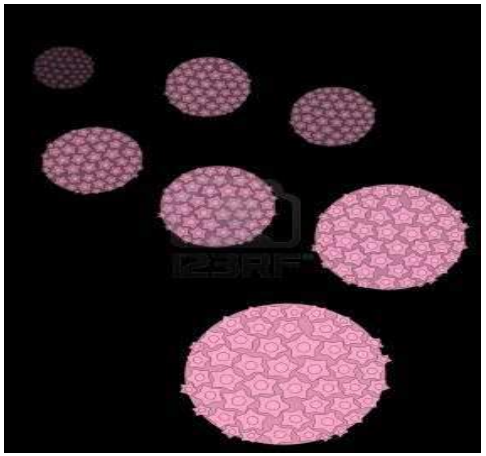
INVITRO LABS – 1ST Annual Scientific Meeting

1η επιστημονική συνάντηση InvitroLabs, 2013

Invited speaker: Dr Ekatherina Charvalos-

Director Central Labs-IASO HOSPITAL



TITLE: HUMAN PAPILLOMA VIRUS (HPV)-Biology, molecular diagnosis and ACOG Cervical Screening Guidelines 2013.

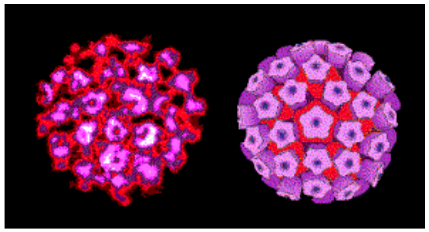
Where? InVitroLabs premises, Leventi 11, Athens, Greece

Presentation outline- HPV

- Biology of the virus: infection, replication, oncogenesis, and some clinical aspects
- Prevention and HPV Molecular diagnosis (FDA approved tests)
- ACOG* screening guidelines (November, 2012)

*American College of Obstetricians and Gynecologists,

Sheila Graham Future Microbiol. 2010;5: 1493-1506, ; Alba et al., The Open Dermatology Journal, 2009, 3, 90-102, (2009)
Naoko Kajitani et al., Front. Microbiol., 2012 | doi: 10.3389/fmicb.2012.



- PART I

Global burden of HPV - Cervical Intraepithelial Neoplasia-Epidemiology

- The first in numbers viral infection worldwide
- Closely associated to Cervical Cancer (almost 100%)

- Cervical cancer =the second most common cancer in women,
- **Worldwide incidence, 530 000 new cases/year.**

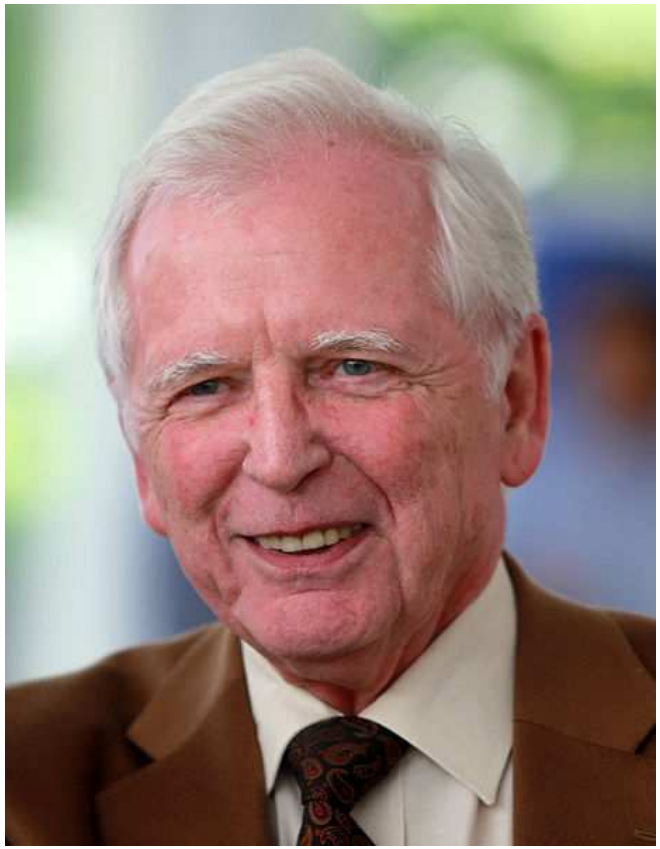
- More than 270 000 women die from cervical cancer/year
- More than 85% of these deaths are in low- and middle-income countries (lack of screening)

HPV is not only …female

CDC: US, in men: 2700 ano-genital and 5700 oropharyngal cancers HPV associated (2013).

- (1)nCervical Cancer Incidence, Mortality and prevalence worldwide in 2008: Summary. http://globocan.iarc.fr/factsheet.asp (Accessed on January 30, 2013)
- (2) WHO/ICO Information Center of HPV and Cervical Cancer (HPV Information Center). Human Papillomavirus and Related Cancers in the World. Summary Report 2010. http://www.who.int/hpvcentre/en/ (Accessed on September 19, 2011)..

Prof Harald Zur Hausen



- *Born in Germany (1936)*
- *Discovered the HPV as the causative agent of cervical cancer* *(Nature 2002)*
- *NOBEL PRIZE in physiology or Medicine (2008)*
- *Januray 2014: Honorary member of Athens University 2014 9to be on the side of Greece).*

…."for his discovery of human papilloma viruses causing cervical cancer"

Risk factors of cervical intraepithelial neoplasia (CIN) and…the cause?

