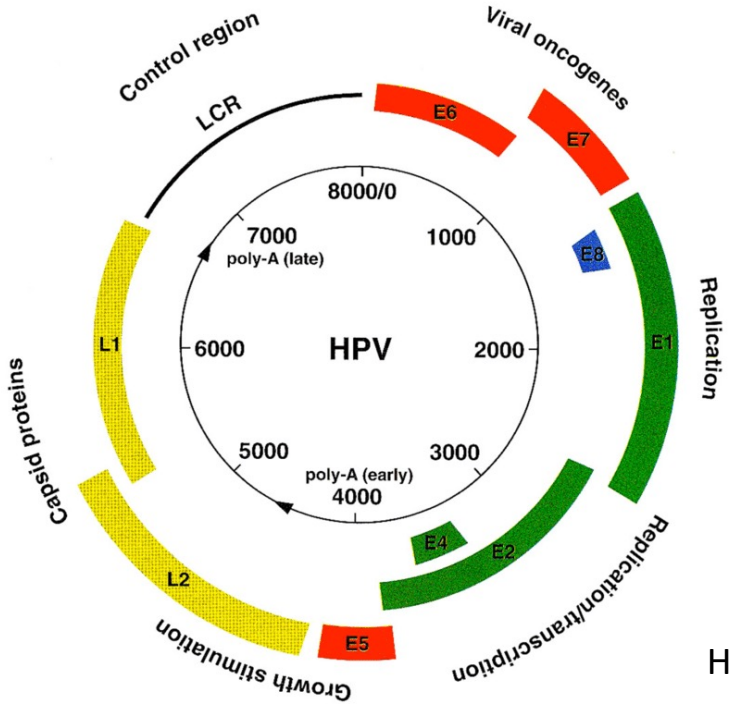
B



J. Doorbar et al.

HPV 16

HPV-PCR
Impfung



HPV-PCR
Tumour suppressor protein p53

Retinoblastoma protein
Rb related pocket prot.
Akt. Cyclins (inh. Cycline kinase Inhibitor)

HPV-PCR

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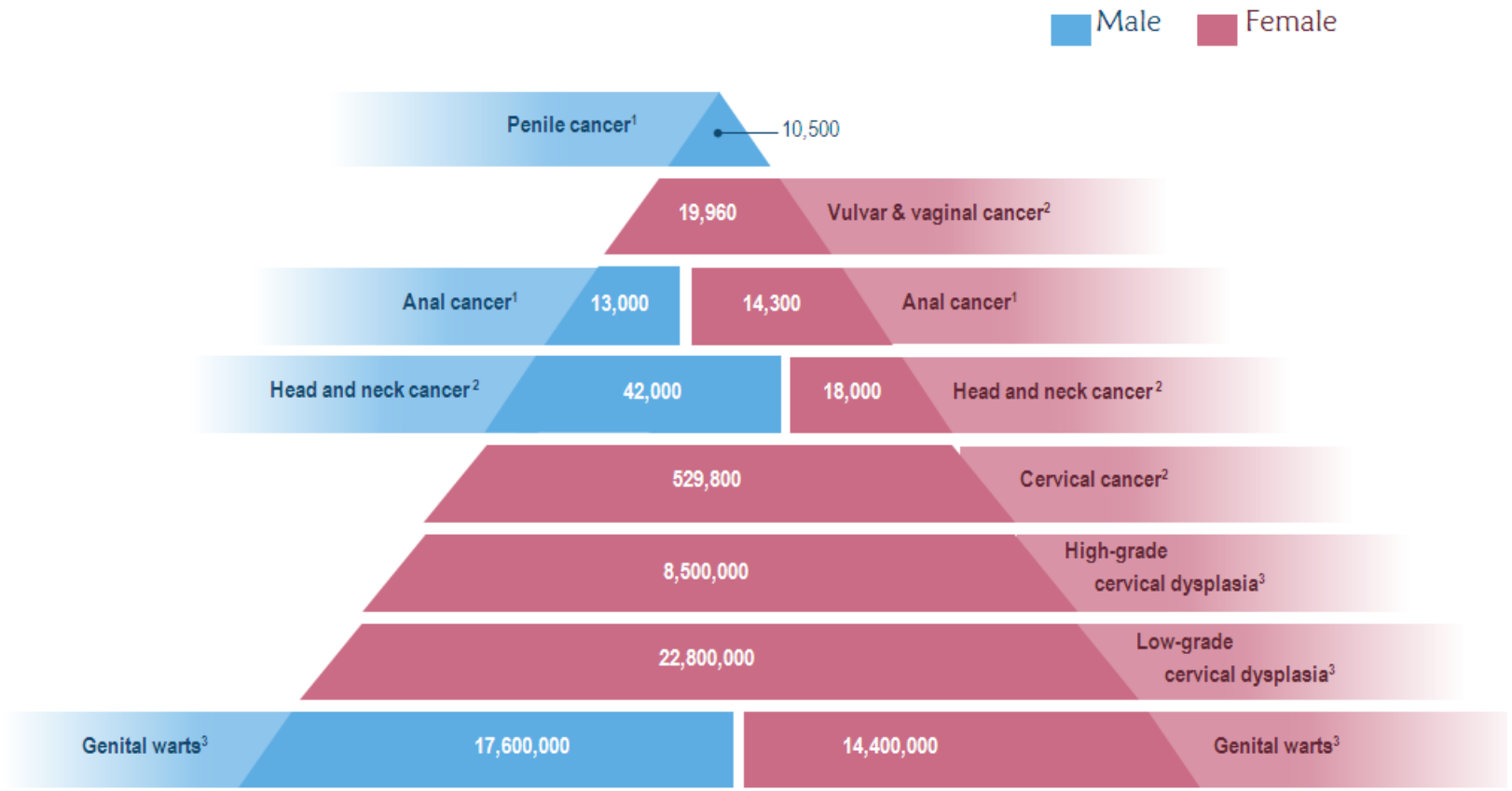
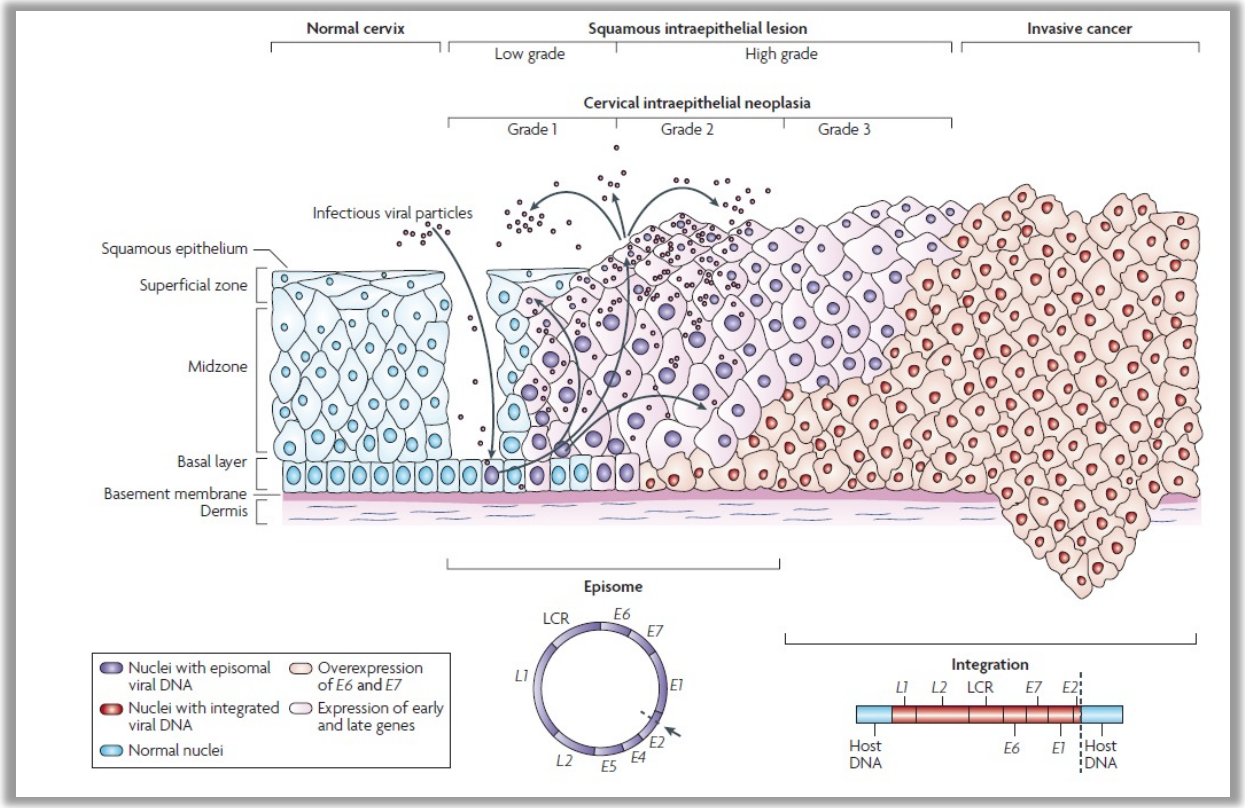
Estimated annual new HPV-related disease cases in males and females globally



Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine*. 2006;24(Suppl 3):S3/11–S3/25.



Ciaran B. J. Woodman, Stuart I. Collins & Lawrence S. Young
Nature Reviews Cancer volume 7, pages 11–22 (2007)

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Grundlegende Eigenschaften eines Screeningtest

- Technische Aspekte
  - Reproduzierbarkeit
  - Durchgängige Identifikation
  - Vertretbarer analytischer Aufwand = Routine Tauglichkeit
- Medizinische Aspekte
  - Ausreichende Sensitivität und Spezifität bzw. NPV und PPV
  - Nachweislicher medizinischer Benefit

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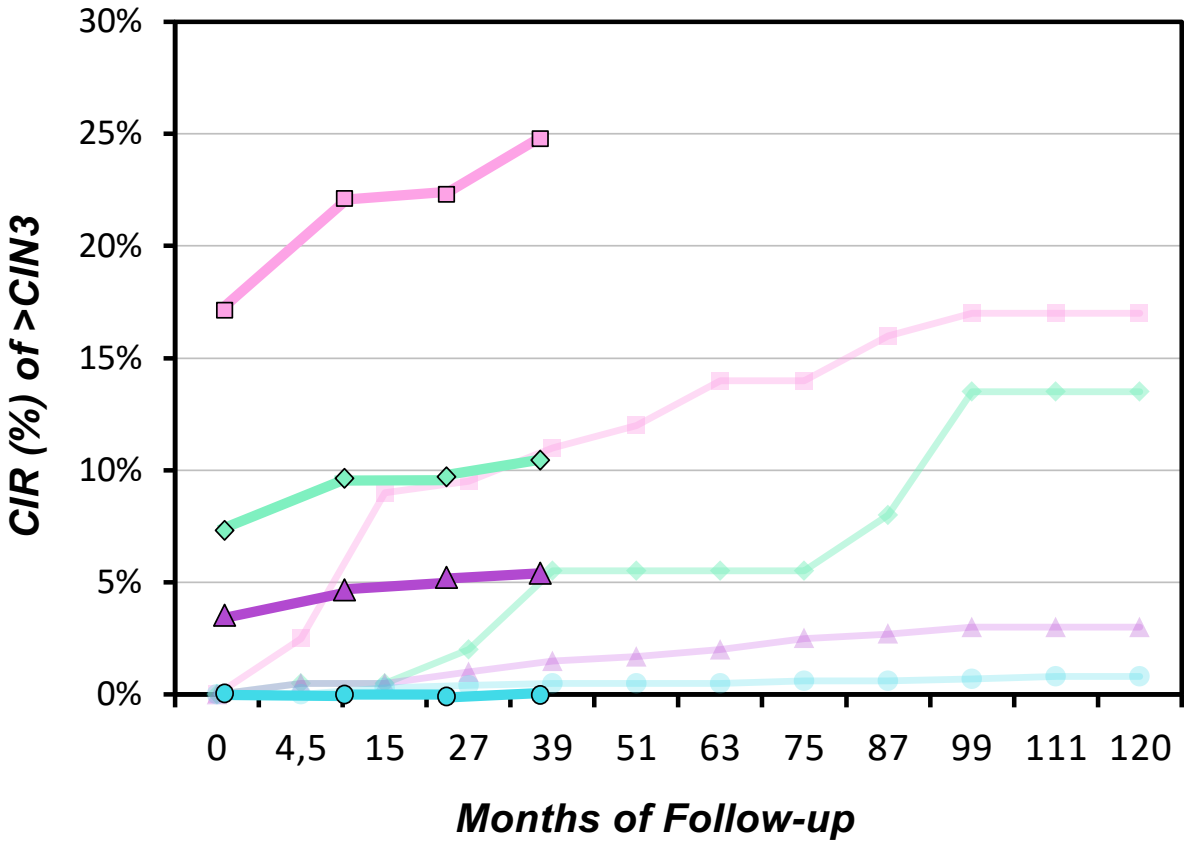
10-yr CIR of ≥CIN3 in ATHENA* vs. Kaiser



***Verification bias adjusted estimates**

All women enrolled shown for both datasets

Khan MJ, *et al. J Natl Cancer Inst* 2005; 97:1072.

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Sensitivität / Spezifität PAP vs. HPV

	Sensitivität	Range	Spezifität	Range
HPV	96,1%	85 – 100%	90,7%	76,5 – 95,5%
PAP	53,0%	18,6 – 76,7%	96,3%	84,2 - 99,5%

Meta – Analyse Screening Studien (Cuzick Int J Cancer 2006)

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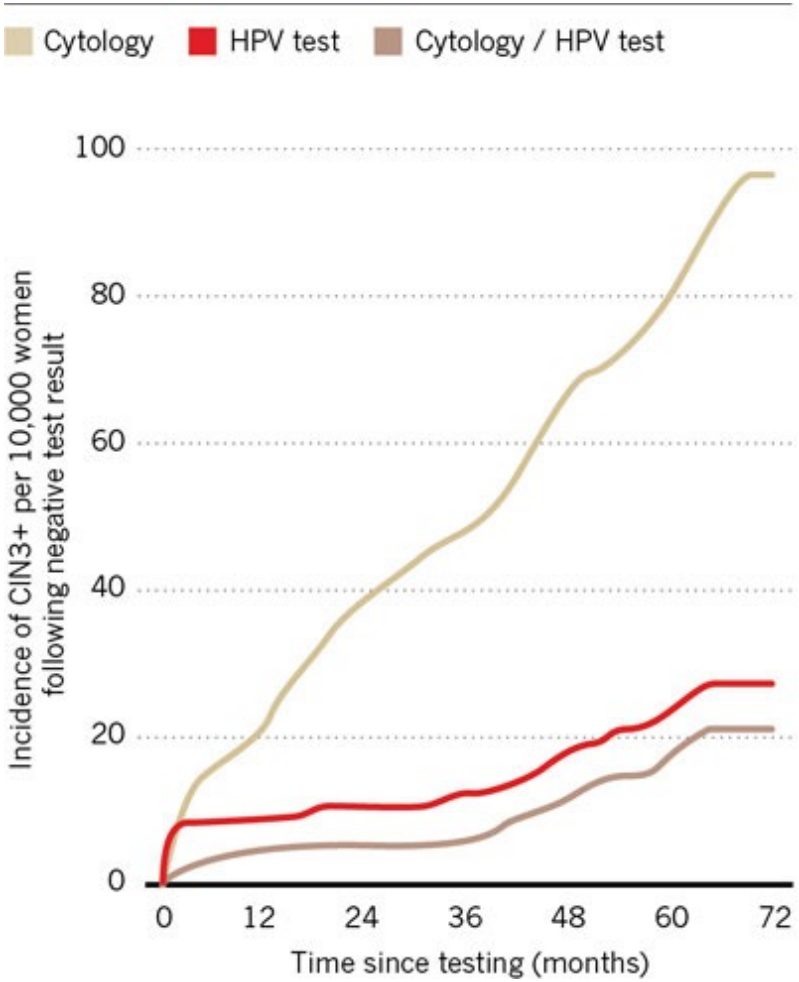
HPV Screening

