From cytology to full molecular cervical screening

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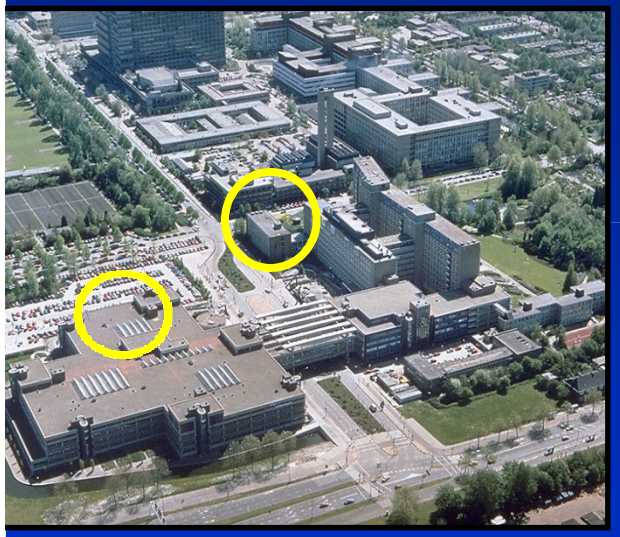


Cervical cancer worldwide

- Worldwide:
  - New cervical cancer cases 530.000/year
  - 3rd cancer in women
  - 275.000 women/year are dying of cervical cancer
  - 80% of cases in low resource countries: Africa, Mid- and south America and Eastern Europe

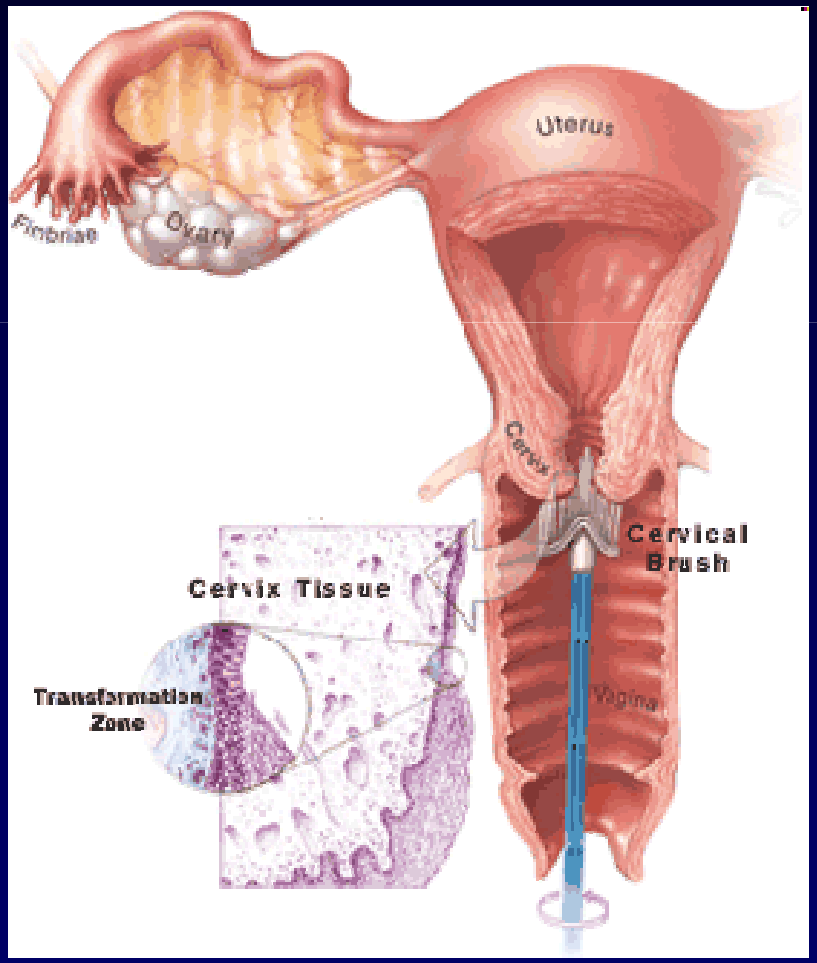
- Netherlands
  - Incidence: ASR/100.000 6.9 Mortality: ASR/100.000 1.6

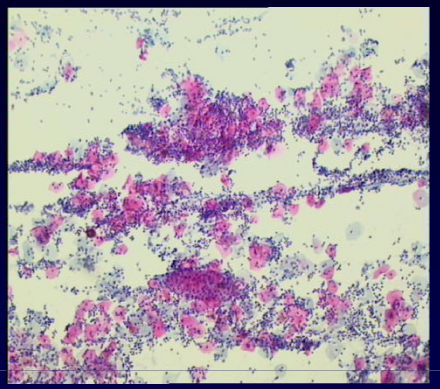
Absolute figures:

- ~700 new cases/year 220 Death/year

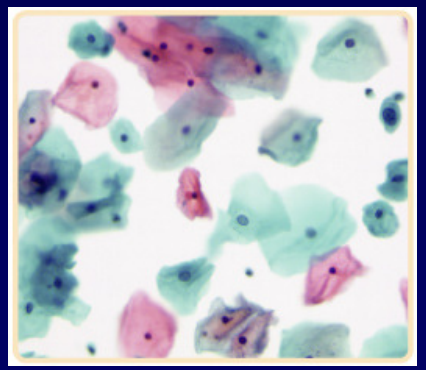
Globocan IARC-WHO 2012

Current cervical screening tool in many countries: Pap test (cytology)





Pap smear



Liquid-based cytology (LBC)