- Risk factors:
  - Smoking
  - Early starting of sexual life
  - Number of sexual partners
  - Immunological disorders and co-infection etc, etc, etc

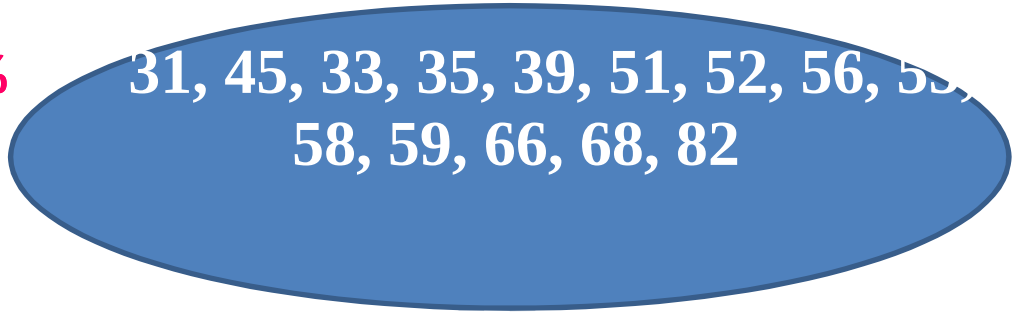
The cause is only one: high risk (hr)HPV

Harald zur Hausen, Nature May 2002, Vol 2, 342-350

Are All HPV oncogenic? NO

Among more than 120 HPVs

- **14 high risk types,** α HPV –epitheliotropic:
- **Types 16, 18 = 70%**



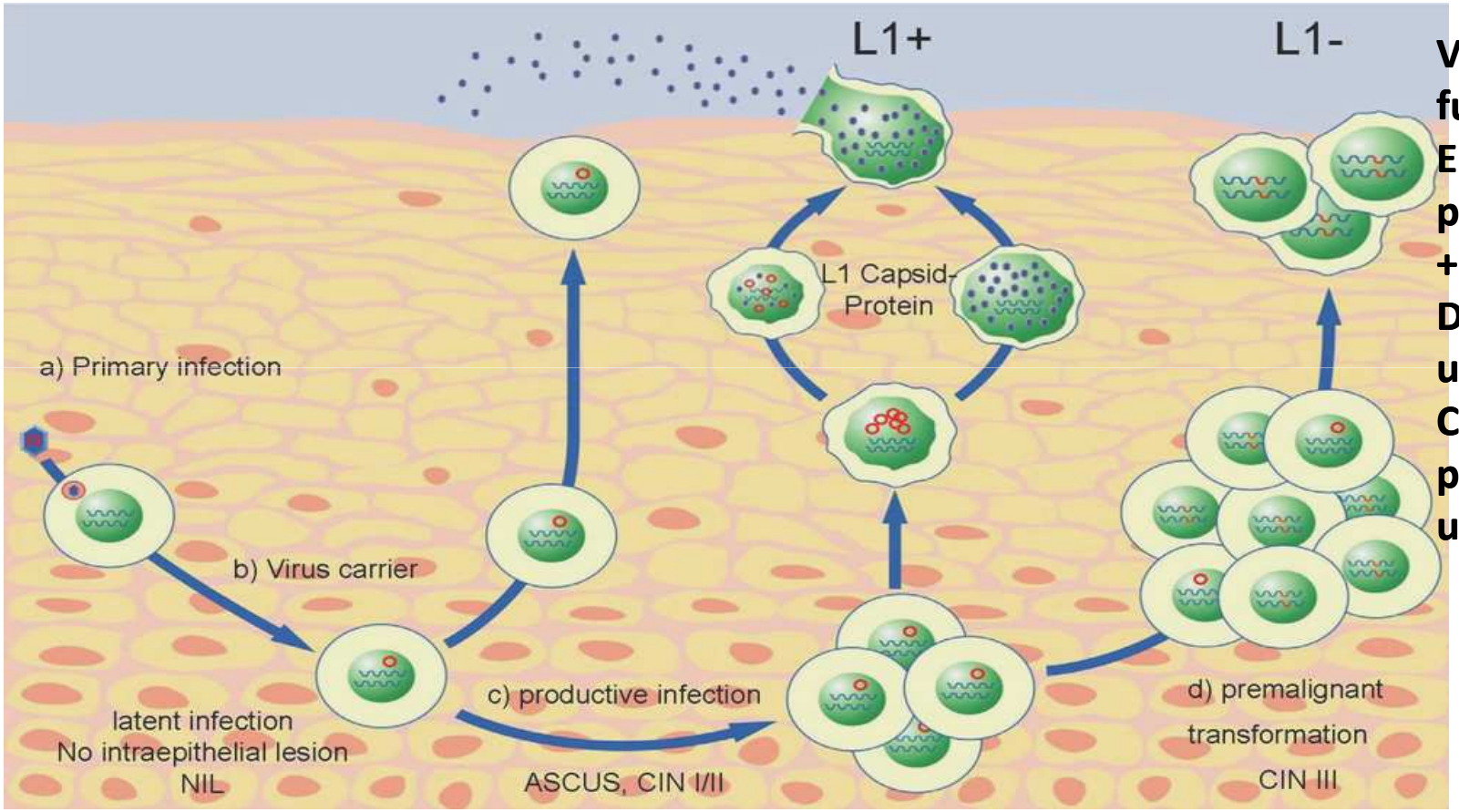
- **Low risk: b-HPVs**, cause **skin, oropharyngal, genital, respiratory tract disease (Warts, Recurrent Respiratory Papillomatosis [RRP], acuminata condylomata): types 6 and 11**

- **HPV 16 life cycle**

- the most frequent, high risk type, the prototype

HPV life cycle

Clearance **Virion assembly/ release** **transformation/ integration**



Viral function:
E regulatory proteins +++ all DNA=mostly upper layers
Capsid proteins= upper layers