- Ein Bild sagt mehr als tausend Worte



L. Hefler

Österreichische Gesellschaft für Gynäkologie und Geburtshilfe

OEGGG

Gemeinsame Leitlinie der OEGGG, AGO, AGK und ÖGZ zur Diagnose und Therapie von Cervikalen Intraepithelialen Neoplasien sowie Vorgangsweise bei zytologischen Befunden mit eingeschränkter Qualität

Table 3. Comparative Effectiveness of the Established Cervical Cancer Screening Methods.*

Methods Compared	Comparison of angesBenefit-to-Harm Balances
HPV DNA testing vs. VIA	HPV DNA testing >> VIA
HPV DNA testing vs. cytology	HPV DNA testing > cytology
HPV DNA testing vs. cotesting†	HPV DNA testing ≥ cotesting

* The symbol >> indicates that the benefits of testing clearly outweigh the harms, the symbol > that the benefits outweigh the harms, and the symbol ≥ that the benefits do not outweigh the harms. VIA denotes visual inspection with acetic acid.
† Cotesting involves screening and cytologic analysis combined.

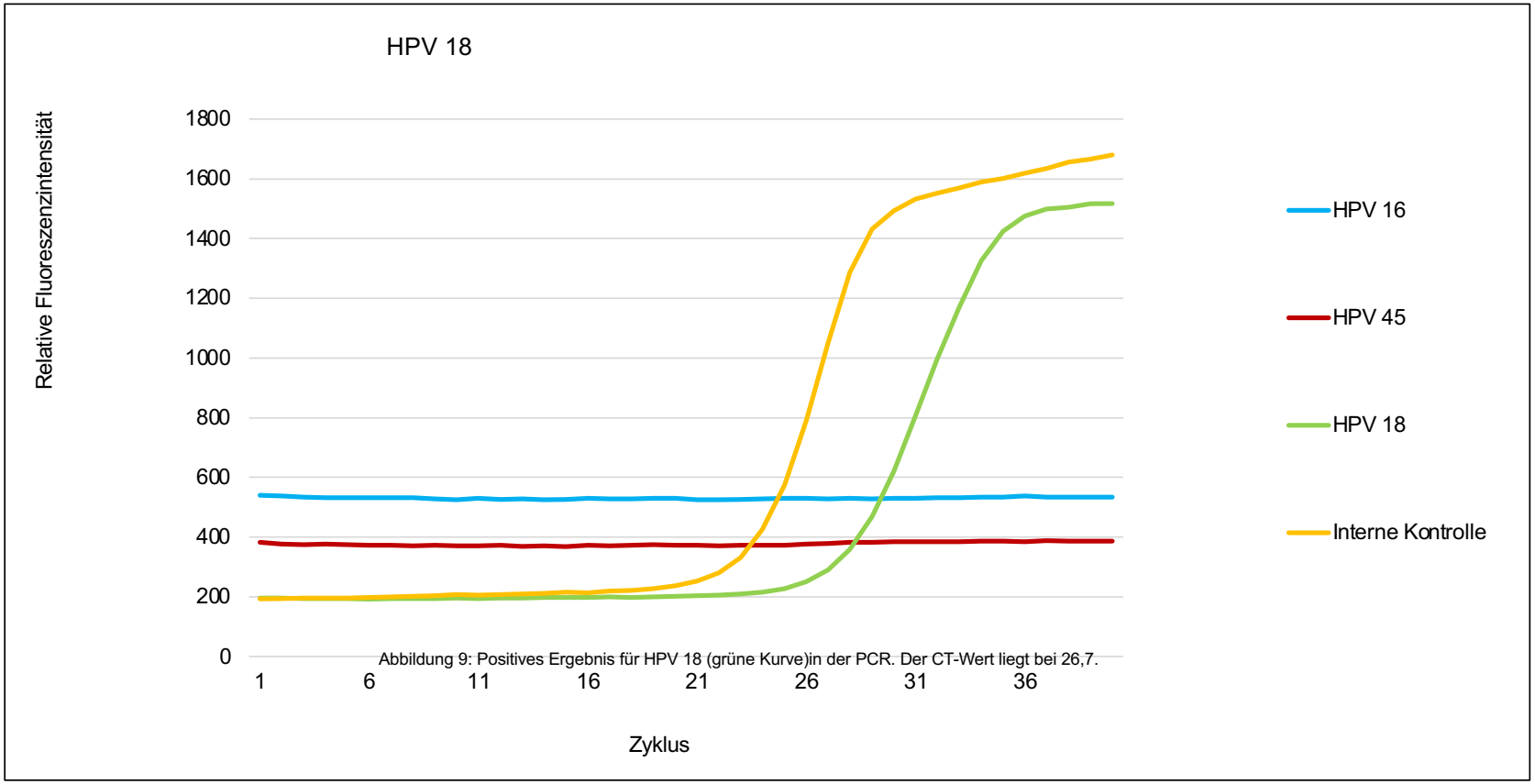
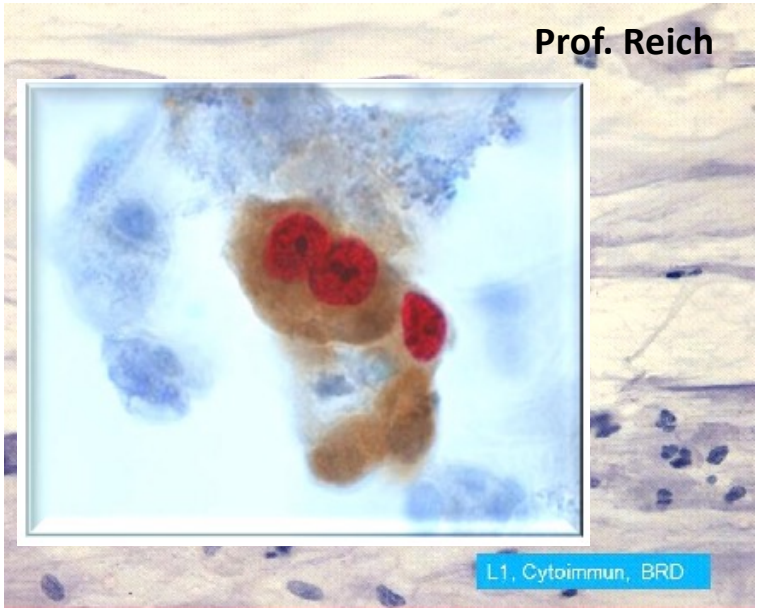


Abbildung 9: Positives Ergebnis für HPV 18 (grüne Kurve)in der PCR. Der CT-Wert liegt bei 26,7.