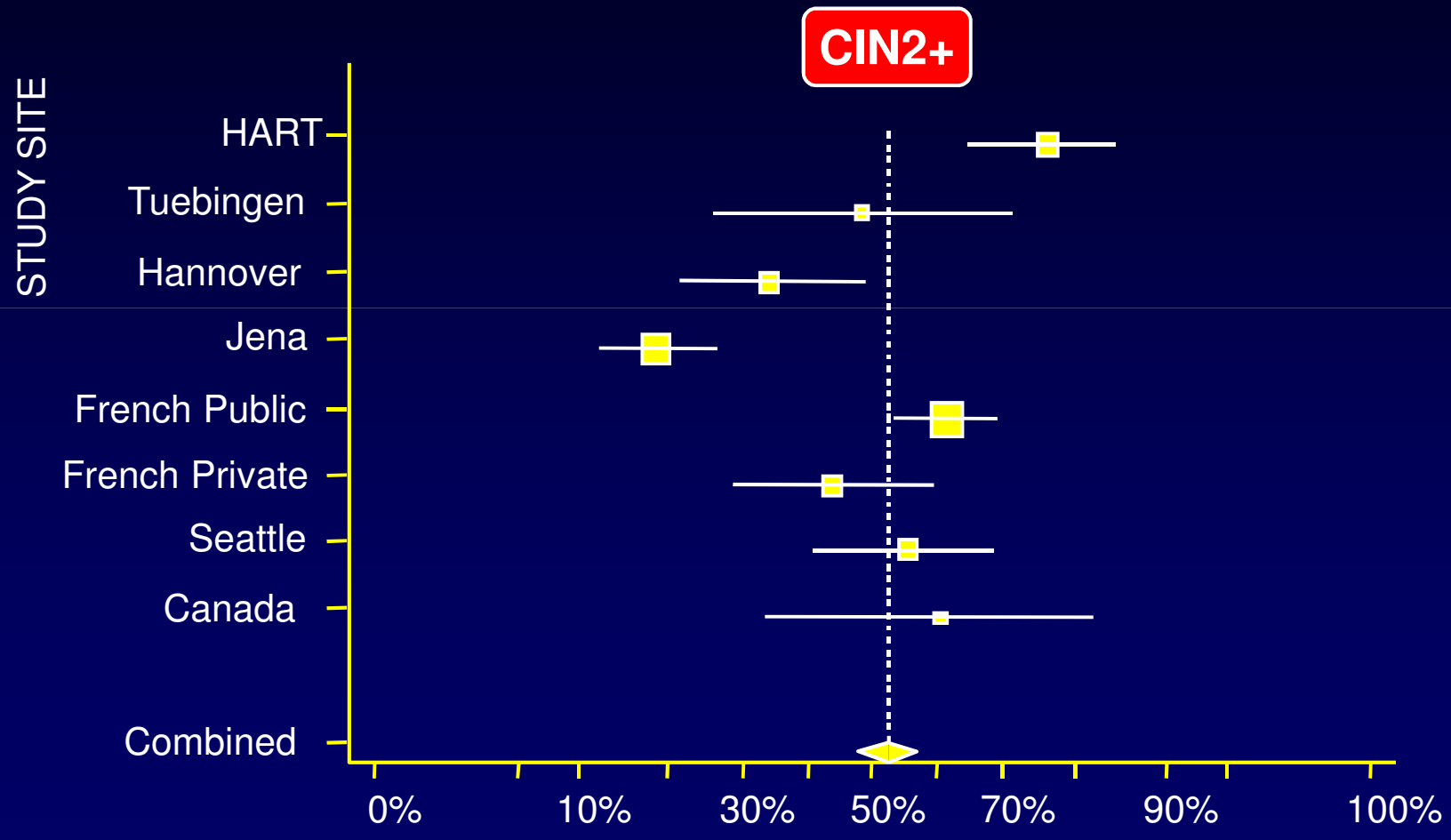
Why should we change from cytology?

Problems in cervical screening by cytology

- Low sensitivity: many false pos. and false neg smears
- Frequent repeat testing necessary
- Subjective; moderate reproducibility
- Require good training of technicians and strong QC
- Not all women are reached for cervical screening

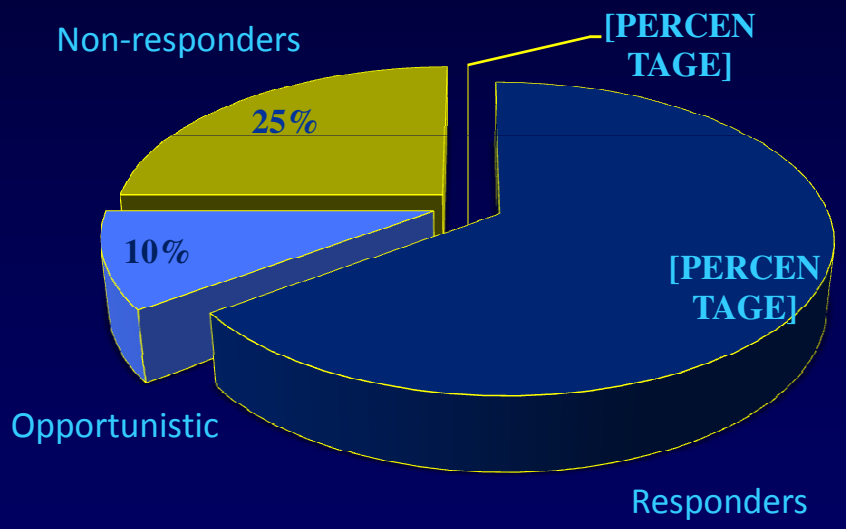
Problems cytology-based cervical cancer screening programmes:

1. Suboptimal sensitivity of the Pap test for cervical precancer



Cuzick et al. Int J Cancer. 2006

2. Not all women are reached for cervical screening



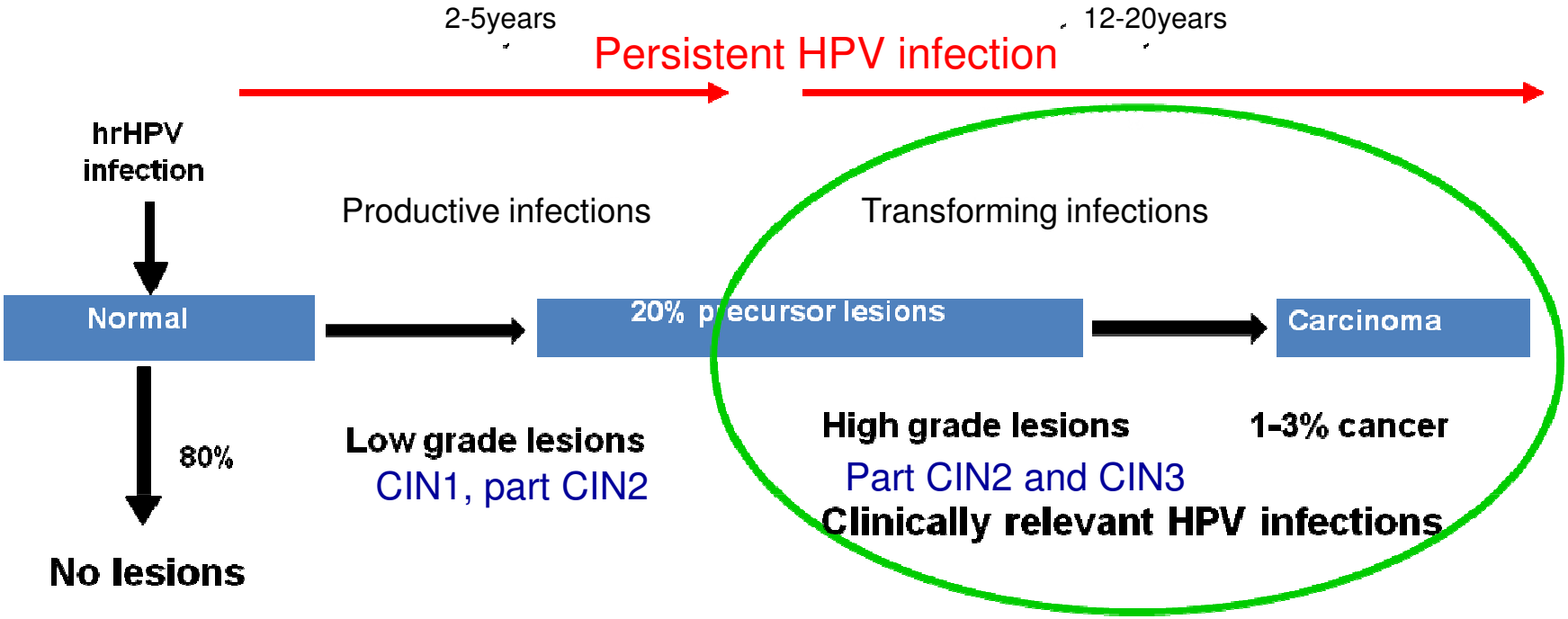
- In the Netherlands: 75% of women is protected (programmed & opportunistic)
- 25% is not screened at all (non-responders)
  - 57% of carcinomas in this group

Novel opportunity for cervical screening:

Testing for hrHPV presence

Q: Role of HPV in cervical carcinogenesis?

Role of HPV in cervical carcinogenesis



1. Persistent infection with hrHPV necessary for cervical carcinogenesis

2. No HPV, no cancer

3. 14 hrHPV types responsible for >99% of allCxCa:
   HPV 16 and 18 cause ~70% of all CxCa

