WORKSHOP

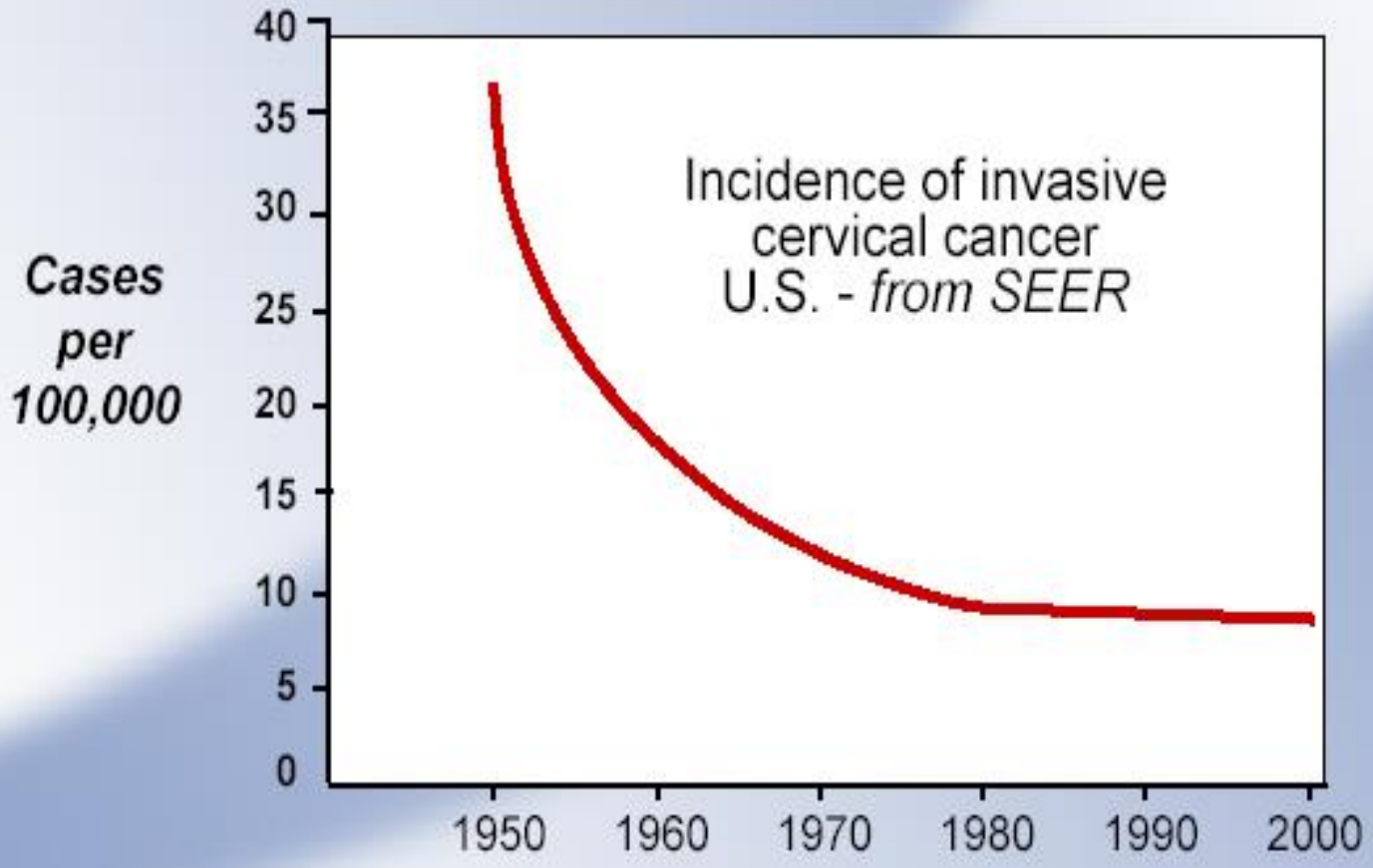
SCREENING DELLA CERVICE UTERINA:
STATO DELL'ARTE

Palazzo Partanna- Unione degli Industriali
P.zza Dei Martiri 56
27 giugno 2018

Il Valore della ricerca scientifica

Gerardo Botti
INT – IRCCS Fondazione Pascale

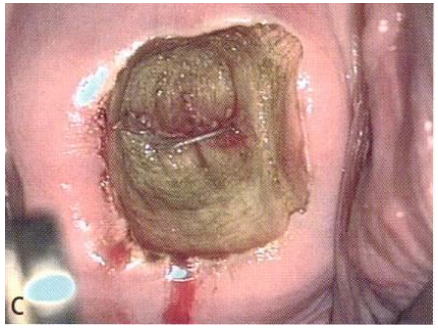
Impact of Cervical Cytology in U.S.



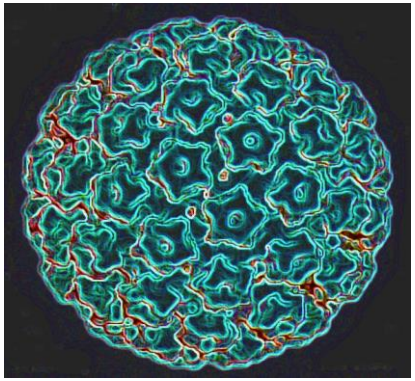
Premio Nobel per la Medicina nel 2008

Harald zur Hausen per la scoperta del ruolo dell'HPV nelle neoplasia umane ed in particolare nelle neoplasie mucosali del tratto ano-genitale

Prevenzione del Carcinoma della Cervice



- Prevenzione Secondaria
  - Diagnosi precoce mediante screening
    - Il trattamento della malattia è soprattutto a livello delle SIL
- Pevenzione primaria
  - Vaccinazione
    - Eradicazione della malattia



Special Report of the Meeting on the IARC Monograph:

A review of human carcinogens – Part B: biological agents

Bouvard et al. The Lancet Oncology 2009

Group	HPV types	Comments
Alpha HPV types		
1	16	Most potent HPV type, known to cause cancer at several sites
1	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Sufficient evidence for cervical cancer
2A	68	Limited evidence in humans and strong mechanistic evidence for cervical cancer
2B	26, 53, 66, 67, 70, 73, 82	Limited evidence in humans for cervical cancer
2B	30, 34, 69, 85, 97	Classified by phylogenetic analogy to HPV types with sufficient or limited evidence in humans
3	6, 11	..
Beta HPV types		
2B	5 and 8	Limited evidence for skin cancer in patients with epidermodysplasia verruciformis
3	Other beta and gamma types	..

Table 2: **Human papillomavirus (HPV) types assessed by the IARC Monograph Working Group**

Carcinogen classification:

- *Group 1 Cancers for which there is sufficient evidence of carcinogenicity in humans*
- *Group2A Probably carcinogenic to humans;*
- *Group 2B Possibly carcinogenic;*
- *Group 3 Not classifiable as carcinogenic*