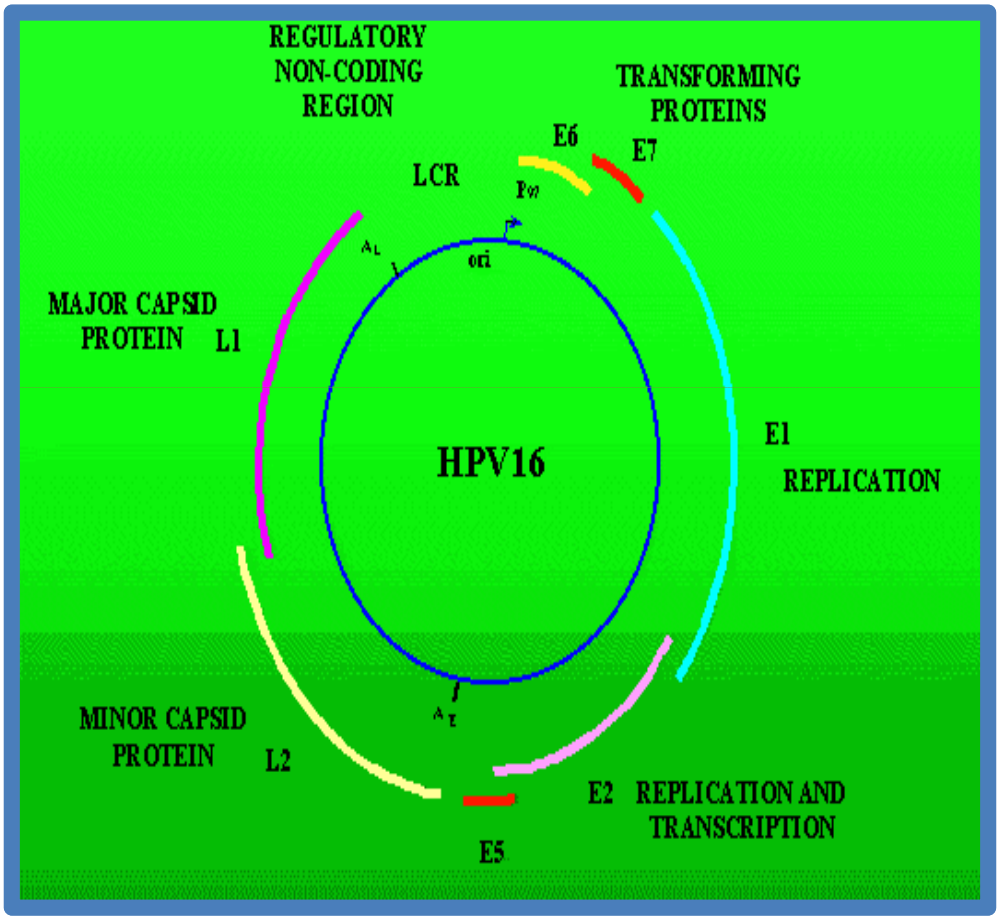
- Adapted: Ozbun, M. A., S. K. Campos, and J. L. Smith. The Early Events of Human Papillomavirus Infections: Implications for Regulation of Cell Tropism and Host Range, In New Strategies for Human Papillomavirus Gene Regulation and Transformation, B. Norrild (Ed.), Research Signpost, Kerala, India, pp 69-122, 2007.

HPV **16 type** GENOME ORGANIZATION

8000 bp, naked dsDNA

Three main regions **(early regulatory, late region** and the LCR) ENCODING:

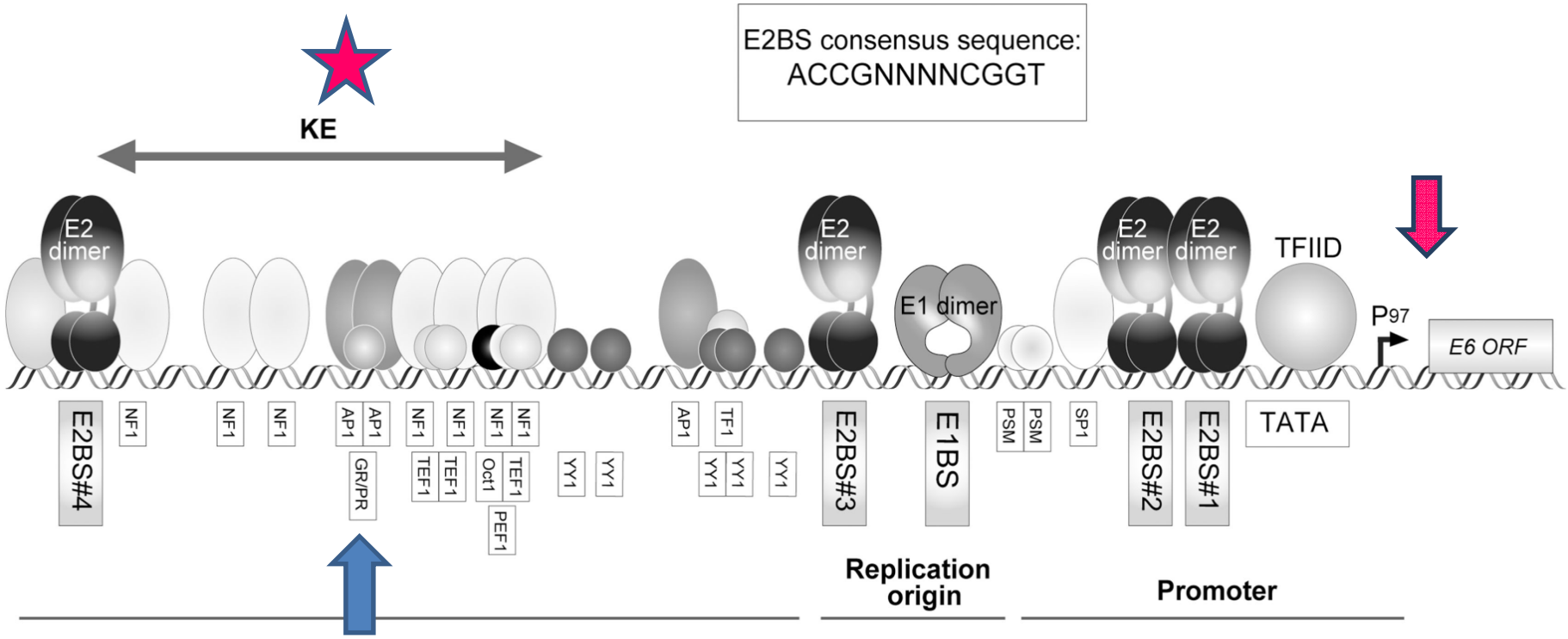
- **(E1-E7)** resides the transformation and immortalization potential.
- **(L)** Two capsid genes, L1,L2.
- **(LCR)** contains all the cis-regulatory elements.
- AE, AL polyadenylation sites
- P97, P670 early, late promotors



What is LCR? : Epitheliotropia and the cross-talking region

- Long Coding Region= is the bridge between the virus and the cell.
- All PVs show high specificity of epithelium infection.
- HPVs epitheliotropia is due to the interaction of numerous transcription cell factors with the LCR.
- In high risk HPVs this region is remarkably different from the other HPVs…