Take home message

HPV testing vs cytology

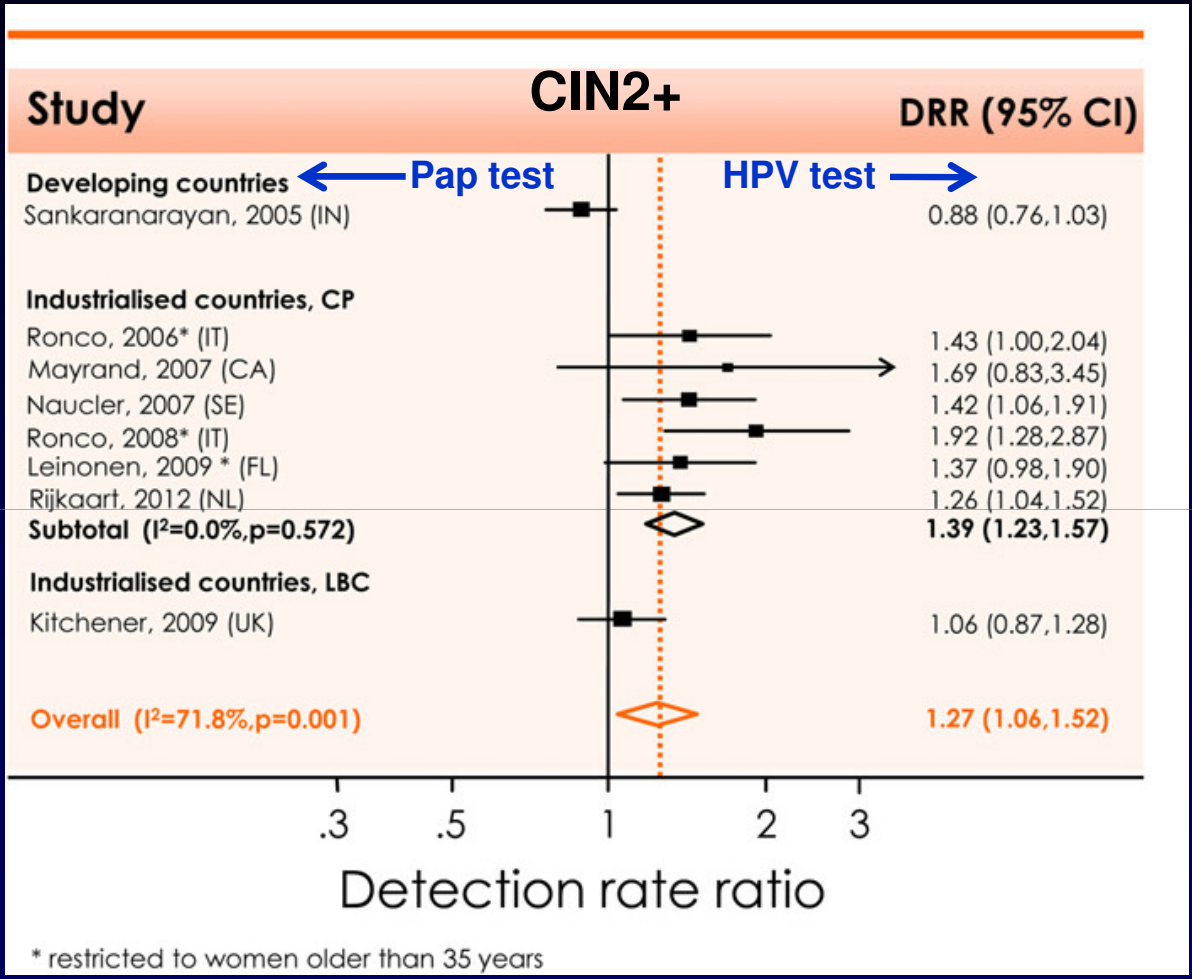
HPV testing is more sensitive for CIN2+ detection than cytology; more objective

HPV provides better protection against CIN3 and cancer than cytology after a screen negative test

For screening purposes HPV testing is as good as HPV & cytology (Combo)

Cuzick 2006 IJC; Bulkmans 2007 Lancet; Rijkaart 2012 Lancet oncology; Ronco 2013 Lancet, Arbyn 2012 Vaccine, Cage 2014 JNCI

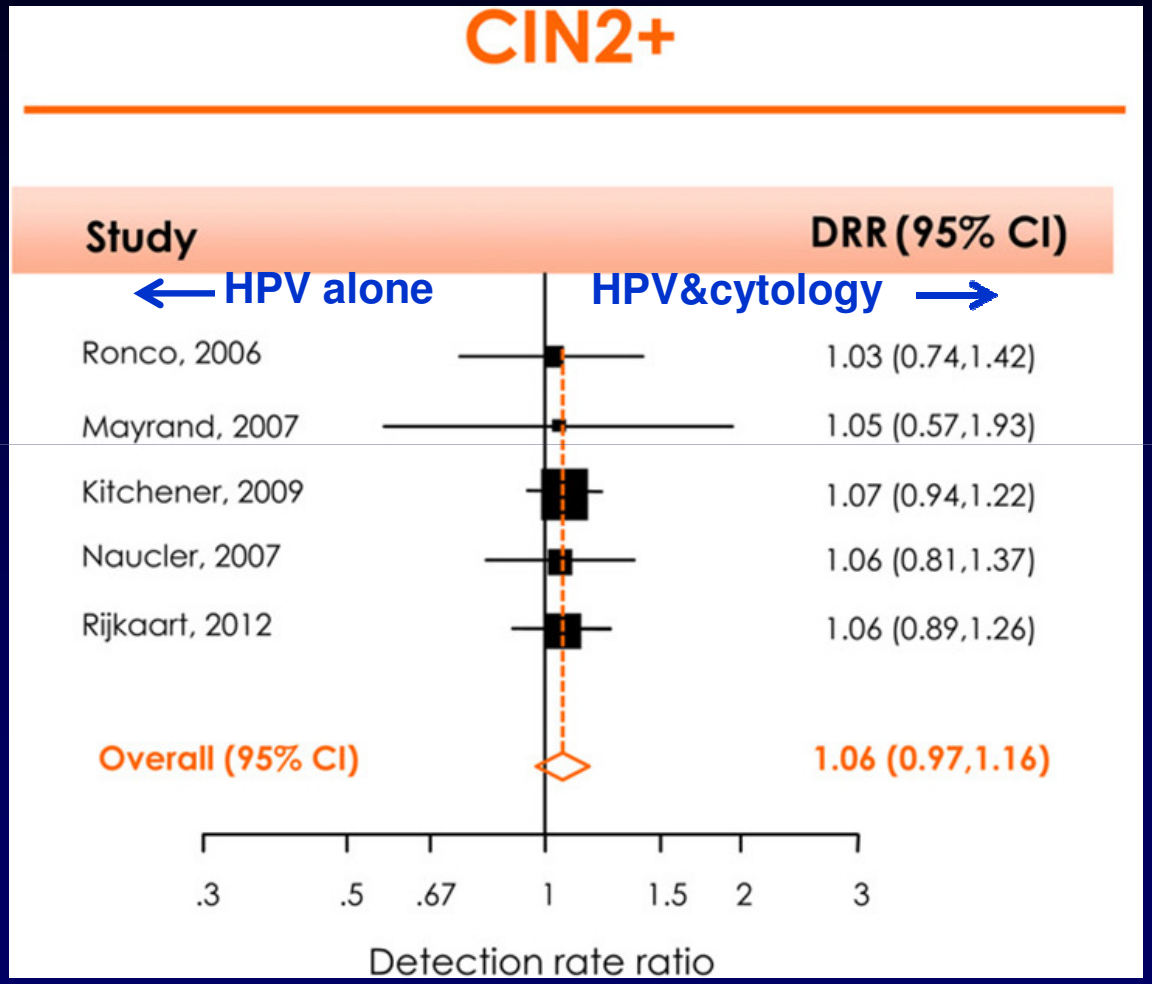
The HPV test is a more sensitive screening tool than the Pap test