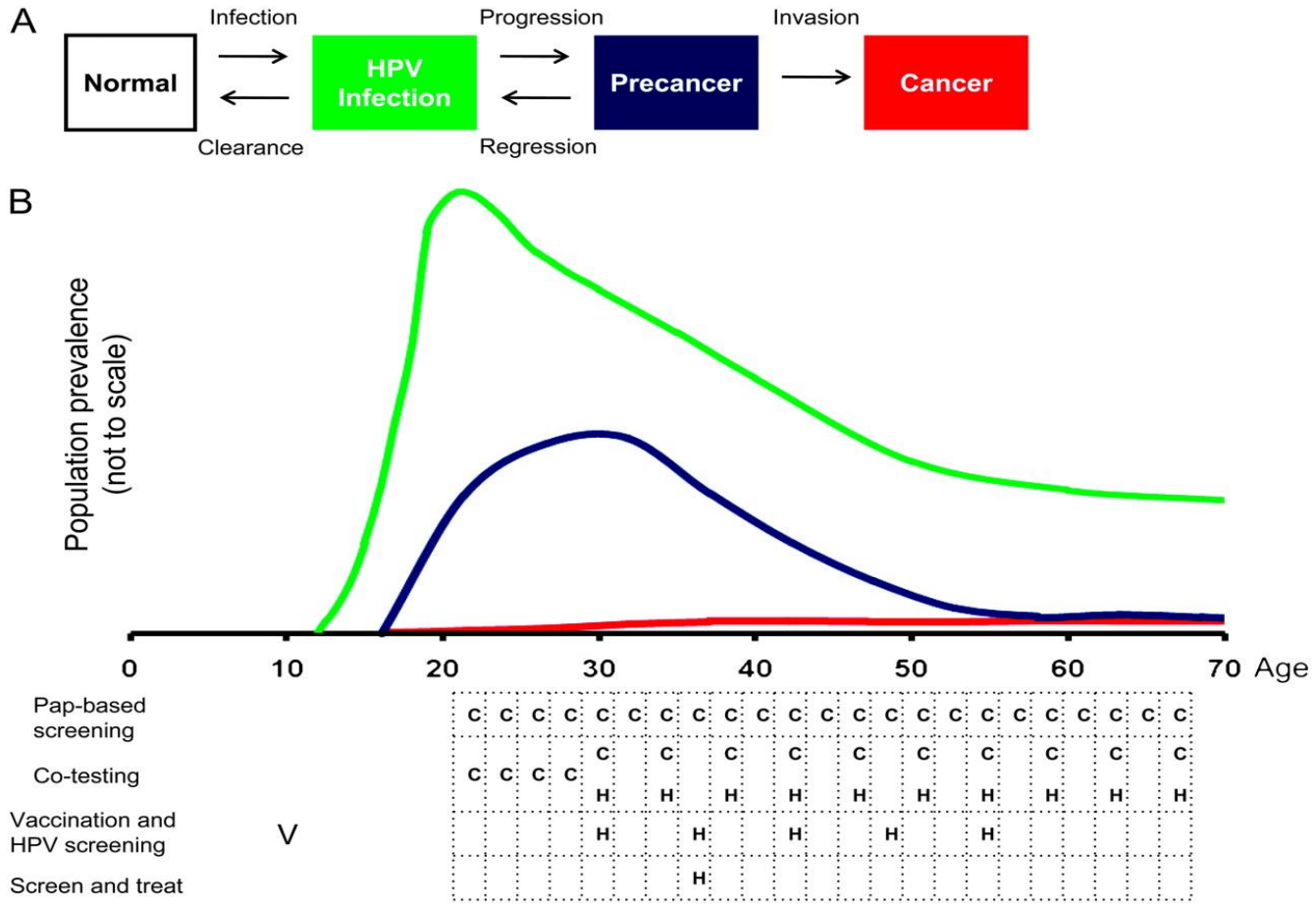
Classificazione degli HPV in alto e basso rischio

1. osservazione epidemiologica di associazione con lesioni benigne o maligne
2. dimostrazione di diversa capacità dei diversi virus di interferire con la proliferazione e la stabilità genomica della cellula infettata

- tipi 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 sono *cancerogeni per la specie umana;*
- tipo 68 *probabilmente cancerogeno,*
- tipi 26, 53, 66, 67, 70, 73, 82 *possibilmente cancerogeni*

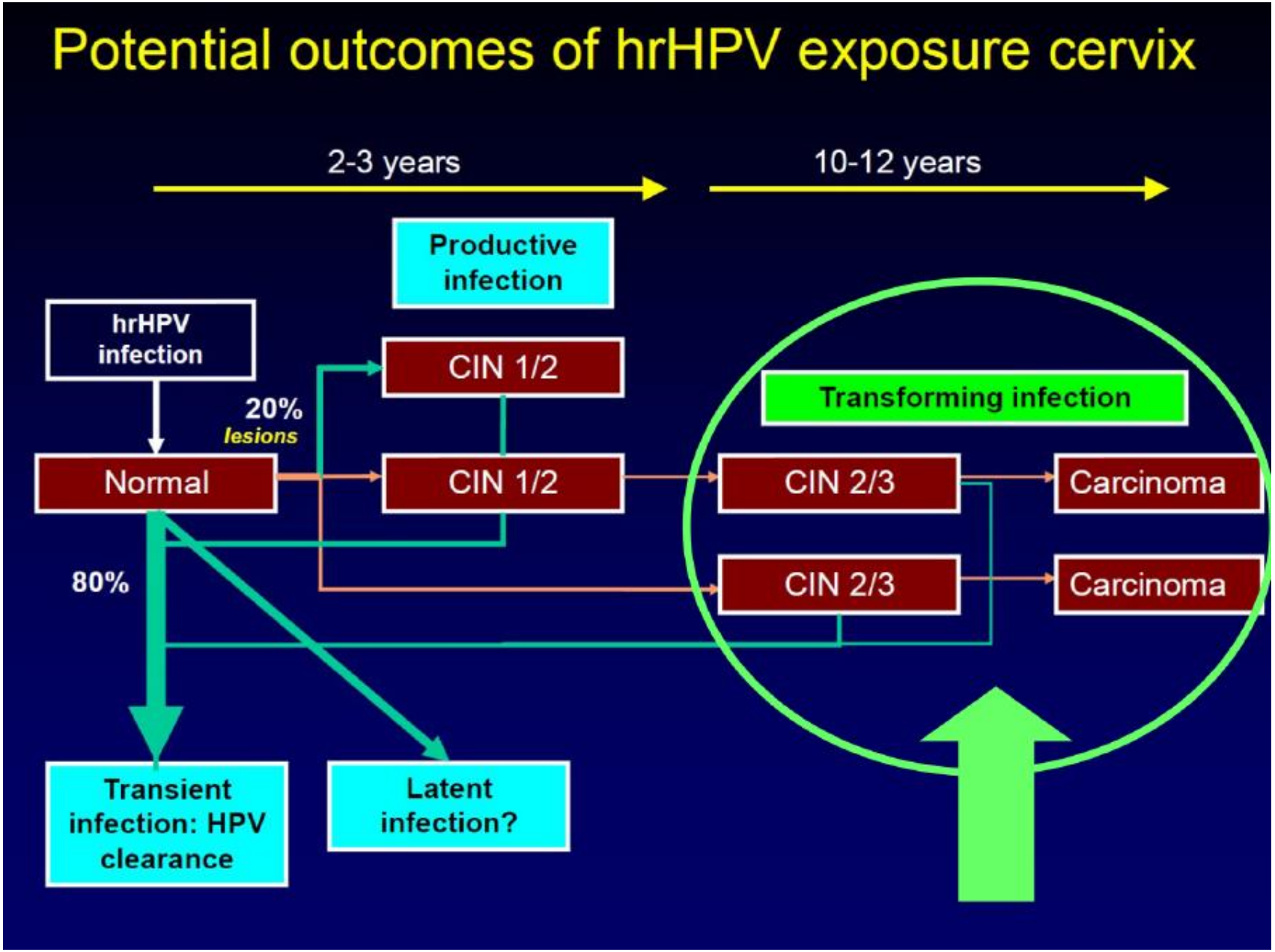
(Bouvard et al. Lancet Oncol 2009;10:321-2)