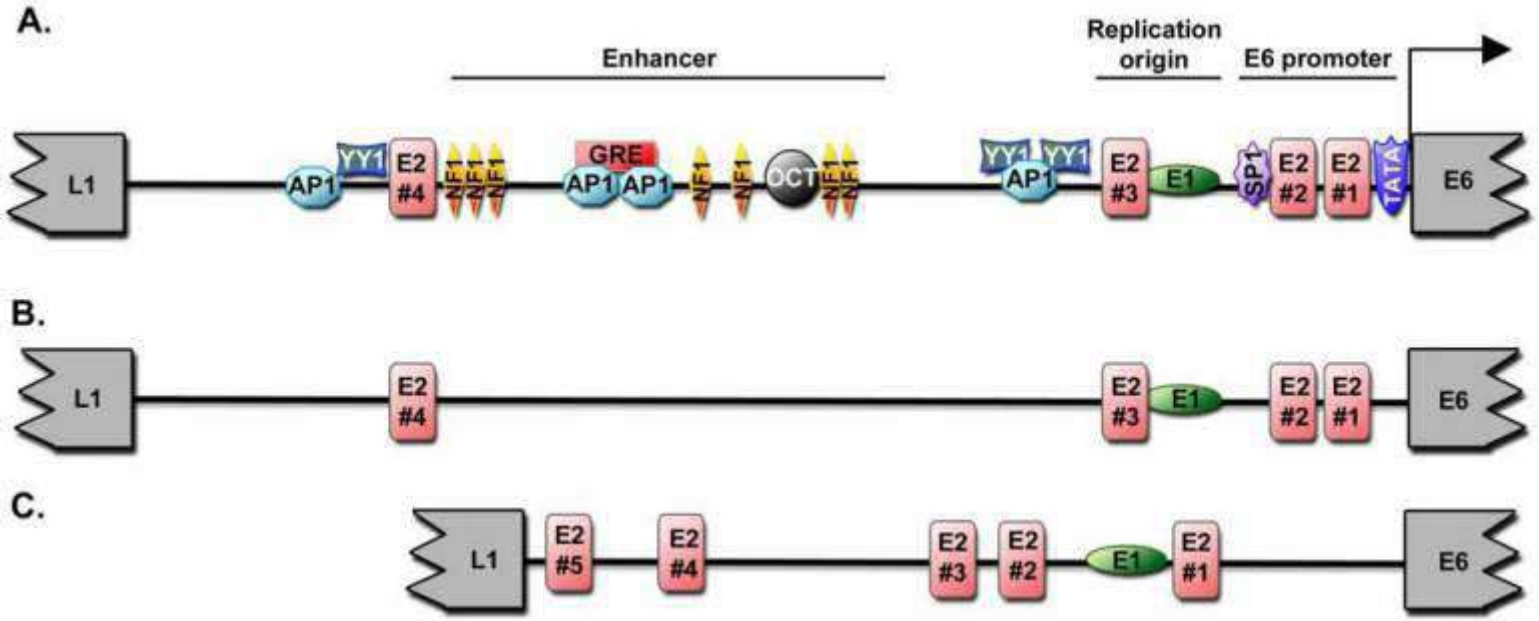
HPV 16 hR -LCR: 15% HPV genome, differs from the LCR HPV genital prototype, or HPV cutaneous prototype.



FIGURE

The structure of HPV16 LCR (region of the control of early promoter P97). The early promoter P97 and replication origin are located in LCR, which are regulated by various cellular factors. Activity of P97 is regulated by AP-1,NF1,SP1,TFIID,TF1,Oct-1,PSM,and the viral transcription factor E2. Four E2-binding sites (E2BS) have been identified in HPV16 LCR and the consensus sequence for E2BS is shown in an inset. A glucocorticoid receptor and progesterone receptor (GR/PR) recognition in LCR. The existence of a keratinocyte-specific enhancer (KE) has been proposed (Desaintes and Demeret, 1996).

HPV 16 LCR


A.
Enhancer
Replication origin
E6 promoter
L1
AP1
YY1
E2 #4
NF1
NF1
NF1
GRE
AP1
AP1
NF1
NF1
OCT
NF1
YY1
YY1
AP1
E2 #3
E1
SP1
E2 #2
E2 #1
TATA
E6
B.
L1
E2 #4
E2 #3
E1
E2 #2
E2 #1
E6
C.
L1
E2 #5
E2 #4
E2 #3
E2 #2
E1
E2 #1
E6

HOW HPV CAUSES CANCER ?

- The procedure is long …..as long as 15 years

- Complexity – cross talking HPV- differentiating Epithelium

- Key events: (a) E2 protein, regulating both the E1 protein for replication and the E6/E7 expression

  and (b) E6/E7 anti apopoptic and transforming activity

(Sheila Graham Future Microbiol. 2010;5: 1493-1506); Ozbun, M. A., S. K. Campos, and J. L. Smith. The Early Events of Human Papillomavirus Infections: Implications for Regulation of Cell Tropism and Host Range, In New Strategies for Human Papillomavirus Gene Regulation and Transformation, B. Norrild (Ed.), Research Signpost, Kerala, India, pp 69-122, 2007.

E2 regulatory protein-key actor in HPV lifecycle

350-500 aminoacids

Functions in viral cell life:

Transcription of viral genes

Replication of viral genome

Maintenance of viral genome

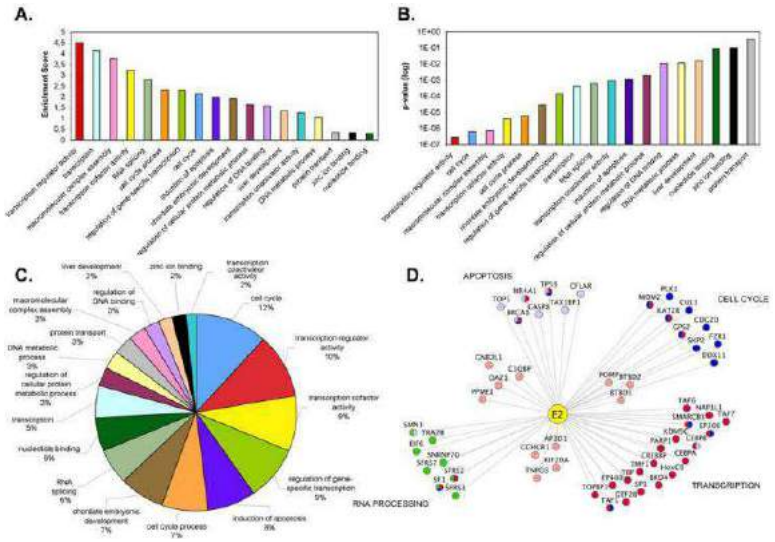
Activities:

Transactivation//transrepression,

DNA-binding activity

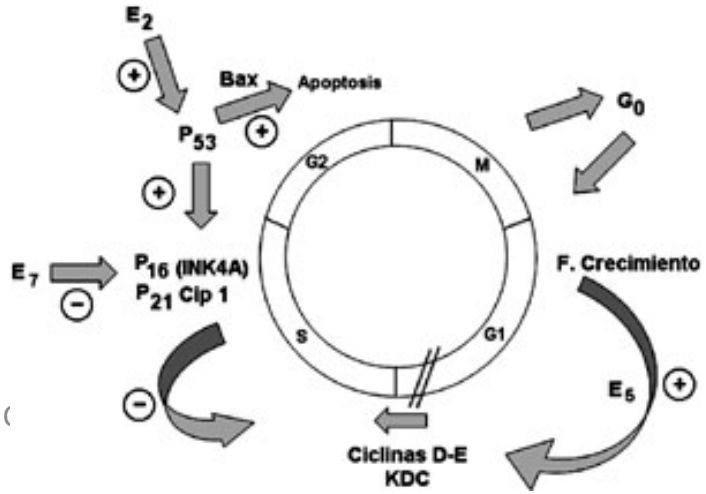
Ability to interact with several family proteins

Apoptotic



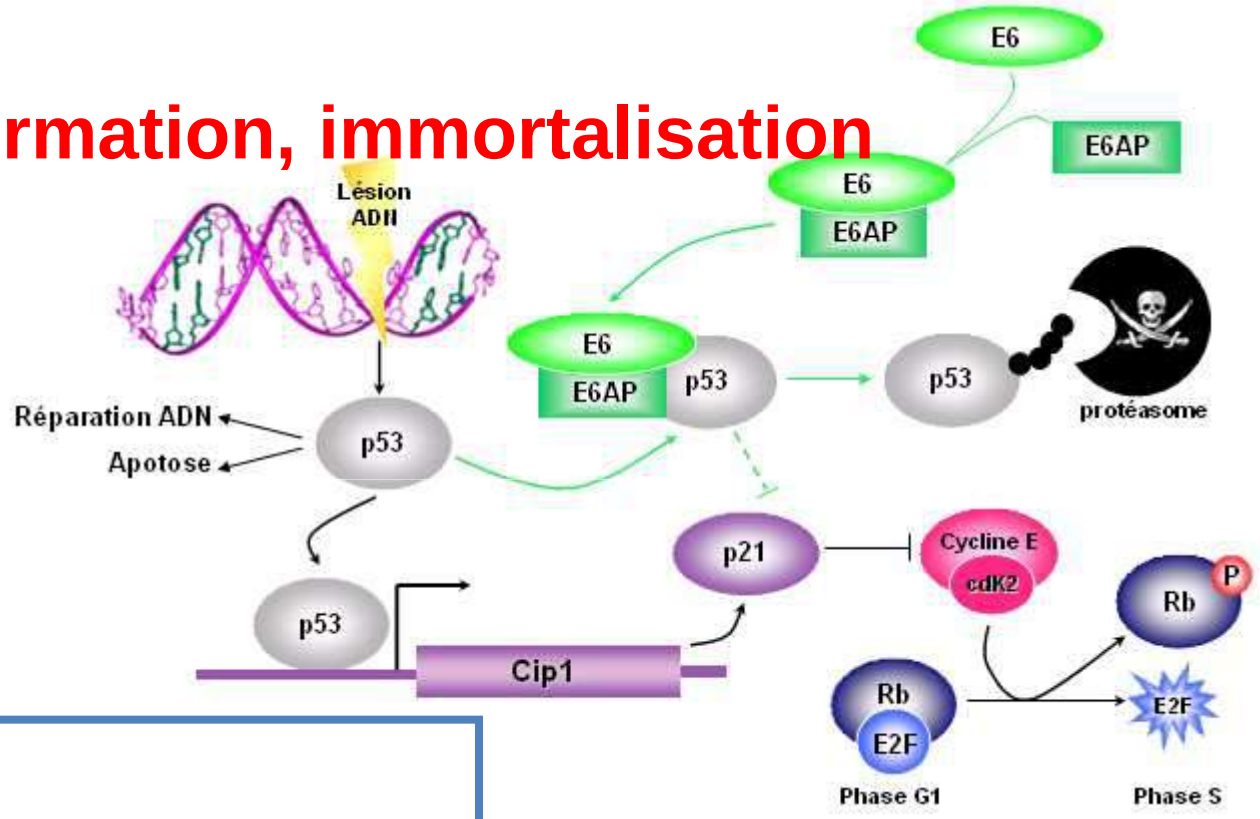
- **Regulates E1 expression for replication (low layers, maintenance/upper layers, proliferation)**
- **Responsible of fine tuning expression of E6/E7 oncoproteins.**
- **DNA segregation and tethering in host cell (transforming)**

E2 INTERACTOM Open Virol J. 2012; 6: 173–189.