Biomarker

- E6/E7 mRNA (PCR)
- L1 p16, Ki-67
- Singel Genotypisierung
- DNA Methylierung



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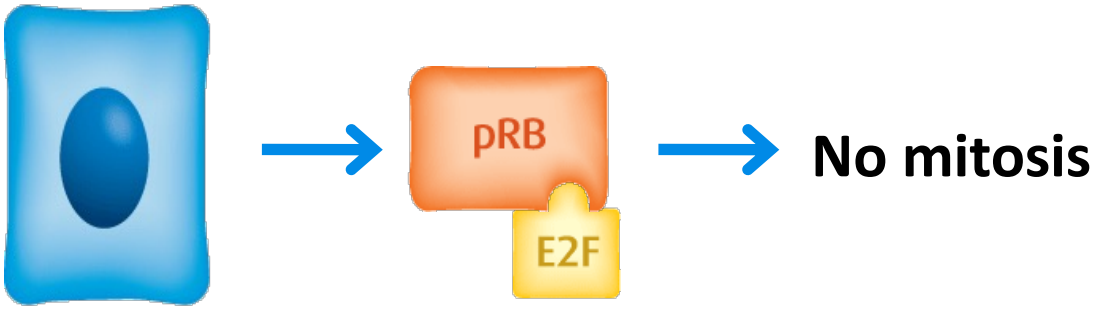
Normal cell cycle arrest (not dividing)



Retinoblastoma protein (pRB) binds to the transcription factor E2F.

The pRB-E2F protein complex blocks transcription of genes that promote cell cycle progression and proliferation.

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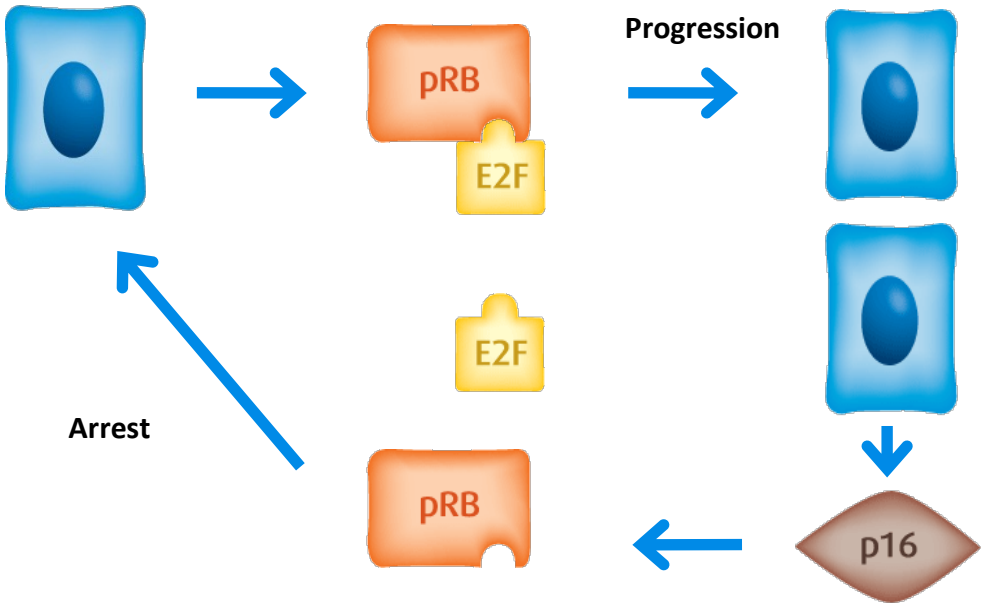
Normal cell cycle dividing



Feedback control mechanism maintaining cell cycle proliferation and arrest.
The release of E2F from pRB results in cell cycle progression, mitotic replication and activation of the p16 gene. This enables p16 protein production and facilitates the re-binding of pRB to E2F, leading to cell cycle arrest.

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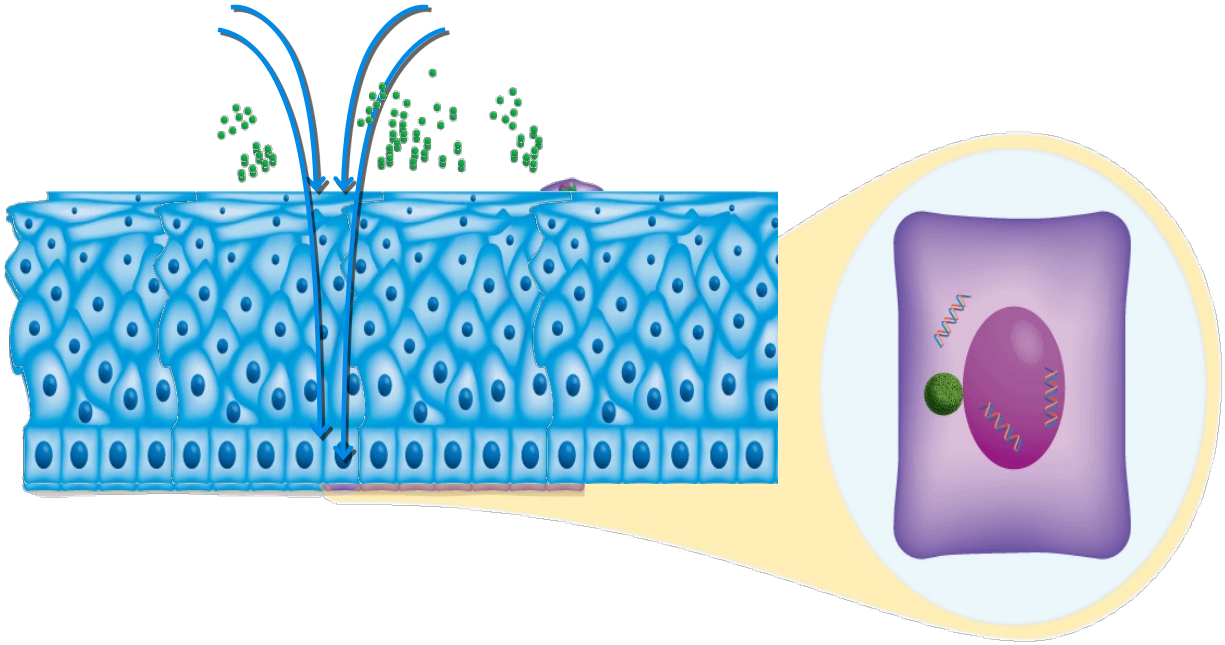
HPV Infection



The onset of HPV-mediated cervical disease occurs when HR-HPV types infect the basal cells of the epithelium. The vast majority of HPV infections are transient and clear within 6-12 months.

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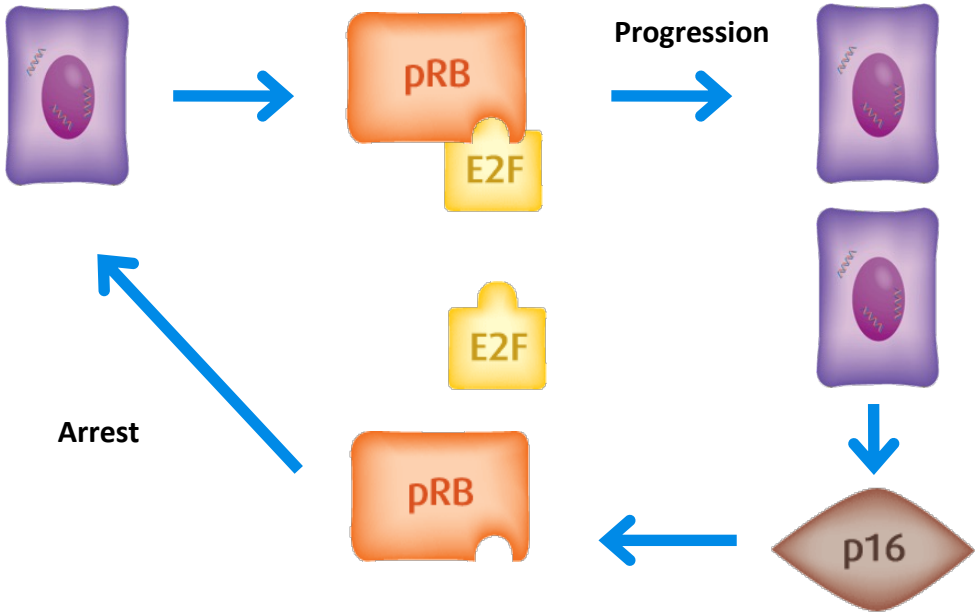
Transient HPV Infection



Although transient HPV infection may result in increased cell proliferation, these infections do not disrupt the balance between pRB and E2F or the control of p16 expression.