Alpha high risk HPVs and Cervical cancer progression model

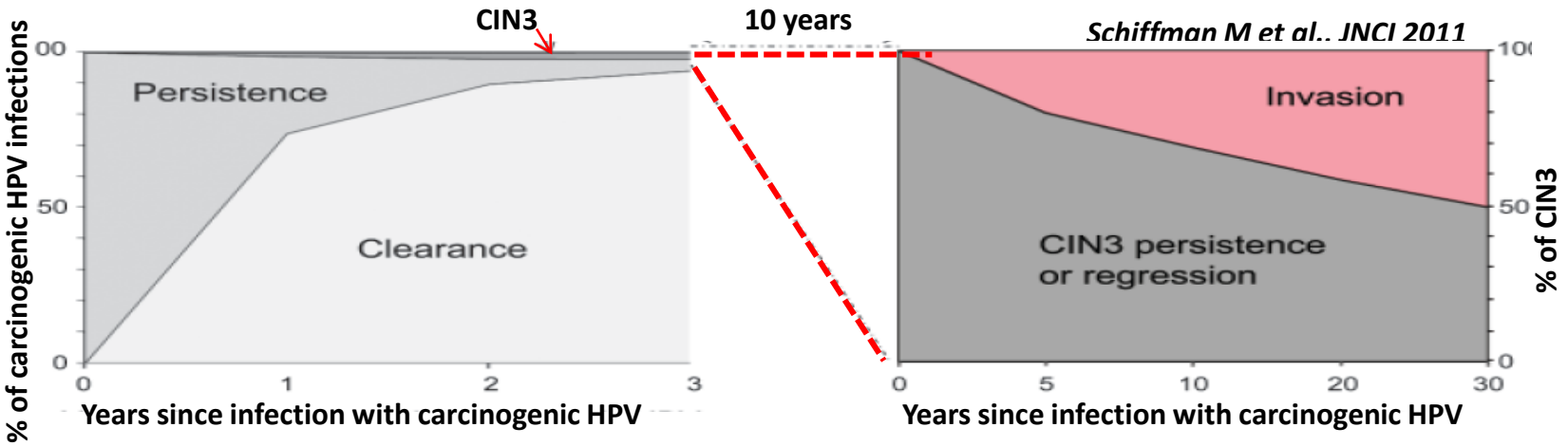


Schiffman M et al., J Natl Cancer Inst 2011

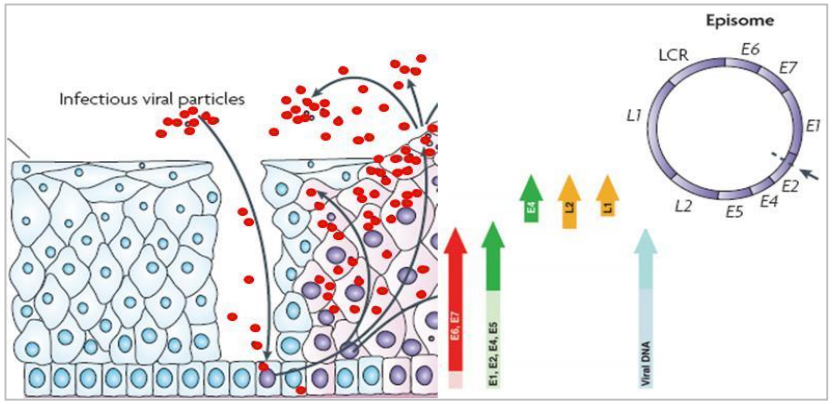
Potential outcomes of hrHPV exposure cervix



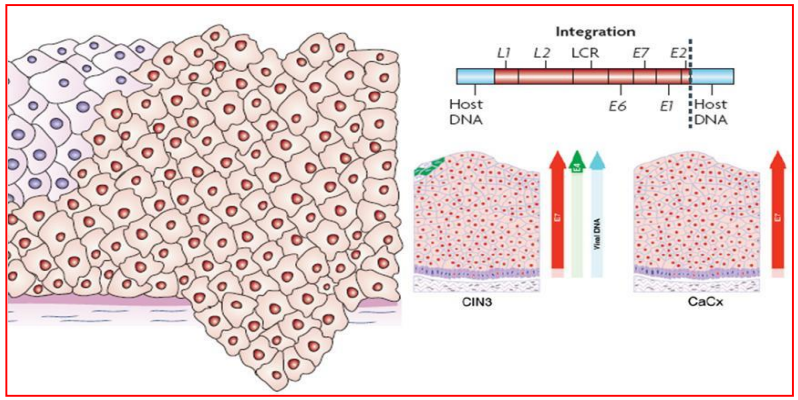
HPV infection, persistence and progression



Productive Infection



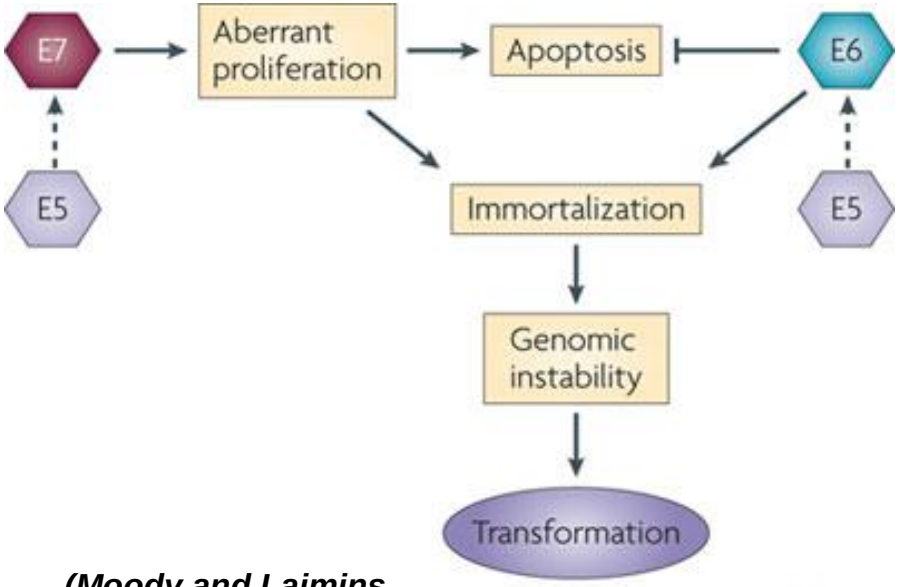
Abortive Infection



HPV TYPE 16 (HPV16) AND CANCER

Group	α-HPV types	Comments
1	16	Most potent HPV type, known to cause cancer at several sites
1	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Sufficient evidence for cervical cancer
2A	68	Limited evidence in humans and *in vitro* evidence for cervical cancer
2B	26, 53, 66, 67, 70, 73, 82	Limited evidence in humans for cervical cancer
2B	30, 34, 69, 85, 97	Classified by phylogenetic analogy to HPV types with sufficient or limited evidence in humans
3	6, 11	--

(IARC, Lancet Oncology 2009)



(Moody and Laimins, 2010)

Protein	Function
E5	↓MHC-I, ↑EGFR
E6	↓p53, ↑TERT, ↓BAX e BAK
E7	↓pRB, ↓p21 e p27, ↑E2F

HTA report italiano:
Luglio 2012.
Contiene
un'anticipazione
ufficiale delle LLGG
EU
Gennaio 2013
documento
Ministero della
Salute di supporto
alla programmazione
regionale.

www.epiprev.it

Quali tipi di HPV è utile rilevare?

Sulla base dei risultati degli studi randomizzati condotti su donne invitate a programmi di screening organizzati

→ tipi ad alto rischio (hrHPV)

→ individuazione come pool senza identificazione dei singoli tipi

Sommario esecutivo Rapporto HTA

Only tests for the DNA of oncogenic HPV, validated according to the European guidelines as for sensitivity and specificity for high-grade lesions, should be applied.

There is no evidence that double testing with cytology and HPV is more protective than stand-alone HPV as primary test,…… For this reason, if HPV is used as primary screening test, it is recommended not to add cytology in parallel.

Nello screening

il test HPV non è un test virale
ma un test di rischio oncogeno

- **Buon bilanciamento fra sensibilità e specificità clinica** per minimizzare le procedure di follow-up non necessarie
- Cautela verso i tentativi di raggiungere una sensibilità clinica del 100% aumentando la sensibilità clinica:
  - **un piccolo guadagno in sensibilità risulterà in una drammatica riduzione della specificità clinica (aumento dei falsi positivi)**

Cosa intendiamo per validazione clinica?