Once E6/E7 overexpressed, they will target p53* and pRb* to induce cell proliferation and to inhibit apoptosis,

➡ transformation, immortalisation



E6 ⊣ **p53** (degradation)

E7 ⊣ **Rb** (binding)

retinoblastoma

Apoptosis Checkpoint G1/S

↓ ↑ P53, RB=Apopotitic antioncogenic proteins

Persistent infection: when both E6, E7 continue expression

- Normal viral life cycle is lost
- Differentiated epithelial cells continue replicating and cumulate mutations

- **Non reversible high E6/E7 mRNA expression** → **Cancer**

Virus expression and complexity of cancer genesis

- Multiple transcription factors communicating with LCR
- Alternative splicing exon sites (exons and introns) and polyadenylation sites.
- Cell differentiation-dependent virus productive life cycle
- Lack of strong topical immune response

Summary of key events in HPV INFECTION

- Infection of undifferentiated basal cells
- Expression of **E2/E1** proteins
- E6/E7 expression at low levels
- low level replication
- Infection of differentiated cells
- Low Layers: Maintenance-proliferation- Low copy numbers (up to 50 copies/cell)
- Upper layers: Maintenance and amplification, more than 1000 copies/cell
- E6/E7 high expression in differentiated cells-persistence
- **E2** segregation and tethering in host cell
- L1, L2 expression (upper layers), escaping the immune response
- Host cell cycle transformation- cumulative mutations
- Host cell immortalisation …NEOPLASIA
- Neoangiogenesis *(Hin1)* and metastasis (body of knowledge remains unkown)

- PART II

Screening =main way to prevent Cervical cancer

CIN- Screening tools

- Cytology (test Pap, Thin Prep)
- Colposcopy
- HPV DNA analysis (in 2000)

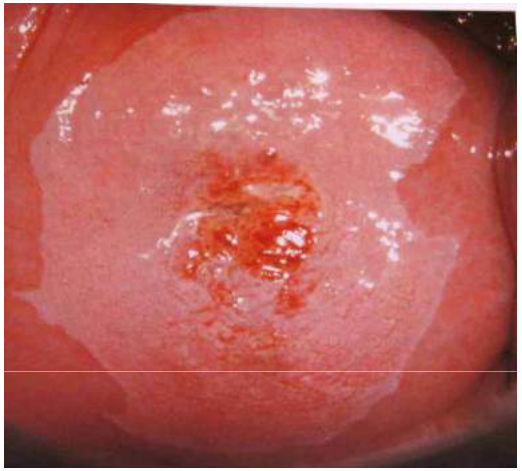
How to translate in clinical practice/Diagnosis?

HPV pathology/colposcopy classification (Bethesda system)

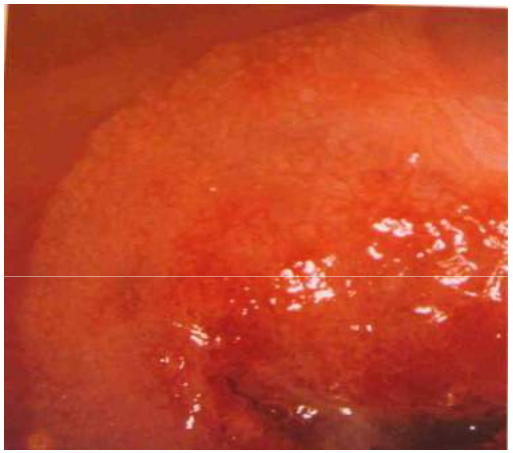
Cytology/ colposcopy (Cervical/ Vaginal/ Vulvar/ Anal/ Penile)

Dysplasia	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia Carcinoma in-situ
Intraepithelial Neoplasia/cyto	CIN I VIN 1 VAIN 1	CIN 2 VIN 2 VAIN 2	CIN 3 VIN 3 VAIN 3
Squamous Intraepithelial Lesion (SIL)/colpo	Low Grade SIL (LSIL)	High Grade SIL (HSIL)	High Grade SIL (HSIL)

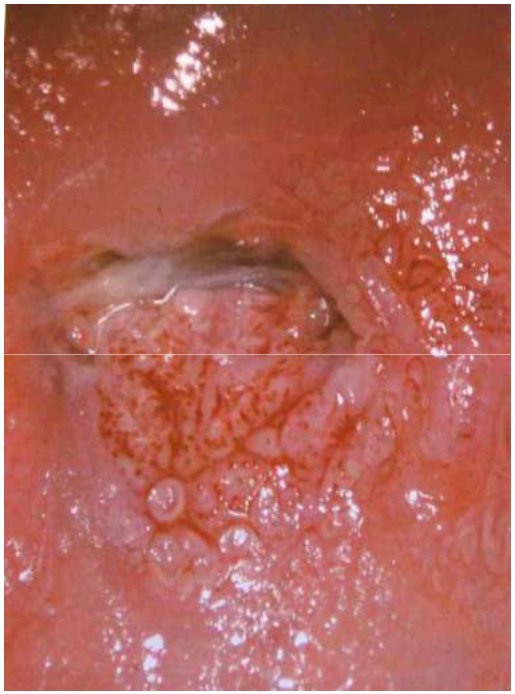
How does it show in colposcopy? HPV lesions: Acetowhite lesions



Acetowhite lesions



punctations



mosaicism+punctation

Once acetic acid is dropped on the cervix

Prevention: based on Pap smear, colposcopy, HPV DNA test ↑ sensitivity

Dr Papanicolaou test Pap

Screening Test Performance
British HART Study

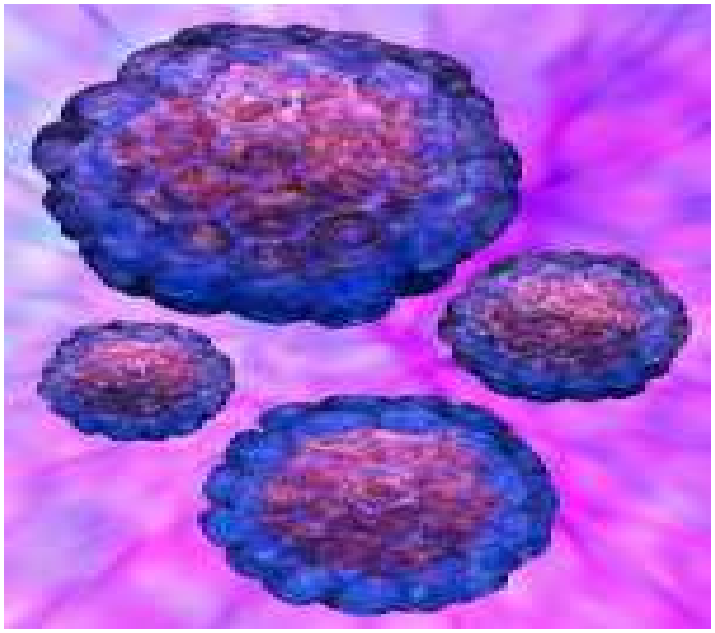
Author	For Detection of CIN 2+ Sensitivity	Specificity
Pap Test *	76.6%	95.8%
HPV DNA Test	97.1%	93.3%