HPV testing detects more CIN2+ than the Pap test

Arbyn et al., Vaccine 2012

Performance HPV & Pap (combo) vs HPV test alone



Sole HPV testing is nearly as sensitive as HPV&Pap:
For screening use sole HPV testing

Arbyn et al., Vaccine 2012

Cumulative detection of invasive carcinoma

Pooled data from POBASCAM, NTCC, Artistic and Swedescreen (>160.000 women)

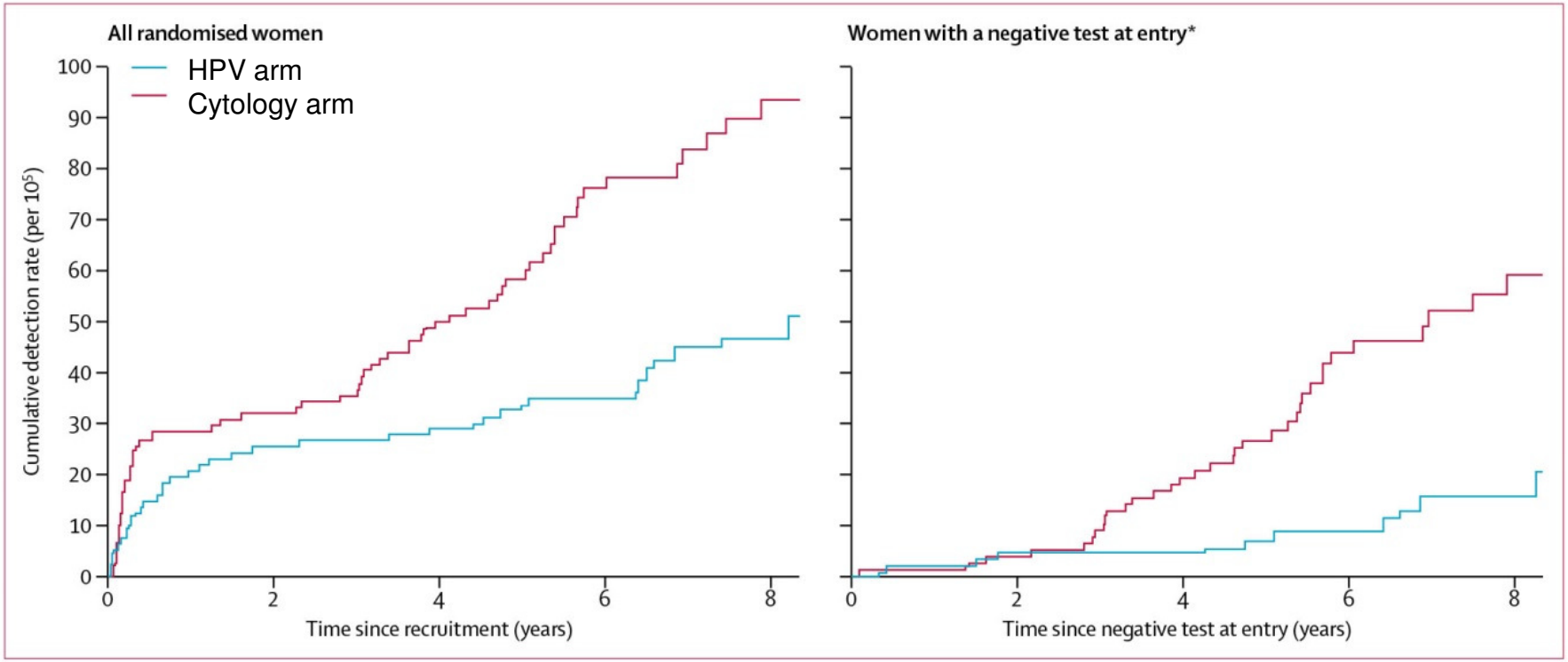


Figure 2: **Cumulative detection of invasive cervical carcinoma**

*Observations are censored 2·5 years after CIN2 or CIN3 detection, if any.

Ronco et al., Lancet 2013

A negative HPV test provides better protection against cancer than cytology

Take home messages

- Women who were at enrolment HPV screen neg, have in the second round 50% less CIN3+ and significantly less cancer compared to women who were cytology screen negative at enrolment