- **Sensibilità e specificità analitica di un test HPV**
  - rileva qualsiasi infezione da hrHPV, incluse le infezioni transienti, che sono clinicamente non rilevanti

- **Sensibilità e specificità clinica di un test HPV**
  - rileva infezioni hrHPV associate con lesioni CIN2+ (infezioni hrHPV clinicamente rilevanti)

➔ **la rilevazione di infezioni hrHPV nello screening cervicale è utile solo quando associata con la presenza o lo sviluppo di lesioni CIN2+**

Vaccine 30S (2012) F100–F106

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Nucleic Acid Tests for the Detection of Alpha Human Papillomaviruses

Mario Poljak[a,*], Jack Cuzick[b], Boštjan J. Kocjan[a], Thomas Iftner[c], Joakim Dillner[d], Marc Arbyn[e]

Identificati almeno 125
diversi test HPV e almeno
84 varianti dei test originali

Per tipi rilevati, metodologia, automazione,
solo alcuni di questi possono essere
considerati come test primario nello screening

5.1 Caratteristiche del test HR-HPV

.....

Per la validazione e introduzione di nuovi test HR-HPV in ambito di screening primario vengono pertanto recepite le indicazioni contenute in un recente articolo di C. Meijer, che stabilisce i criteri per la validazione di nuovi test consentendo il confronto del 'nuovo test' rispetto al 'test validato' *(11)*. **I dati relativi alle validazioni di nuovi test devono essere pubblicati su riviste in Medline.**

Dal DNA all'RNA.
Dall'infezione di per sé a marcatore di progressione

PMC full text: J Clin Microbiol. Jul 2009; 47(7): 2136–2141.
Published online Apr 29, 2009. doi: 10.1128/JCM.01733-08

J Clin Microbiol

TABLE 7.

Cytology and mean RNA/DNA copy number ratios

Cytology result	Mean RNA/DNA copy no. ratio for positive samples				
	HPV types				All types[a]
	16	18	31	33	
Normal	2.6	2.3	0.8		1.92
ASCUS	2.6	3.5		0.5	2.21
LSIL	12.9	5.8	12.0	7.5	9.56
HSIL	22.2	11.6	7.2	10.2	12.8

Dal DNA all'RNA.
Dall'infezione di per sé a marcatore di progressione

TABLE 6
Comparison of Human Papillomavirus 16 E6 and E7 DNA and RNA Expression in High-Grade Intraepithelial Lesion ThinPrep Samples

	HPV-16 E6			HPV-16 E7		
Sample no.	Conventional PCR (DNA)[a]	Real-time PCR (DNA)[b]	Real-time RT-PCR (RNA)[b]	Conventional PCR (DNA)[a]	Real-time PCR (DNA)[b]	Real-time RT-PCR (RNA)[b]
2	Negative	1.77×10^2	36.1	p++	0	18
11	p+++	4.11×10^4	4.76×10^5	p+++	11.7	1.71×10^4
15	p++	2.24×10^9	N/A	p++	2.04×10^3	N/A
17	p+	7.66×10^4	1.05×10^5	p++	5.1	3.20×10^4
20	p++	1.58×10^2	1.84×10^4	p+++	39.5	1.16×10^4
24	p+++	1.39×10^3	2.54×10^4	p+++	58.3	5.38×10^4
31	p++	2.63×10^2	8.11×10^4	p+++	73.7	5.22×10^4
32	p+++	42.3	3.62×10^3	p++	10.9	2.60×10^3
37	p+++	3.44×10^4	4.67×10^5	p++	1.61×10^4	5.50×10^5
80	p++++	8.08×10^4	4.25×10^5	p+	6.54×10^3	1.65×10^6
85	p++	6.7	1.10×10^4	p?	980	8.77×10^5
97	p+	2.9	2.4	Negative	0	1.20×10^2
101	p++++	1.53×10^5	2.00×10^5	p++++	2.18×10^4	2.04×10^5

CANCER April 15, 2002 / Volume 94 / Number 8

$10\text{-}10^9$ $10^3\text{-}10^5$

A seguito di integrazione, fino al 60% del genoma virale può essere perso

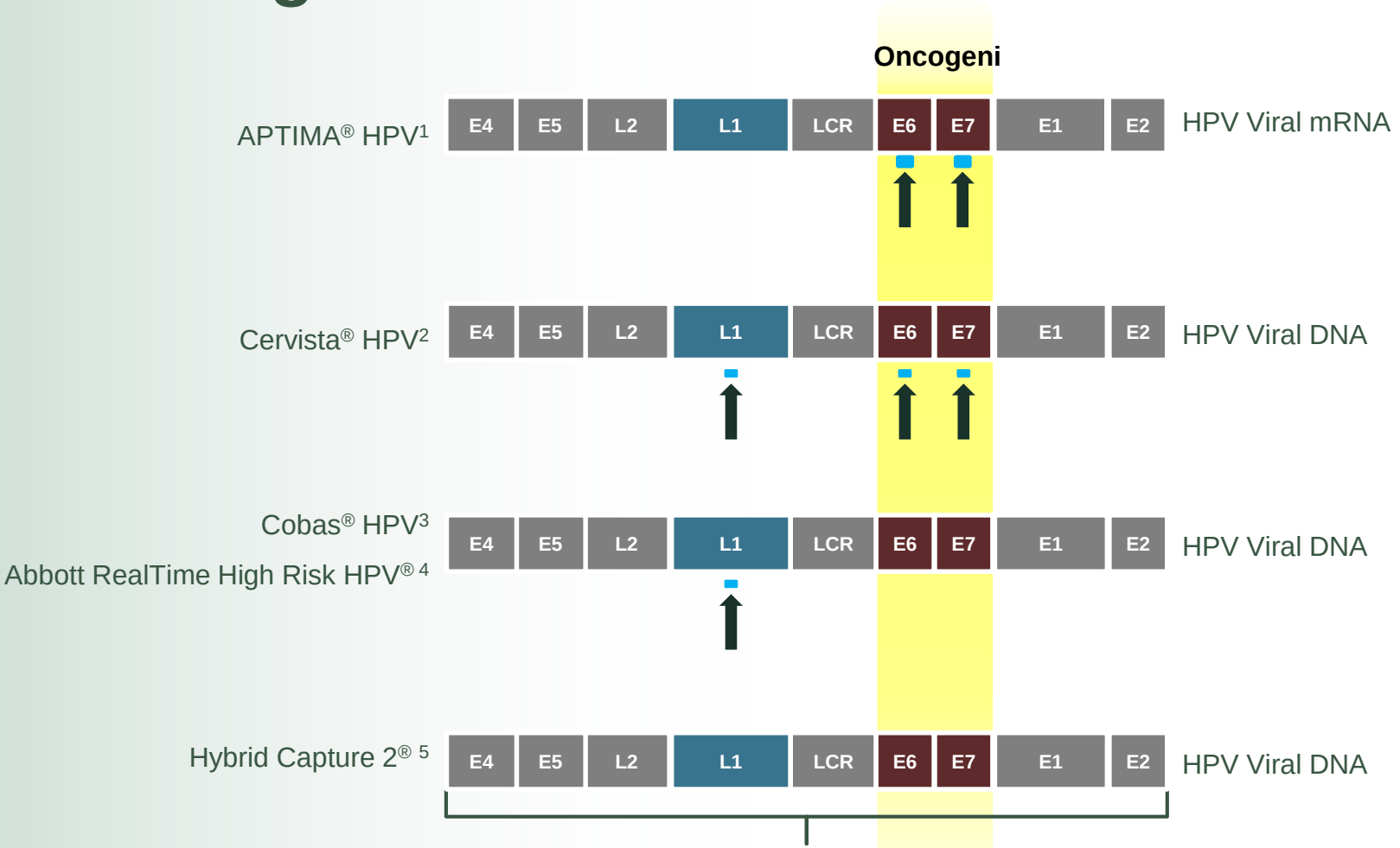
	L-SIL	H-SIL	CC	ICC	SNPs
L1			5-10	41	High Grade
E1/E2	15.4	37.1	91*		
E2		always			
E6/E7		“never”	sempre presente !		Low Grade