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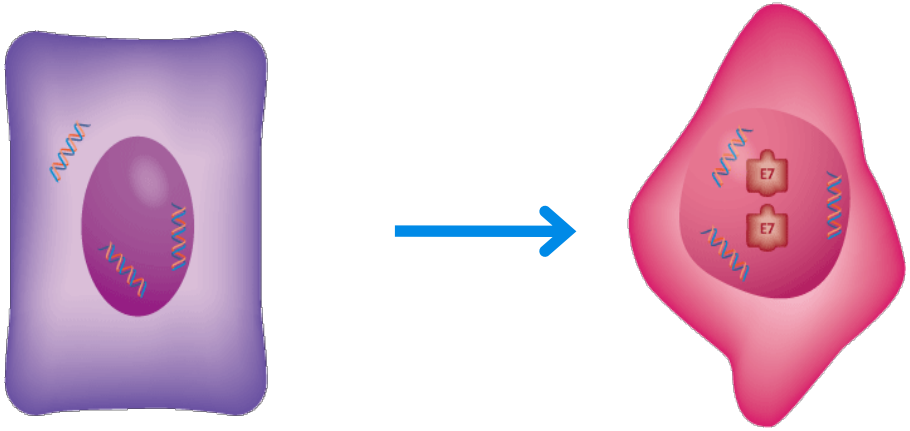
Transforming HPV Infection



Some HR-HPV infections persist and produce levels of viral E6 and E7 oncoproteins that can mediate oncogenic transformation by disrupting the cell cycle regulatory mechanism.

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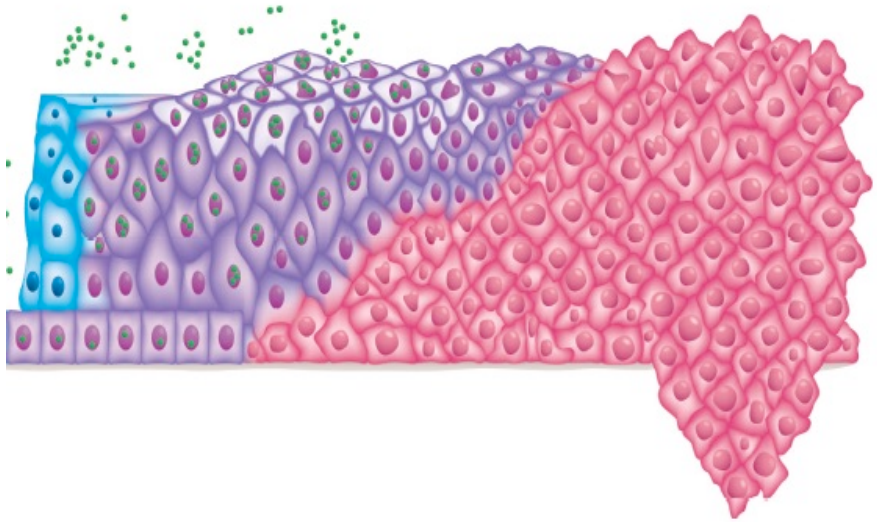
Transforming HPV Infection



Fully transformed cells are characterized by unregulated cell cycle progression, disrupted maturation and the ability to invade underlying cervical stroma, resulting in cervical cancer.

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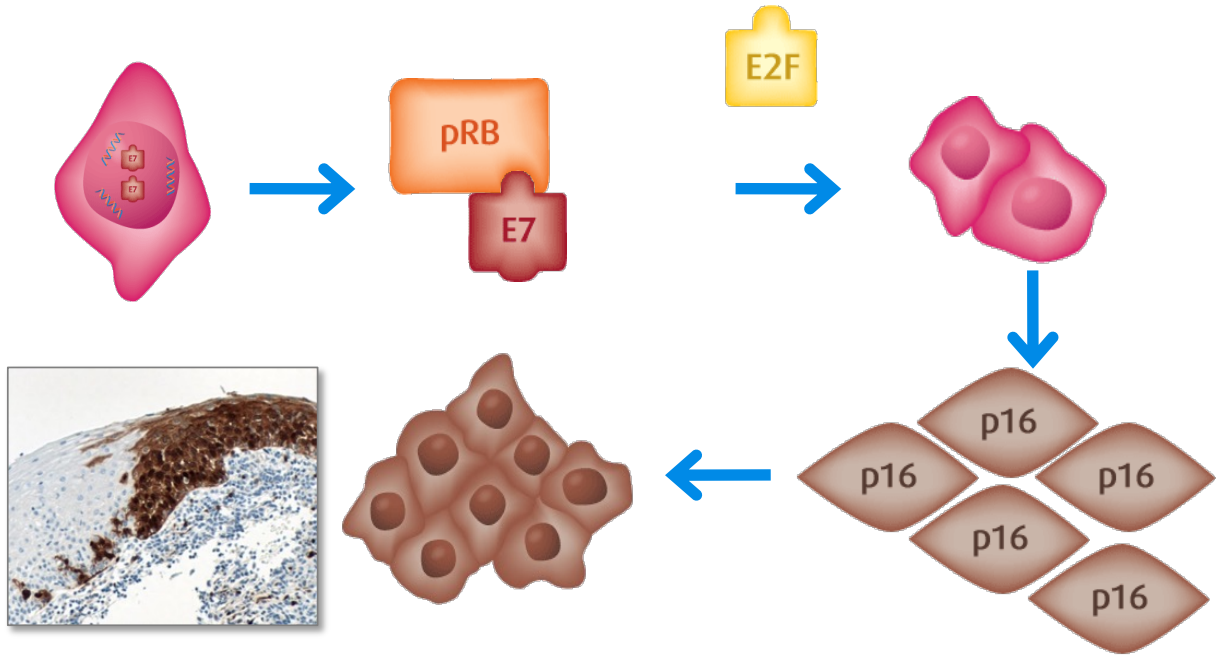
Transforming Infection: Oncogenesis



In cells with transforming HPV infections, HPV viral oncoprotein E7 impairs the function of pRB, disrupting its ability to bind to E2F. This leads to deregulated cell proliferation, genetic instability and p16 over-expression. Detectible by immunohistochemistry and immunocytochemistry staining.

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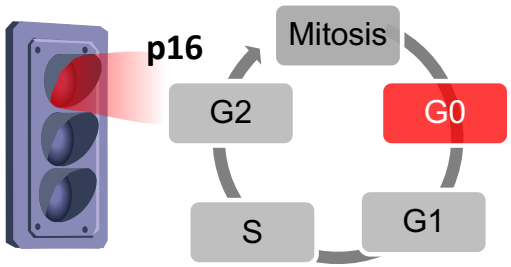
p16 Protein Expression

p16, negative cell cycle regulator

Anti-proliferative effect in physiologically normal cells

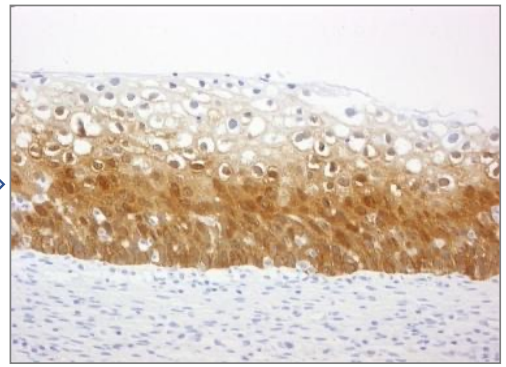
p16 restricted to the G0 Phase

In cervical dysplasia, p16 cannot facilitate cell cycle arrest; the HPV viral E7 oncoproteins inactivate the tumor suppressor pRb upstream from p16