➢ HPV testing provides better protection against CIN3+ and CxCa than cytology

Other advantage of HPV testing

- HPV testing can be done on self-collected cervico/vaginal material

Offering self-sampling for HPV testing to non-attendees

1. Can offering self-sampling for HPV testing increase compliance to screening?

2. Is this approach effective in detecting CIN2+?

Offering self-sampling for HPV testing re-attracts non-attendees

Reference	Study design	Method (self vs clinician)	Attendance rate
Gok et al. (2010)	Self-sampling vs recall letter (99:1) 28,073 non-responders	Self-sampling (Delphi Screener) vs cervical smear	**Self: 27.7%** Recall letter: 16.6% **P<0.001**
Gok et al. (2011)	Self-sampling vs recall letter (99:1) 26,409 non-responders	Self-sampling (VibaBrush) vs cervical smear	**Self: 30.8%** Recall letter: 6.5% **P<0.001**
Bais et al. (2007)	Self-sampling vs recall letter (9:1) 2830 non-responders	Self-sampling (VibaBrush) vs cervical smear	**Self: 34.2%** Recall letter: 17.6% **P<0.001**
Sanner et al. (2009)	Self-sampling (no control group) 2829 non-responders	Self-sampling (Qvintip) on demand	**Self: 39.1%**
Virtanen et al. (2011)	Self-sampling vs recall letter (1:2.7) 4160 non-responders	Self-sampling (Delphi Screener) vs cervical smear	**Self: 29.8%** Recall letter: 26.2% **P = 0.02**
Virtanen et al. (2011)	Self-sampling vs recall letter (1:2.7) 8699 non-responders	Self-sampling (Delphi Screener) vs cervical smear	**Self: 31.5%** Recall letter: 25.9% **P<0.001**
Szarewski et al. (2011)	Self-sampling vs recall letter (1:1) 3000 non-responders	Self-sampling (cotton swab, Qiagen) vs cervical smear	**Self: 10.2%** Recall letter: 4.5% **P<0.001**
Giorgi Rossi et al. (2011)	Self-sampling vs recall letter. 2480 non-responders	Self-sampling (Delphi Screener) vs cervical smear	**Self: 19.6%** Recall letter: 13.7% **P=0.007**
Wikström et al. (2011)	Self-sampling (n=2000) vs recall letter (n=2060)	Self-sampling (Qvintip) vs cervical smear	**Self: 39.0%** Recall letter: 9.0% **P<0.001**

Snijders et al Int J Cancer 2012

Two different self-sampling devices
(used for hrHPV testing)

PROHTECT 1
N=~ 28,703 (age: 29-60 years)
Year of non-attendance: 2005

Delphi screener (cervico-vaginal lavage)

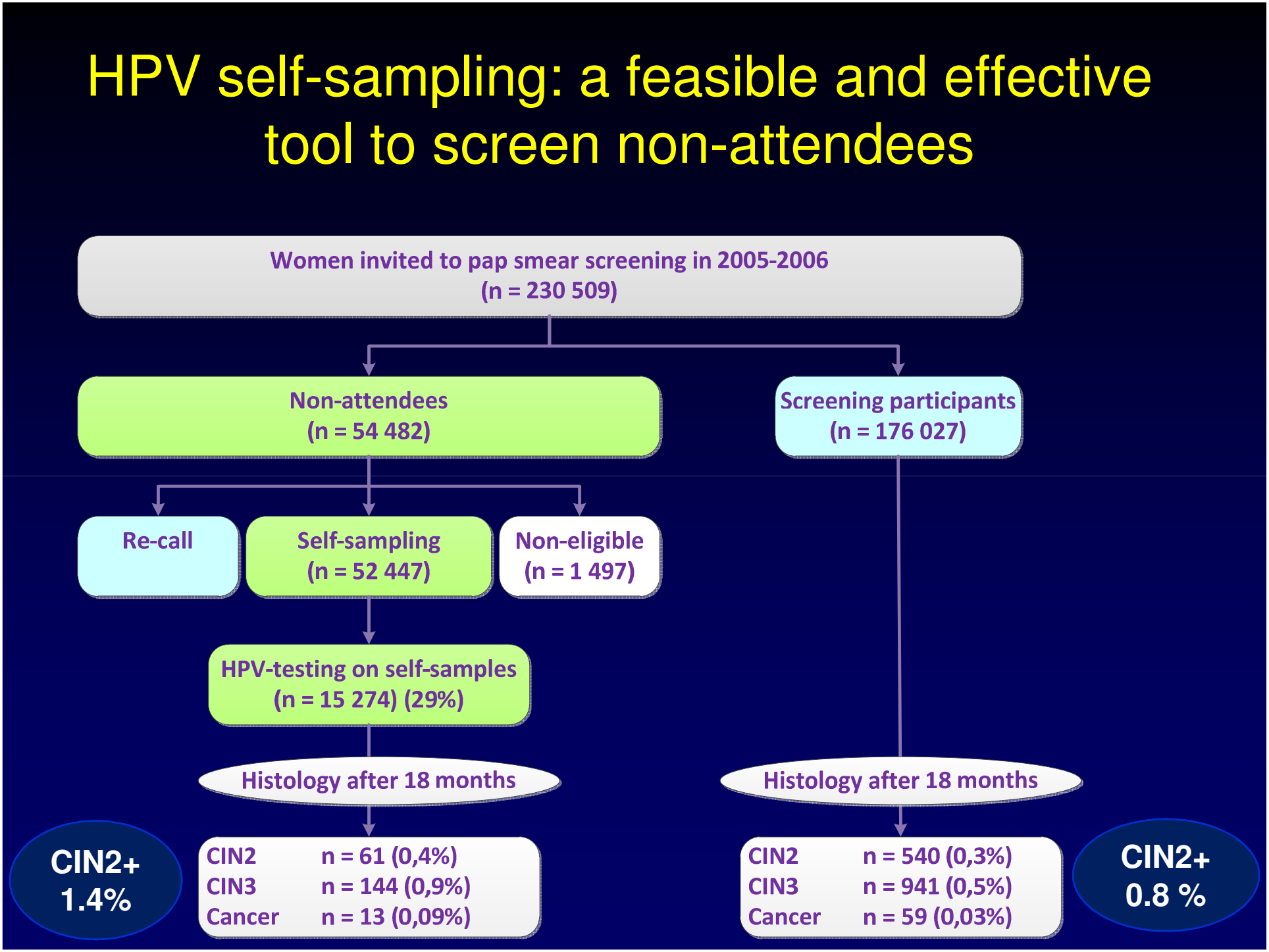
Gök et al., BMJ 2010

PROHTECT 2
N=~ 26,409 (age: 29-60 years)
Year of non-attendance: 2006

Viba brush (vaginal brush)