* E1 – human genome fusion

Karlsen F., *et al*; J.Clin.Microbiol., Spet 1996, p.2095-2100
Walboomers *et al*. J Pathol. 1999;189:12-19.
Morris J.B.; Clin.Chem.Lab.Med 2005;43(11):1171-1177
De Marco L. *et al*,J Clin Virol 2007;38(1):7-13
Cricca M., *et al*; J.Virol.Methods, 2009 Jun: 158(1-2);p.180-3

HPV

Strategie di rilevazione



1. APTIMA HPV Assay package insert #502170 Rev A 2011
2. Cervista HPV HR package insert #15-3053 2010
3. Cobas 4800 HPV Test US Package Insert #05641268001-01EN Rev 1.0 2011
4. Abbott Real Time High Risk HPV package insert #2N09 Rev 49-2028/R3
5. Hybrid Capture 2 High-Risk HPV DNA Test® package insert #L00665 Rev. 2 2007

1992

Detection of Cervical Cancer by HPV L1 DNA Tests (PCR)

Reference	Study Scope /Location	HPV Test	CxCA Cases (n)	CxCA Cases Detected	False-Negative Rate, (%)
Guerrero, J Clin Micro 1992	Columbia, Spain	PCR L1	302	208	31.1
Williamson, J Med Virol 1994	South Africa	PCR L1	68	55	19
Karlsen, Eur J Cancer 1995	Norway	PCR L1	146	130	11
Burger, J Nat Can Inst 1996	US (California)	PCR L1	287	245	14.6
Baay, J Clin Micro 1996	Netherlands	PCR L1	162	118	27.2
Torroella-Kouri, Gynecol Oncol 1998	Mexico	PCR L1	69	60	13
Schwartz, J Clin Oncol 2001	US (Washington State)	PCR L1	399	342	14.3
Shellekens, Gyn Onc 2004	Indonesia	PCR L1	74	71	4.1
Hoyer, Int J Cancer 2005	Germany	PCR L1	9	6	33.3
Sowjanya, BMC Int Dis 2005	India	PCR L1	41	36	12.2
Herrero, J Inf Dis 2005	Guanacaste Costa Rico	PCR L1	35	34	2.9
Stevens, Int J Gyn Can 2006	Australia	PCR L1	191	166	13.1
Peedicayil, Int J Gyn Can 2006	India	PCR L1	119	113	5
Castellsaque, J Nat Can Inst 2006	Worldwide	PCR L1	167	156	6.6
Keita, Br J Cancer 2009	Guinea	PCR L1	77	70	9.1
Wheeler, J Nat Can Inst 2009	US (New Mexico SEER)	PCR L1	2,021	1,913	5.3

Detection of Cervical Cancer by HPV L1 DNA Tests (PCR)

Reference	Study Scope /Location	HPV Test	CxCA Cases (n)	CxCA Cases Detected	False-Negative Rate, (%)
Illades-Aguiar, Cancer Det Prev 2009	Mexico	PCR L1	133	133	0
Roa, Int J Gyn Obstet 2009	Chile	PCR L1	312	294	5.8
Raza, Br J Cancer 2010	Pakistan	PCR L1	91	83	8.8
Okolo, Inf Agents Cancer 2010	Nigeria	PCR L1	68	67	1.5
de Sanjose, Lancet Oncology 2010	38 countries world-wide	PCR L1	10,575	8,977	15.1
Odida, Inf Agts Cancer 2010	Uganda	PCR L1	171	154	9.9
Mariani, BMC Cancer 2010	Italy	PCR L1	134	121	9.7
Dreier, Virology 2011	Austria	PCR L1	36	32	11.1
Tornesello, Gyn Onc 2011	Italy	PCR L1	171	140	18.1
Basu, J Clin Micro 2011	India	PCR L1	192	166	13.5
Waldstom, Cytopathology 2011	Denmark	PCR L1	1	1	0
Biernat-Sudolska, Adv Med Sci 2011	Poland	PCR L1	49	45	8
Coutlee, J Med Virol 2011	Canada	PCR L1	252	223	11.5
Castle, Can Ep Biom Prev 2011	US	PCR L1	97	94	3.1
Castle, Lancet Oncol 2011	ATHENA (Roche US Trial)	PCR L1	20	18	10
Total		L1 DNA	16,469	14,271	13.3

Detection of Cervical Cancer by Hybrid Capture 2

Reference	Study Scope /Location	HPV Test	CxCA Cases (n)	CxCA Cases Detected	False-Negative Rate (%)
Brummer, Gyn Oncol 2002	Germany	HC2	15	15	0
Sankaranarayanan, Int J Cancer 2004	India	HC2	51	38	25.5
Derchain, Gyn Oncol 2004	Brazil	HC2	16	13	18.8
Lie, Gyn Oncol 2005	Norway	HC2	20	18	10
Atkins, Cancer Cytopathol 2006	US (ALTS)	HC2	7	7	0
Wu, J Clin Virol 2006	China	HC2	541	487	10
Ekalaksananan, Gyn Oncol 2006	Thailand	HC2	2	2	0
Jastania, Am J Clin Pathol 2006	US	HC2	1	0	100
Moreira, Int J Gyn Cancer 2006	Brazil	HC2	279	250	10.4
Hong, Int J Gyn Cancer 2008	China	HC2	181	172	5
Kang, Int J Gyn Can 2009	Korea	HC2	198	185	6.6
Yoon, Eur J Cancer 2009	Korea	HC2	2	2	0
Wang, West Ind J Med 2010	China	HC2	66	62	6
Zhao, Lancet Oncol 2010	China	HC2	51	50	2
Katki, Lancet Oncol 2011	US (Kaiser Screening Trial)	HC2	87	60	31
Ratnam, J Clin Micro 2011	Canada	HC2	13	12	7.7
Clad, J Clin Micro 2011	Germany	HC2	13	11	15.4*
Monsonego, Int J Cancer 2011	France	HC2	5	5	0
Coquillard, Gyn Oncol 2011	US	HC2	8	7	12.5
Rijkaart, Lancet Oncol 2011	Netherlands (POBASCAM)	HC2	18	16	11.1
Ibanez, BMC Inf Dis 2012	Spain	HC2	2	2	0