Cell cycle arrest

p16 over-expression in CIN



Cervical intraepithelial neoplasia

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Proliferation Marker Ki-67

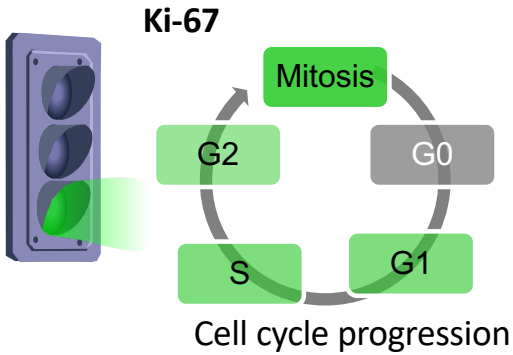
Ki-67 protein can be detected within nuclei of normal proliferating cells

Expression restricted to the G1, S, G2 and M phase of the cell cycle

- Marker of cell proliferation

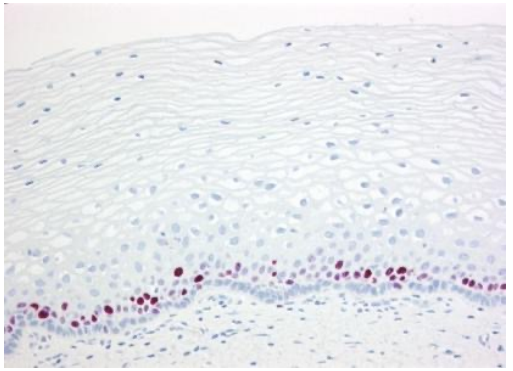
No expression in non-dividing cells

- Absent in G0 phase of the cell cycle



Cell cycle progression

Proliferating, normal squamous epithelium, expressing Ki-67


Normal cervical squamous epithelium

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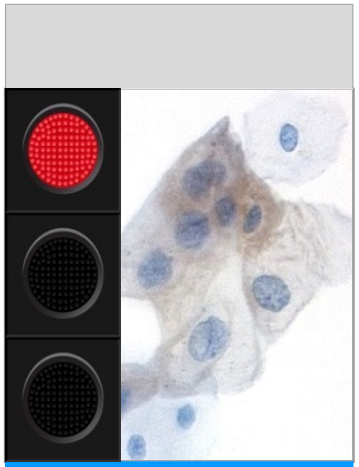
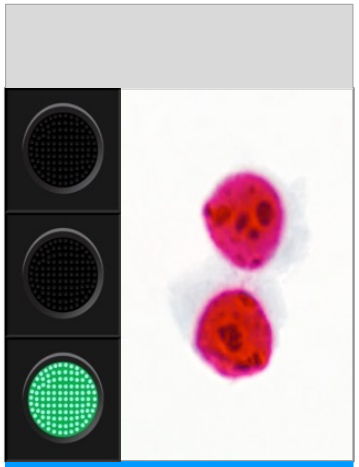
p16, Ki-67 and Co-expression



Negative

Expression of p16 (brown) signals halting of cell division



Negative

Expression of Ki-67 (red) signals progression of cell division

Disease

Co-expression of p16 & Ki-67 (brown & red) indicates abnormality

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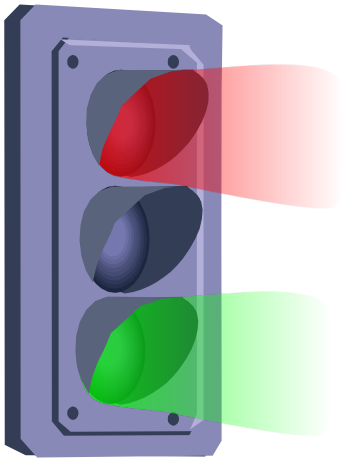
p16, Ki-67 and Co-expression

Expression of p16 and Ki-67 is **mutually exclusive of each other** in cells with intact cell cycle control.

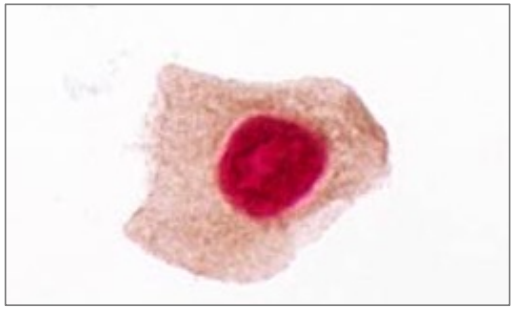
p16/Ki-67 dual-staining in the same cell indicates **cell cycle deregulation.**

Identification of double-immunoreactive cells in cervical cytology preparations can be an **indicator** for the presence of **high-grade cervical dysplastic lesions.**