Cuzick et al. (2003) Lancet

* *at a cut-off of ASC-US*

ANTICANCER RESEARCH *29: 3401-3410 (2009)*
Comparison of Cytology, Colposcopy, HPV Typing and Biomarker Analysis in Cervical Neoplasia

Authors: Adamopoulou M, Kalkani E, Charvalos E, Avgoustidis D, Haidopoulos D and Yapidjakis C



Molecular diagnosis/the tests-SCREENING

Approved as as method of diagnosis in 2000
FDA 2010 approved for screening:

4 DNA TESTS

1 RNA TEST

FDA guidelines for molecular screening HPV approval (2010)

- To detect at least 13 high risk HPV
- With clinical specificity at least 85% - suitable for PPV (positive predictive value) for CIN 3+
- With clinical sensitivity more than 92% +/- 3% for CIN 3+
- Data available for evaluation (peer review)
- Interlaboratory repeatability (kappa > 0.7)

http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&cntvwrPtlt%7BactionForm.contentReference%7D=committees%2Ftechnology%2FHPV.html&_pageLabel=cntvwr

HPV MOLECULAR TEST FDA APPROVED

- ## Digene Hybrid Capture
  ### (13 pool) +2 High-Risk HPV DNA Test

- High-Risk HPV DNA Test
  Manufacturer: Digene Corporation
  Address: 1201 Clopper Road, Gaithersburg, MD 20878
  Approval Date: March 31, 2003
  Approval Letter:
  http://www.accessdata.fda.gov/cdrh_docs/pdf/p890064s009a.pdf

- ## Cervista™ HPV 16/18

- **Product Name:** Cervista™ HPV 16/18
  PMA Applicant: Third Wave Technologies
  Address: Third Wave Technologies, Hologic, Inc., 502 South Rosa Road, Madison , WI 53719
  Approval Date: March 12, 2009
  Approval Letter:
  http://www.accessdata.fda.gov/cdrh_docs/pdf8/P080015a.pdf

- ## Cervista™ HPV HR

- ### 3rd wave-technologies -Hologic

- **roduct Name:** Cervista™ HPV HR and Genfind™ DNA Extraction Kit
  PMA Applicant: Third Wave Technologies
  Address: Third Wave Technologies, Hologic, Inc., 502 South Rosa Road, Madison , WI 53719
  Approval Date: March 12, 2009

- *http://www.accessdata.fda.gov/cdrh_docs/pdf/p890064s009a.pdf*

- ## Cobas HPV ROCHE - P080015 Test - P100020

Approval Date: April 19, 2011
Approval Letter:
http://www.accessdata.fda.gov/cdrh_docs/pdf10/p100020a.pdf

APTIMA® HPV Assay - P100042

Approval Date: October 28, 2011

- **Approval Letter:** *http://www.accessdata.fda.gov/cdrh_docs/ pdf10/p100042a.pdf*

TIGRIS DTS System DETECTING **E6/E7 mRNA**

- PART III

- ACOG screening guidelines: established to show how to use the approved methods

ACOG CERVICAL SCREENING GUIDELINES -PREVENTION

ACOG Cervical screening guidelines: Key changes, November 2012, (Medscape, May 2013)

Kudos to the Pap test :1928

Dr Papanicolaou first cytology observations on stained cervical smears associated with CIN!

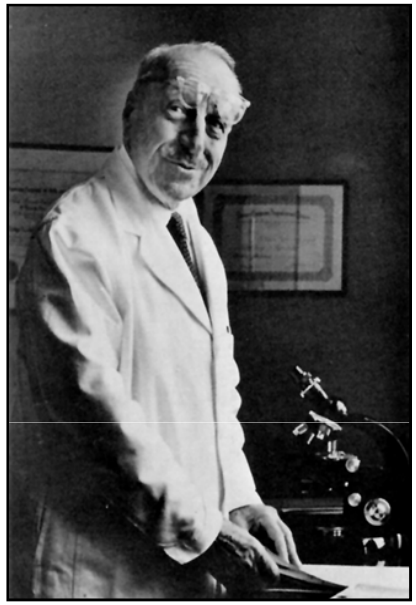
Sandra Adamson Fryhofer, MD, Medscape, 24/05/2013: announced the guidelines in only 4,7 min!

ACOG CIN Screening Guidelines: Cervical Cancer Prevention

Established Study groups

To analyze:

History of prevention methods vs epidemiological data



1940	1996	2000	2006	2014
Pap test	LBC	HPV/DNA	VACCIN	New Drugs?

Approved Liquid Based Cytology

ACOG Cervical screening guidelines: I Key changes, May 2013, Sandra Adamson Fryhofer, MD, Medscape, 22/05/2013

- 1941 the paper:

  Papanicolaou GN, Traut HF. "The diagnostic value of vaginal smears in carcinoma of the uterus". *American Journal of Obstetrics and Gynecology*. 1941;42:193.

- **Because of the pap test as screening method, Cervical cancer mortality decreased 50% for the last 30 years**

ACOG Cervical screening guidelines: II Key changes, May 2013, key discovery dates Sandra Adamson Fryhofer, MD, Medscape, 24/05/2013

However….

ACS* announced 12000 new cases and 4000 deaths in 2013….