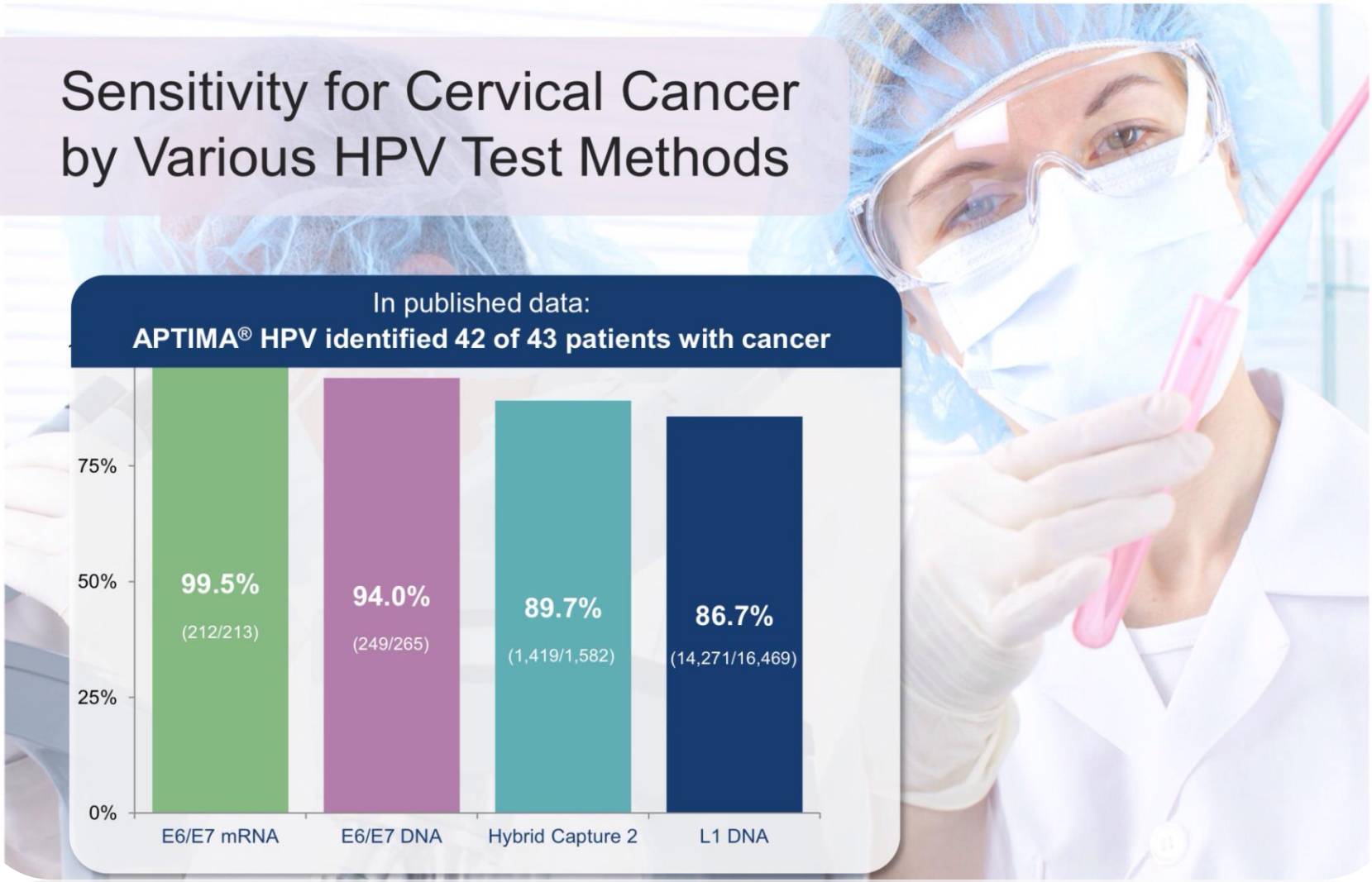
Detection of Cervical Cancer by HPV E6/E7 mRNA Tests

Reference	Study Scope /Location	HPV Test	CxCA Cases (n)	CxCA Cases Detected	False-Negative Rate (%)
Rose, Gyn Oncol 1995	Australia	RT-PCR E6/E7	28	28	0
Nakagawa, J Med Virol 2000	Japan	RT-PCR E6/E7	31	31	0
Andersson, J Clin Virol 2012	Sweden	RT-PCR E6/E7	11	11	0
de Boer, Clin Cancer Res 2007	Netherlands	RT-PCR E6/E7	72	72	0
Coquillard, Gyn Oncol 2011	US	FISH-Flow Cytometry	8	8	0
Lie, Gyn Oncol 2005	Norway	NASBA	20	20	0
Castle, Clin Can Res 2007	US	APTIMA HPV	5	5	0
Ratnam, J Clin Micro 2011	Canada	APTIMA HPV	13	13	0
Clad, J Clin Micro 2011	Germany	APTIMA HPV	13	12	7.7*
Waldstrom, Cytopathology 2011	Netherlands	APTIMA HPV	1	1	0
Monsonego, Int J Cancer 2011	France	APTIMA HPV	5	5	0
Nieves, Int J Gyn Cancer 2013	Mexico	APTIMA HPV	2	2	0
Stoler, AJOG 2013, APTIMA HPV Assay package insert	US (CLEAR trial)	APTIMA HPV	4	4	0
Total		E6/E7 mRNA	213	212	0.5

Reference	Study Scope /Location	HPV Test	CxCA Cases (n)	CxCA Cases Detected	False-Negative Rate (%)
de Boer, Clin Cancer Res 2007	Netherlands	PCR E6/E7	74	70	5.4
Gheit, Vaccine 2009	India	PCR E6/E7	180	168	6.7
Andersson, J Clin Virol 2012	Sweden	PCR E6/E7	11	11	0
Total		E6/E7 DNA	265	249	6.0

2013

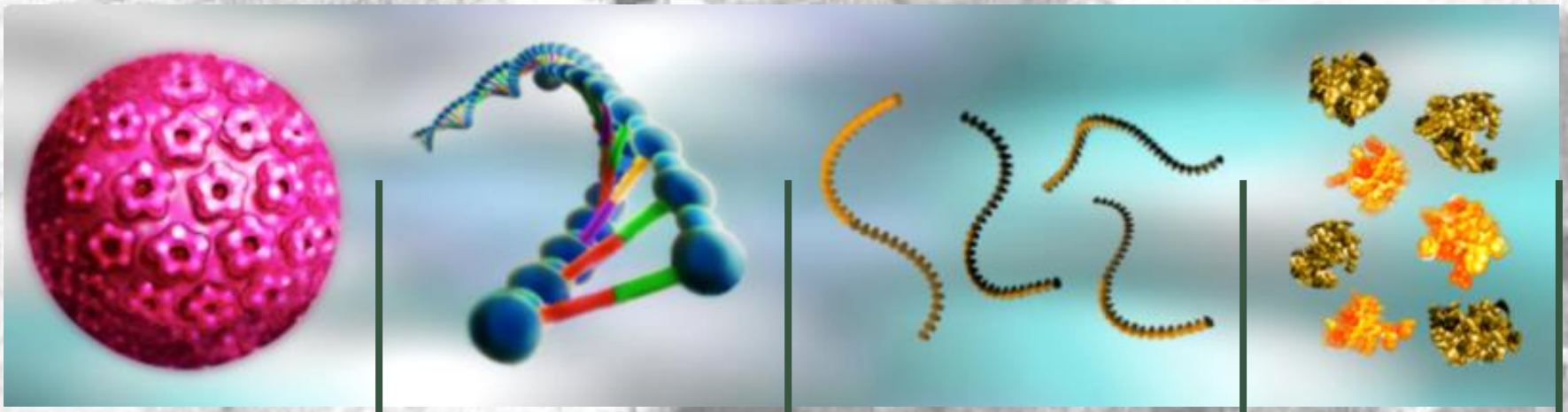
Sensitivity for Cervical Cancer by Various HPV Test Methods



DNA Detects '*Presence*'
E6/E7 mRNA Detects '*Activity*'