p16

Ki-67



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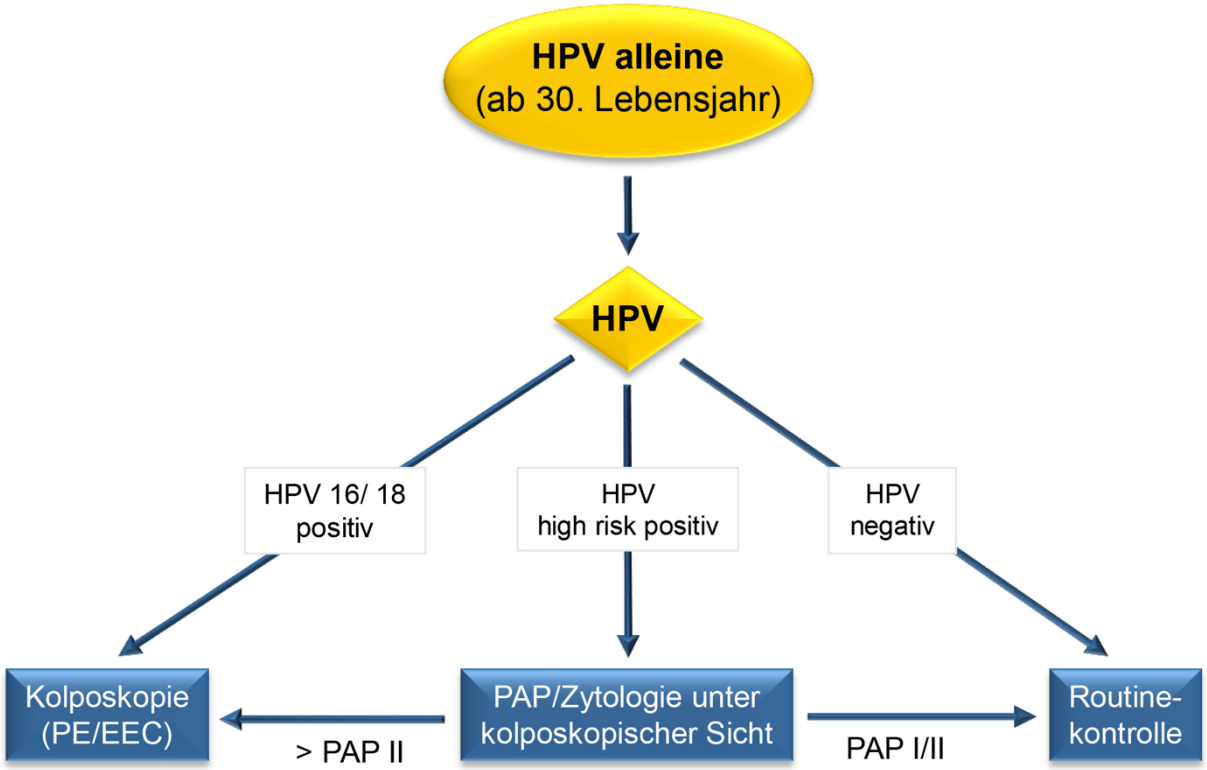
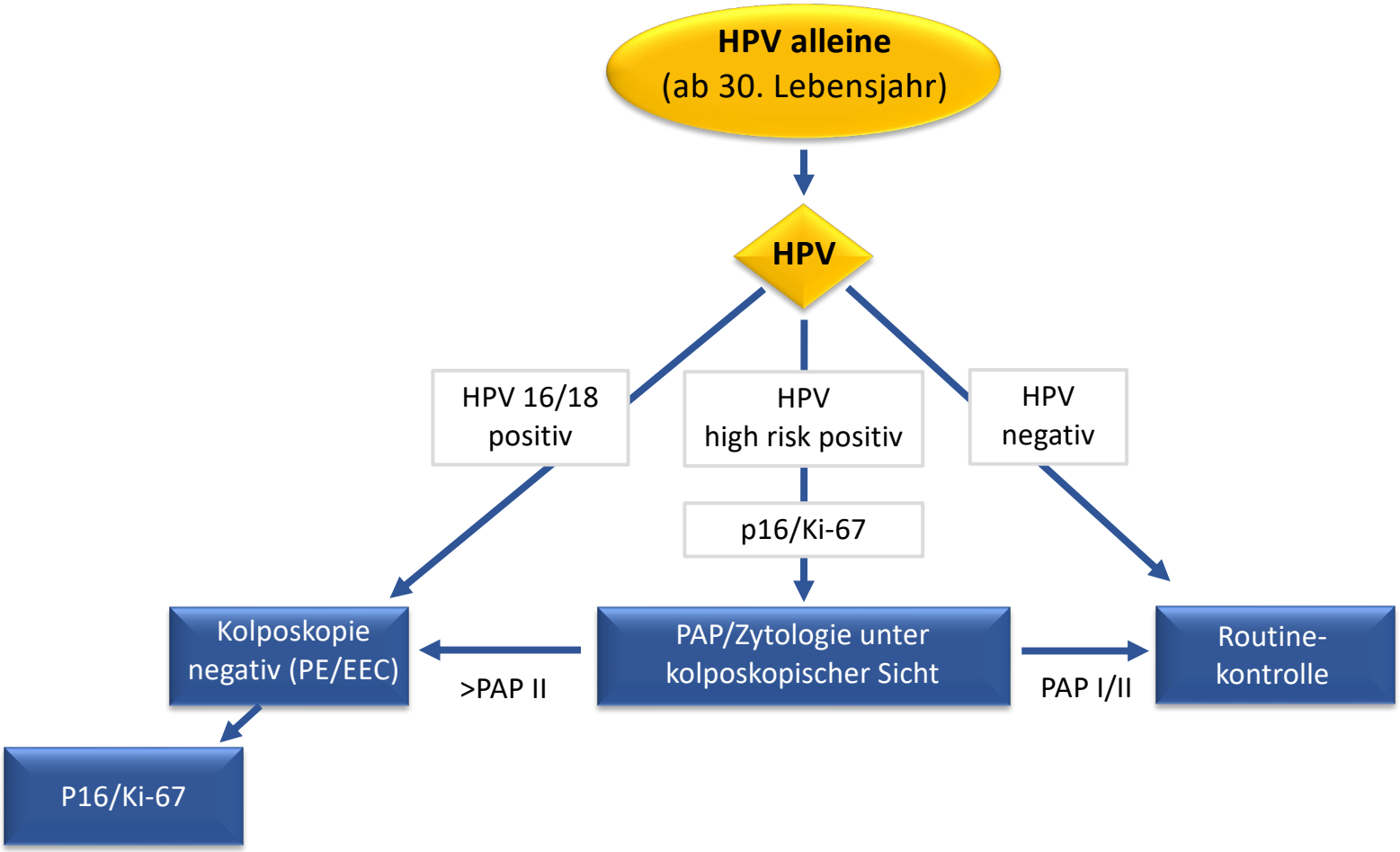


Die kolposkopische und zytologische Kontrolle soll in einem Zeitraum von bis zu 6 Monaten durchgeführt werden (S3 Leitlinie Prävention Zervixkarzinom 2017).



Die kolposkopische und zytologische Kontrolle soll in einem Zeitraum von bis zu 6 Monaten durchgeführt werden (S3 Leitlinie Prävention Zervixkarzinom 2017).

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Triage

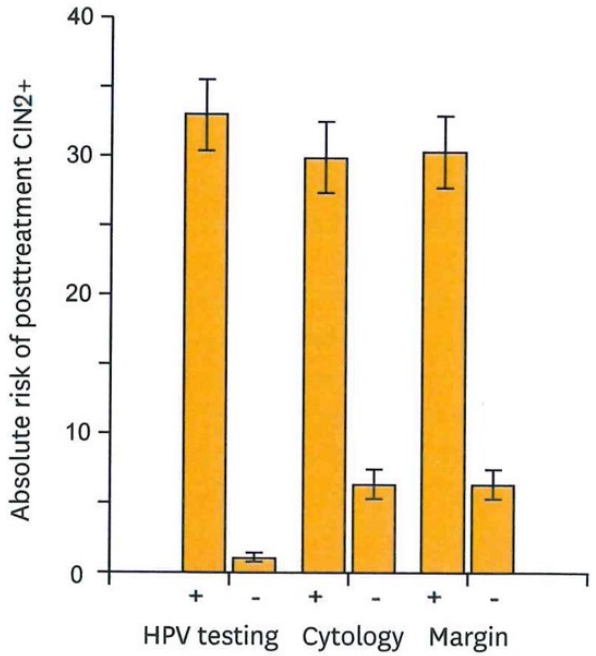


Fig. 2. Risk stratification of posttreatment cervical intraepithelial neoplasia grade 2+ (CIN 2+) provided by carcinogenic human papillomavirus (HPV) testing, cytology, and surgical margin histology. In each test method of high-risk HPV testing, cytology (atypical squamous cells of undetermined significance+) or surgical margin histology, the absolute risks of having recurrent or residual CIN 2+ lesions (■) and 95% confidence intervals (error bars) were calculated for women testing positive or negative for each test. HPV testing provided the greatest risk stratification between test-positive and -negative women.

Mamiko Onuki et al., Posttreatment human papillomavirus testing for residual or recurrent high-grade cervical intraepithelial neoplasia: a pooled analysis. J Gynecol Oncol. 2016 Jan;27 (1):5

TABLE 2 Type-specific HPV recurrence/progression rates in the surveillance group

	HPV at initial conization		Recurrent CIN2+		Progression to invasive cancer	
	n	(%)	n	(%)	n	(%)
Total	493	(100)	84	(17.0)	6	(1.2)
HPV–	28	(5.7)	0	(0)[c,*]	0	(0)[c,**]
HPV+	465	(94.3)	84	(18.1)[c,*]	6	(1.3)[c,**]
Single	368	(74.6)	62	(16.8)	5	(1.4)
Multiple	97	(19.7)	22	(22.7)	1	(1.0)
HPV16[a]	156	(31.6)	36	(23.1)	4	(2.6)
HPV18[a]	27	(5.5)	4	(14.8)	1	(3.7)
HPV31[a]	28	(5.7)	9	(32.1)	0	(0)
HPV33[a]	32	(6.5)	9	(28.1)	0	(0)
HPV35[a]	6	(1.2)	1	(16.7)	0	(0)
HPV39[a]	13	(2.6)	1	(7.7)	0	(0)
HPV45[a]	5	(1.0)	0	(0)	0	(0)
HPV51[a]	25	(5.1)	3	(12.0)	0	(0)
HPV52[a]	127	(25.8)	19	(15.0)	1	(0.8)
HPV56[a]	12	(2.4)	4	(33.3)	0	(0)
HPV58[a]	77	(15.6)	12	(15.6)	1	(1.3)
HPV59[a]	3	(0.6)	0	(0)	0	(0)
HPV68[a]	6	(1.2)	2	(33.3)	0	(0)
HPV82[a]	7	(1.4)	0	(0)	0	(0)
Lr-HPVs[b]	55	(11.2)	12	(21.8)	0	(0)

Abbreviation: HPV, human papillomavirus.