Gök et al., IntJCancer 2011

HPV self-sampling: a feasible and effective tool to screen non-attendees



HPV testing in cervical screening

- HPV testing on self-collected c/v specimen is more sensitive than cytology in detecting CIN2+

- HPV testing on self-collected c/v specimen is as sensitive as HPV testing on physician taken smears, provided a clinically validated combination of a self-sampling device and a hrHPV test is used

Snijders Int J Cancer 2013;Arbyn Lancet oncology 2014

HPV testing in cervical screening

- HPV vs cytology
- Clinical validation of HPV tests
- Triage of HPV pos women

HPV tests vary in their property to detect the various types of HPV infections

Important distinctions:

- Analytical sensitivity and specificity
  - ➢ Detect all hrHPV infections: both transient (irrelevant) and transforming infections
- Clinical sensitivity and specificity
  - ➢ Detect mainly HPV infections associated with CIN2+/3+ (clinically relevant hrHPV infections):

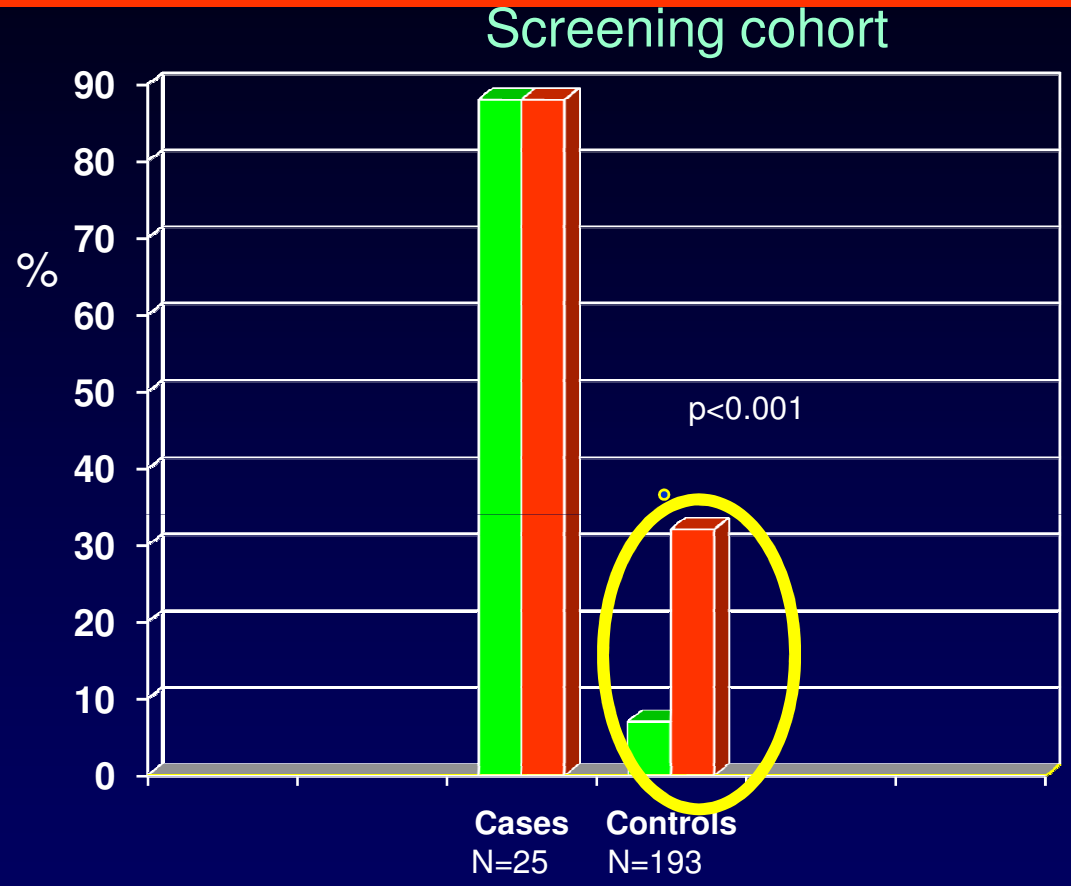
For HPV testing in cervical screening clinical validation is necessary

For screening purposes it is imperative to detect transforming HPV infections associated with (pre)cancer i.e CIN2,CIN3,CxCa and ignore the transient HPV infections

Otherwise too many women without lesions enter into diagnostic evaluation. Increase COSTS!