DNA
Indicates *Presence* of HPV

Expression of E6/E7mRNA
Indicates *Activity* of HPV



HPV Viral Particles

Viral DNA Genome

E6/E7 mRNA Expression by Active Virus

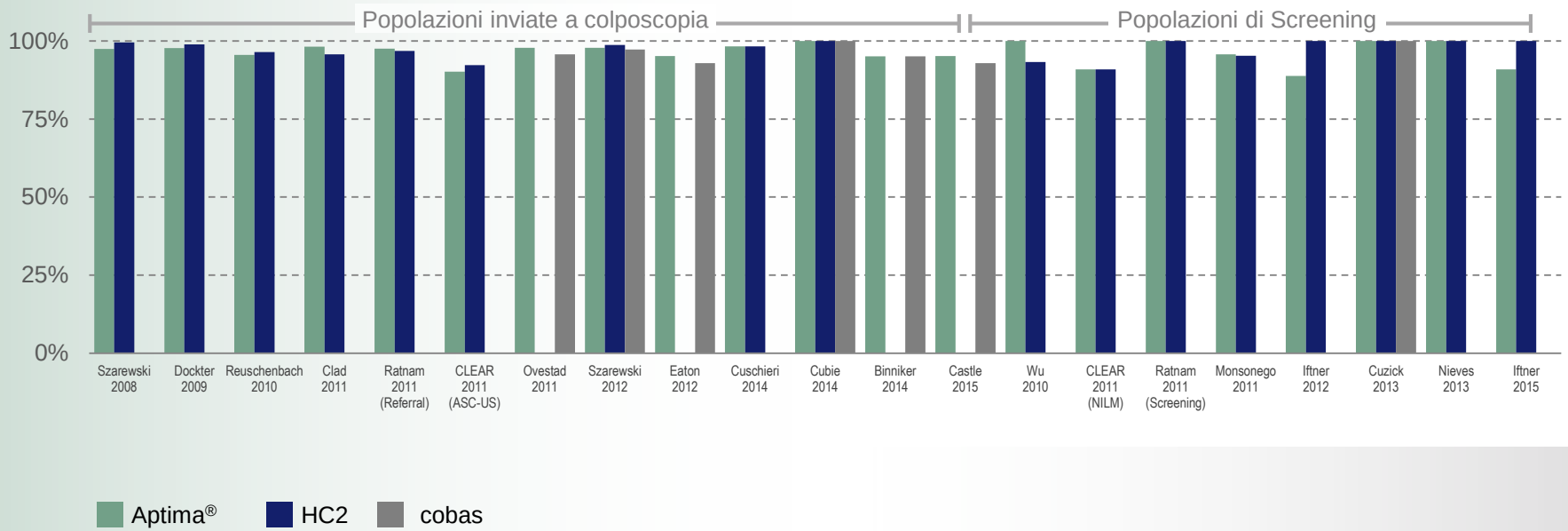
E6/E7 *Oncoproteins* Induce Carcinogenesis

Doorbar. Clinical Science 2006. 110(5):525-41

Aptima® HPV

La stessa eccellente sensibilità clinica dei test HPV DNA

Sensibilità clinica per CIN3+

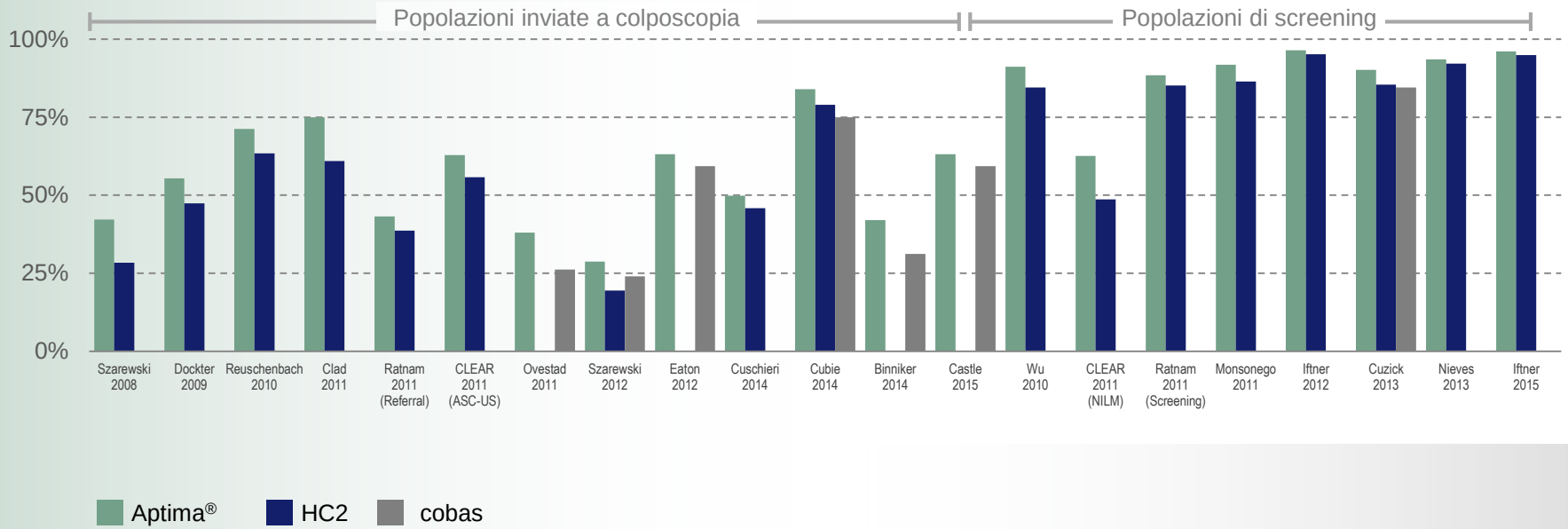


Aptima HPV è **altrettanto sensibile** rispetto ai test HPV DNA nelle popolazioni di screening e in quelle inviate a colposcopia

Aptima® HPV

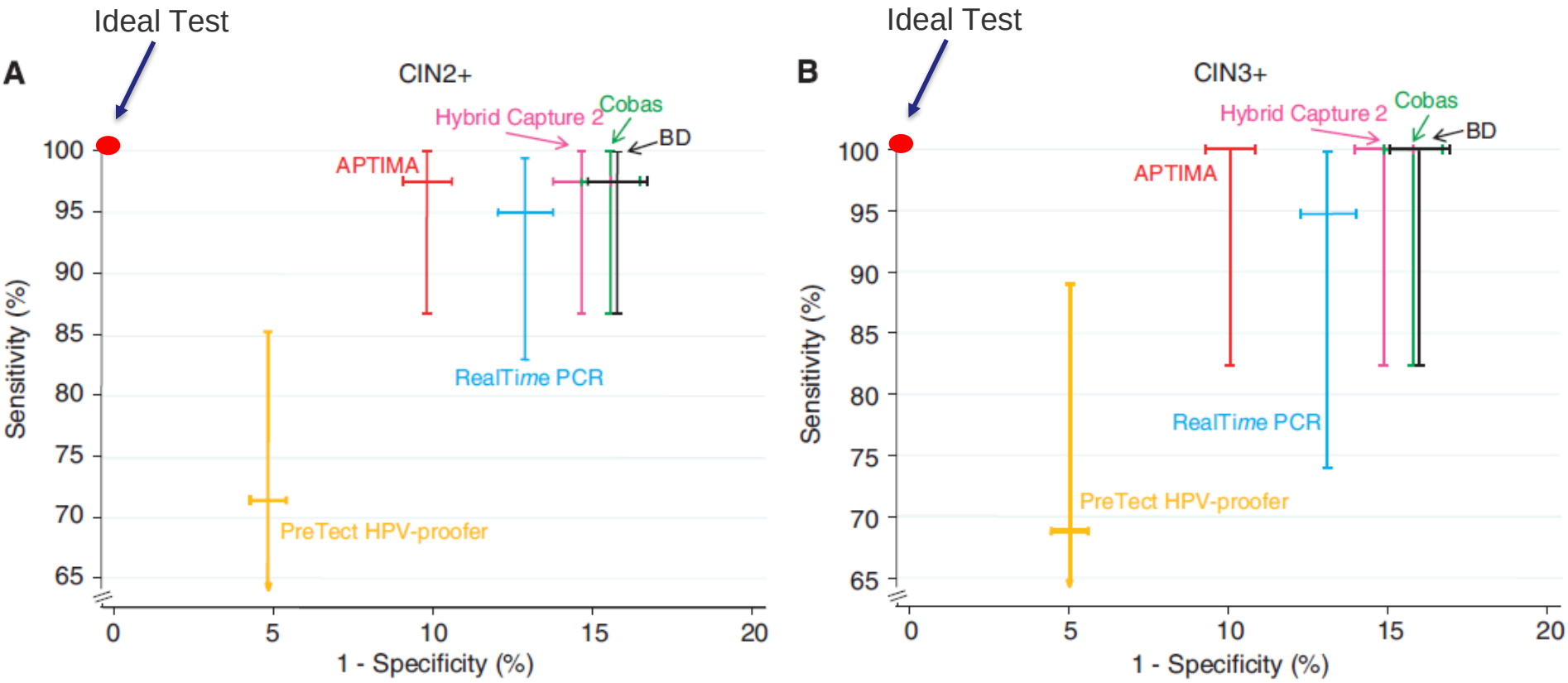
Specificità superiore rispetto ai test HPV DNA