[a]Same woman can be counted more than once because of multiple infections.

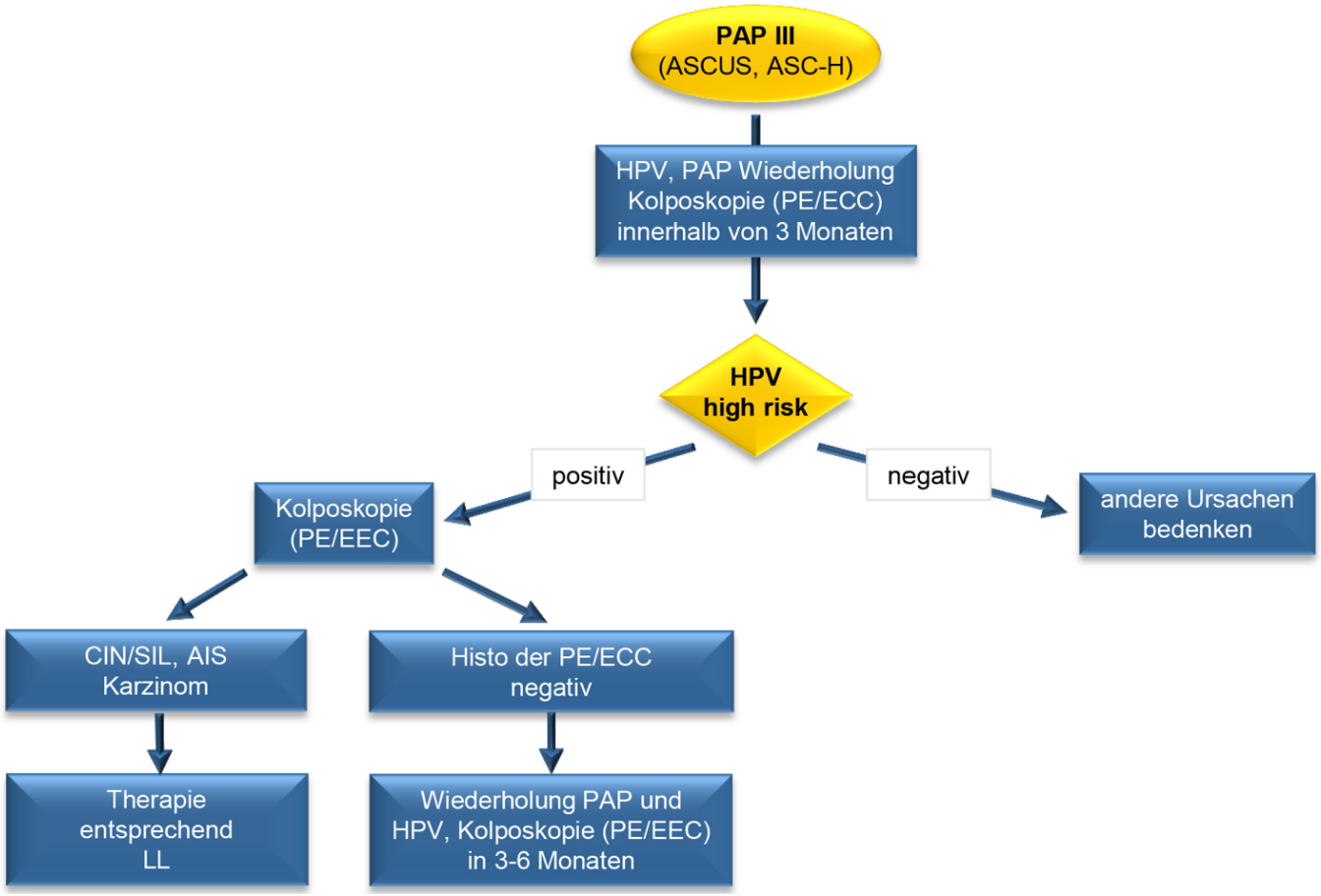
[b]Probable hr-HPVs (HPV 26, 53, and 66) were grouped with low-risk types as lr-HPVs (6, 11, 26, 32, 37, 42, 43, 44, 53, 54, 55, 61, 62, 66, 67, 69, 70, 71, 72, 74, 81, 83, 84, and L1AE5) based on low prevalence of HPV 26, 53, and 66 in HG-CIN and cervical cancer in Taiwanese data.[4,8,10,13]

[c]*P* values for comparisons annotated by * and ** are .008 and >.999 respectively, by Fisher's exact test.

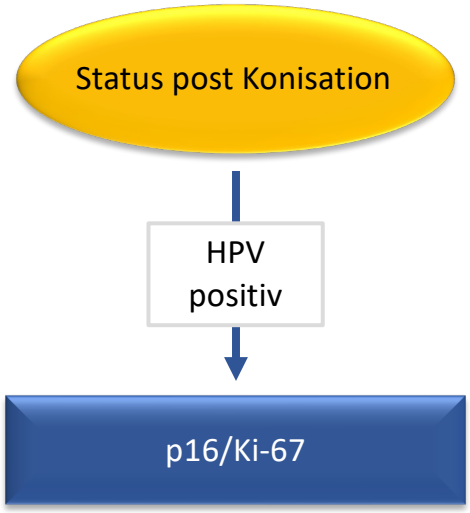
Huei-Jean Huang et al., Role of human papillomavirus status after conization for high-grade cervical intraepithelial neoplasia. Int. J Cancer. 2021;148:668

The pooled posttreatment risk of cervical intraepithelial neoplasia (CIN) 2+ in all studies was 4.8% (95% CI = 3.4%- 6.8%), ranging from 0.4%-19.5% (τ^2 = 0.57) in individual studies. Among individuals testing negative for human papillomavirus (HPV) posttreatment, the risk of CIN 2+ was 0.69% (95% CI= 0.3%-1.5%); among individuals testing positive for HPV posttreatment, the risk of CIN 2+ was 18.3% (95% Cl= 12.1 %-26.6%) in all studies. All risk estimates were substantially higher for liquid-based cytology.

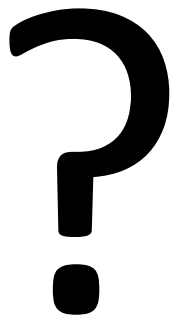
Megan A. Clarke et al., A Systematic Review of Tests for Postcolposcopy and Posttreatment Surveillance. Journal of Lower Genital Tract Disease. 2020;Vol. 24, Nr. 2:148


PAP III
(ASCUS, ASC-H)
HPV, PAP Wiederholung
Kolposkopie (PE/ECC)
innerhalb von 3 Monaten
HPV
high risk
positiv
negativ
Kolposkopie
(PE/EEC)
andere Ursachen
bedenken
CIN/SIL, AIS
Karzinom
Histo der PE/ECC
negativ
Therapie
entsprechend
LL
Wiederholung PAP und
HPV, Kolposkopie (PE/EEC)
in 3-6 Monaten

medilab


Status post Konisation
HPV
positiv
p16/Ki-67

Screening einer geimpften Population



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PAP vs Zytologie

- In der Früherkennung ist das HPV – Screening einem Zytologischen Screening vorzuziehen
- Ein Duales Screening (HPV+PAP) bringt keine Vorteile
- In der Triage spielen neben HPV und Zytologie auch Biomarker wie p16 Ki 67 eine Rolle
- In der Vorsorge ist die Impfung das wichtigste Instrument

Vielen Dank für Ihre Aufmerksamkeit

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