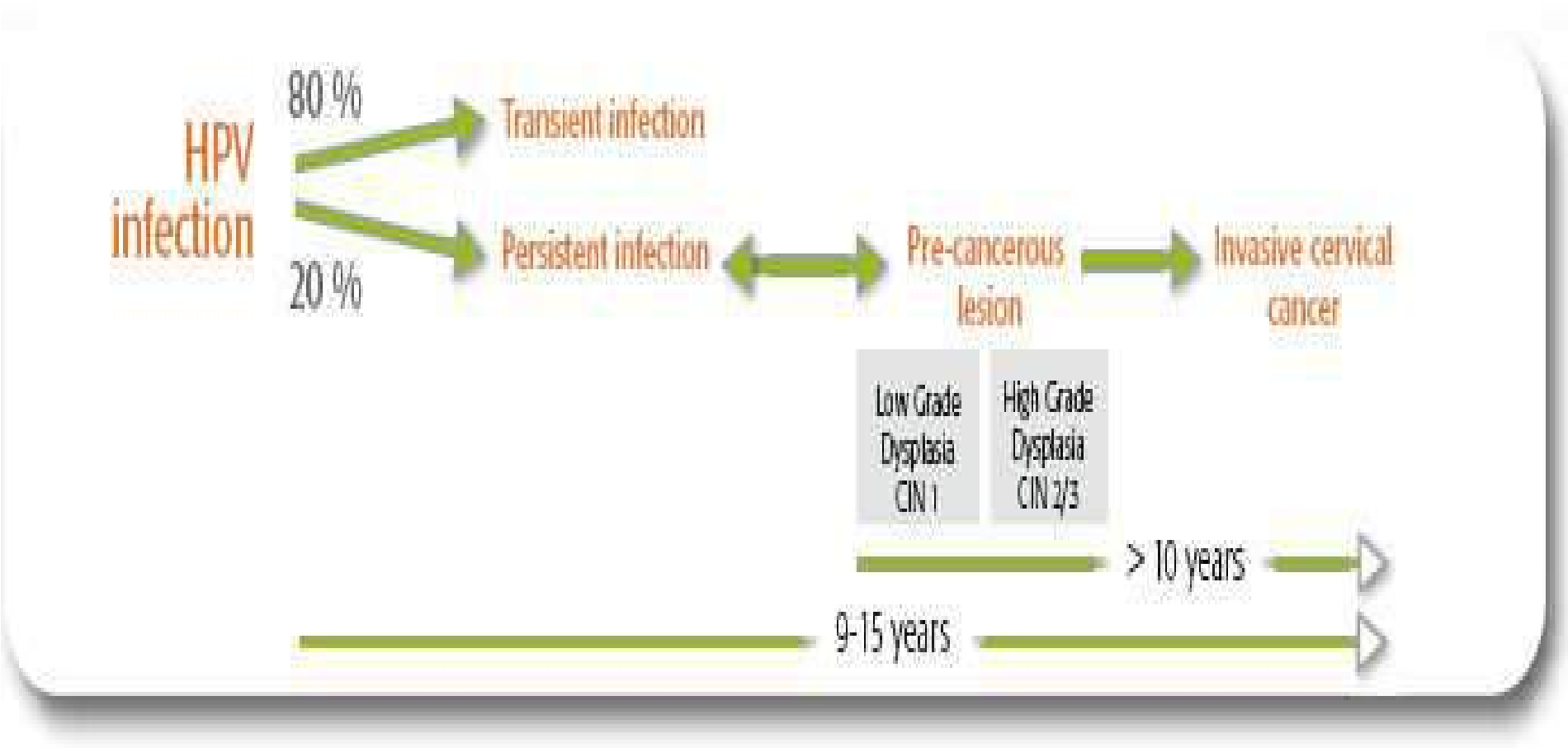
***American Cancer Society**

They recommend….:

SCREENING, SCREENING, SCREENING

ACOG Cervical screening guidelines: III Key

changes, May, 2012 are based on:



- *The American Congress of Obstetricians and Gynecologists. ACOG Practice Bulletin. Clinical Management Guidelines for Obstetrician-Gynecologists: Screening for Cervical Cancer. November, 2012.*

ACOG Cervical screening GUIDELINES
IV: Key changes

When to start SCREENING?

At 21 years

Independently of any epidemiological factor, sexual life style

ACOG Cervical screening GUIDELINES: Key changes V

- **Women age group 21-29 years, the Pap test every three years, no HPV DNA testing**
- **Age group 30-65 "co-testing" Pap +HPV DNA tests every:**
  - **5 years!**
  - **-PAP only, every 3 y**
  - **-NO HPV DNA test without PAP**
- **Women with CIN II or adenocarcinoma do not stop screening at 65 years old**

ACOG Cervical screening GUIDELINES: Key changes VI

- What after hysterectomy? Without CIN II history or cervical surgery –ok

- With high grade lesions before hysterectomy, continue screening with PAP test

  every 3 years for 20 years*.

*(The role of HPV test not yet elucidated)

ACOG Cervical screening GUIDELINES VII : Key changes, 2013

- Routine guidelines do not refer to:

✓Immunocopromised women

✓HIV +

✓Diethylostilbestrol in utero exposure

✓With CIN

ACOG Cervical screening GUIDELINES VIII: Key changes

Cervical cancer screening consists of an excellent paradigm on how a screening test saves lives

Dr Ekatherina Charvalos Director Central Labs-IASO

High progress research on viral omics including INTERACTOMICS, but, where are we now?

- *This is not the end*
- *This is not the beginning of end*
- *…but this is the end of the beginning!*

Sir Winston Churchill

North Egypt Dec 1942

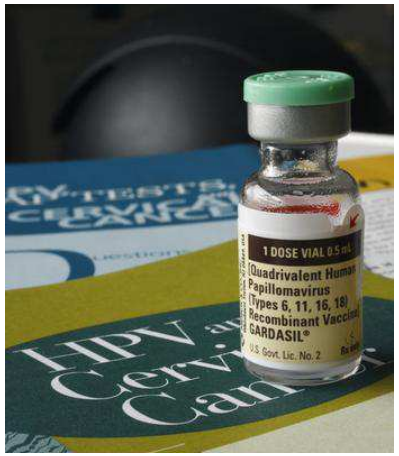
J of Proteome Res. 2013 May 3;12(5):2078-89. doi: 10.1021/pr301067r. Epub 2013 Apr 22.

Dr Ekatherina Charvalos Director Central Labs-IASO

Research focuses on HPV topical therapy (1)

- Developing drugs that target E6-p53 interaction
- Anti-sense nucleotides, ribozymes
- Intrabodies
- Anti E2 compounds for low grade lesions
- Proteasome inhibitors?
- L1 – blocking agents.

GOOD NEWS: Some of them are in phase I or II clinical study.



and vaccine evaluation (2)

VACCINS? To be evaluated
The new vaccines to protect girls against HPV prompt debate about why it's necessary at such a young age…and they still need evaluation

CERVARIX AND GARDASIL

ACOG Cervical screening GUIDELINES: Key changes, 2013

Thank you Doctor Papanikolaou!

Dr Ekatherina Charvalos Director Central Labs-IASO