Specificità clinica per <CIN2+



Aptima HPV è **più specifico** dei test HPV DNA nelle popolazioni di screening e in quelle inviate a colposcopia

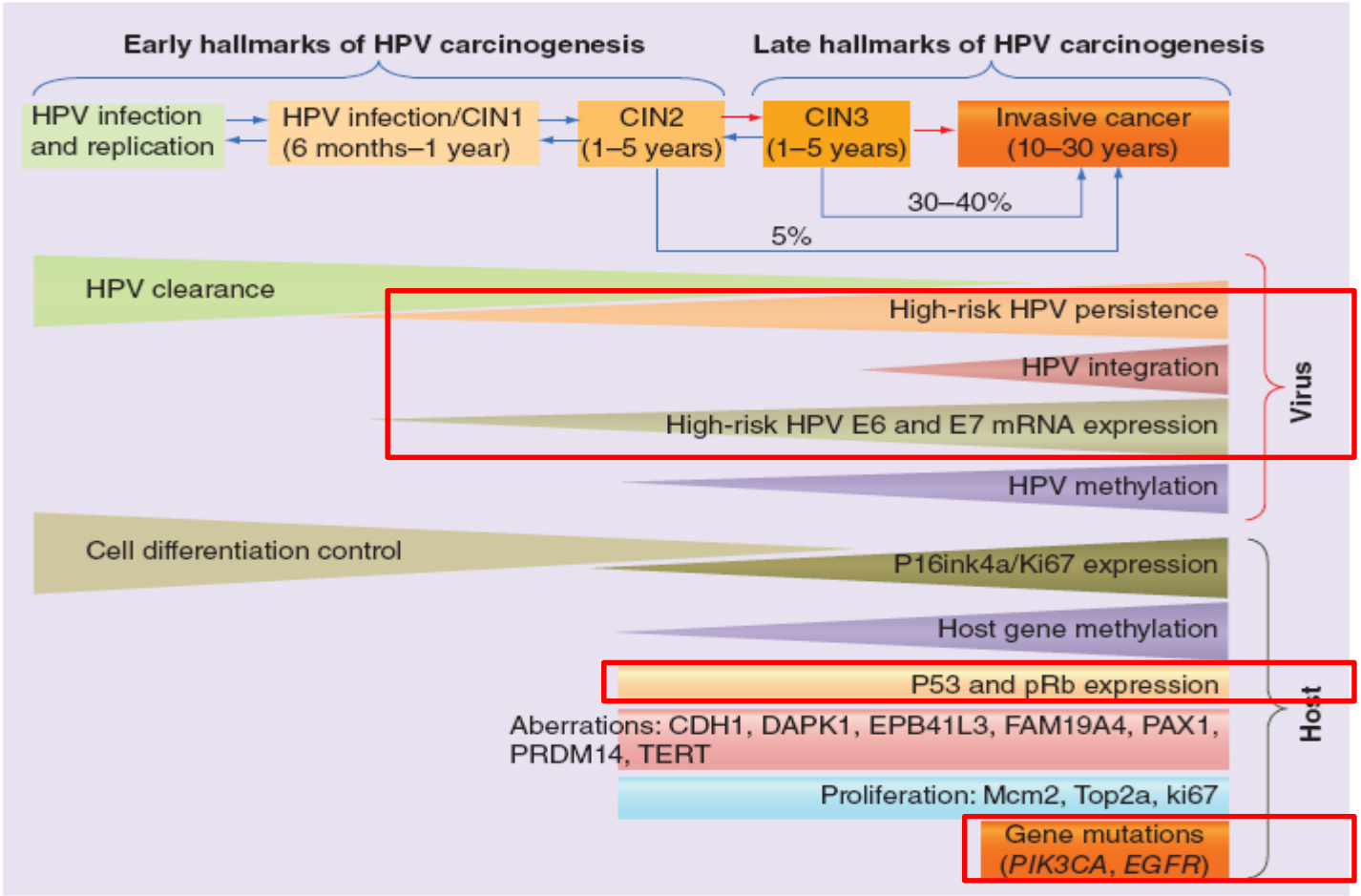
Predictors 3 Study



Of the highly sensitive tests, the Gen-Probe APTIMA assay was the most specific, with ~5% fewer 'false positives'. On a relative basis, this comes to about 15% fewer false positives than seen for the other highly sensitive DNA tests.

Szarewski et al. BJC 2013

Tumor progression markers in cervical cancer



Tornesello & Buonaguro, Future Oncol 2015

Int J Cancer. 2018 Mar 31. doi: 10.1002/ijc.31412.

IJC
International Journal of Cancer

Distinct profiles of *TERT* promoter mutations and telomerase expression in head and neck cancer and cervical carcinoma

Clorinda Annunziata[1], Francesca Pezzuto[1], Stefano Greggi[2], Franco Ionna[3], Simona Losito[4], Gerardo Botti[4], Luigi Buonaguro[1], Franco M. Buonaguro[1] and Maria Lina Tornesello[1]

[1] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Napoli 80131, Italy

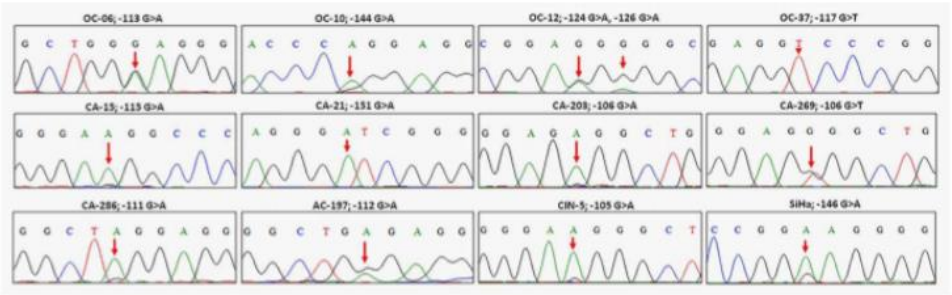


Figure 1. Uncommon mutations identified upstream the ATG start site of *TERT* promoter in oral SCC (OC), cervical squamous cell carcinoma (CA), cervical adenocarcinoma (AC) and cervical intraepithelial neoplasia (CIN) as well as hot spot mutation −146G to A in SiHa cell line. [Color figure can be viewed at wileyonlinelibrary.com]

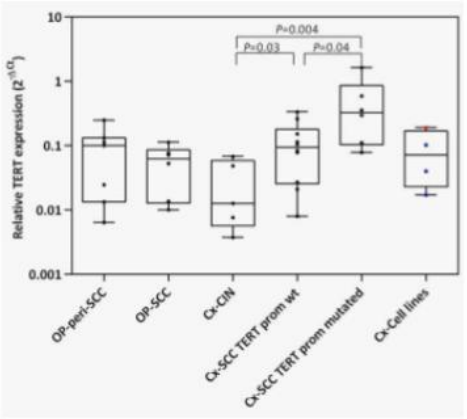


Figure 2. Relative expression values of *TERT* mRNA, normalized to

to A transition at position −146 in the HPV16-positive SiHa DNA. Quantitative RT-PCR of *TERT* mRNA from the four cervical cancer cell lines showed *TERT* mRNA levels 10-fold and 6-fold higher in the mutated SiHa cells compared to CaSki and HeLa cells, respectively, and 2-fold higher expression than C-4I cells (Fig. 3*b*). There was no correlation between HPV16 E6 mRNA levels and *TERT* expression. Interestingly HeLa and C-4I cell lines expressing highest levels of E6 showed lower level of *TERT* mRNA compared to SiHa and CaSki cell lines (Fig. 3*a*).

Discussion

The main risk factors for head and neck SCC are tobacco use and alcohol consumption which alone or in combination exert a cytotoxic and mutagenic effect on the mucosal epithelia of upper aerodigestive tract.[34–36] The most common molecular alterations in head and neck SCCs are somatic nucleotide changes in the exonic regions of *TP53*, *NOTCH1* and *IRF6* genes as well as homozygous deletion and muta-