- Clinical validation of HPV tests obligatory!
- International guidelines have been formulated

Example: Case-control study: women with CIN3 vs women with normal cytology (≥30 years) and no CIN2+ in next 2 years



Cases: CIN3

Controls: ≤CIN1

- **Clinically Validated test: GP5+/6+PCR**
- **clinically non-validated test: SPF10**

Clinically validated: HC2 and GP5+/6+

- In women with normal cytology false positivity rate of a clinically non-validated test was significantly higher than that of a clinically validated test; true positive CIN3+ rate is similar
- Result: Unnecessary F-up, expensive, harmful, and overtreatment of women

Hesselink et al., 2008

Clinical validation of other HPV assays

- In order to become validated for use in cervical screening candidate HPV assays should prove:
  - their value in large prospective screening studies

    or
  - non-inferiority to validated reference assays (HC2 or GP5+/6+-PCR) in cross-sectional clinical equivalence studies

- Consensus guidelines for test requirements have been developed by an international consortium
- *(Meijer et al. : Int J Cancer, 2009)*

Clinically validated HPV assays for cervical screening

Avaliable HPV detection assays

Many (>40)

- Hybrid Capture 2
- Diassay (GP5+/6+-PCR)
- COBAS4800
- APTIMA
- HPV RealTime
- SPF10
- Amplicor
- Cervista
- PapilloCheck
- PGMY
- … (and so on)

→

HPV tests validated for cervical screening (cervical scrapings)

- Hybrid Capture 2*
- Diassay (GP5+/6+-PCR)*
- *COBAS4800***
- *HPV RealTime***
- *PapilloCheck***
- *APTIMA**#*
- *HPV-Risk assay***

→

HPV tests validated for cervical vaginal lavages (Delphi-screener)

- Diassay (GP5+/6+-PCR)
- HPV-Risk assay

*Based on longitudinal studies

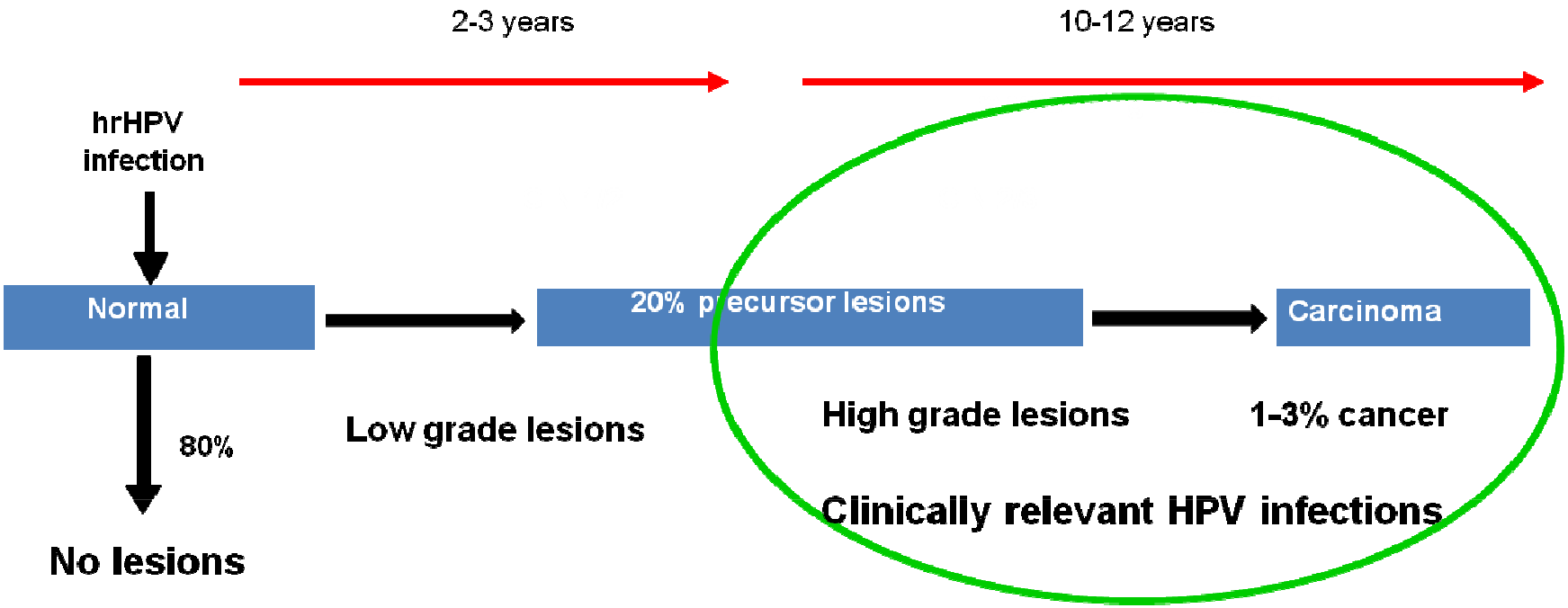
**Based on equivalence analysis according to guidelines

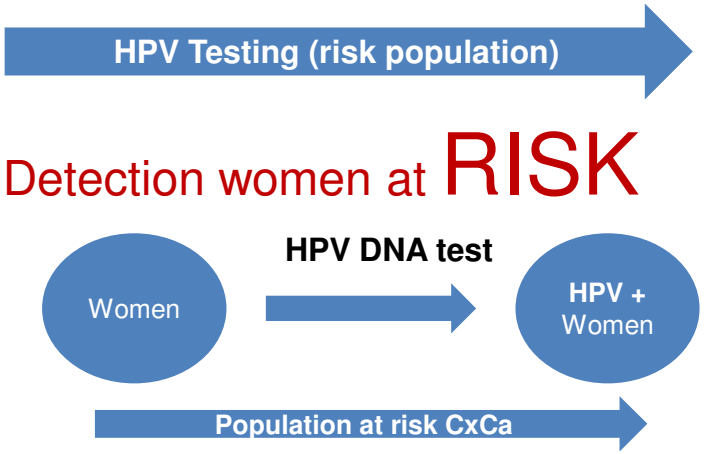
Provided that data of long term NPV of mRNA testing become available

HPV testing in cervical screening

- HPV vs cytology
- Clinical validation of HPV tests
- Triage of HPV pos women

HPV testing recognizes viral infection, but we need to detect disease

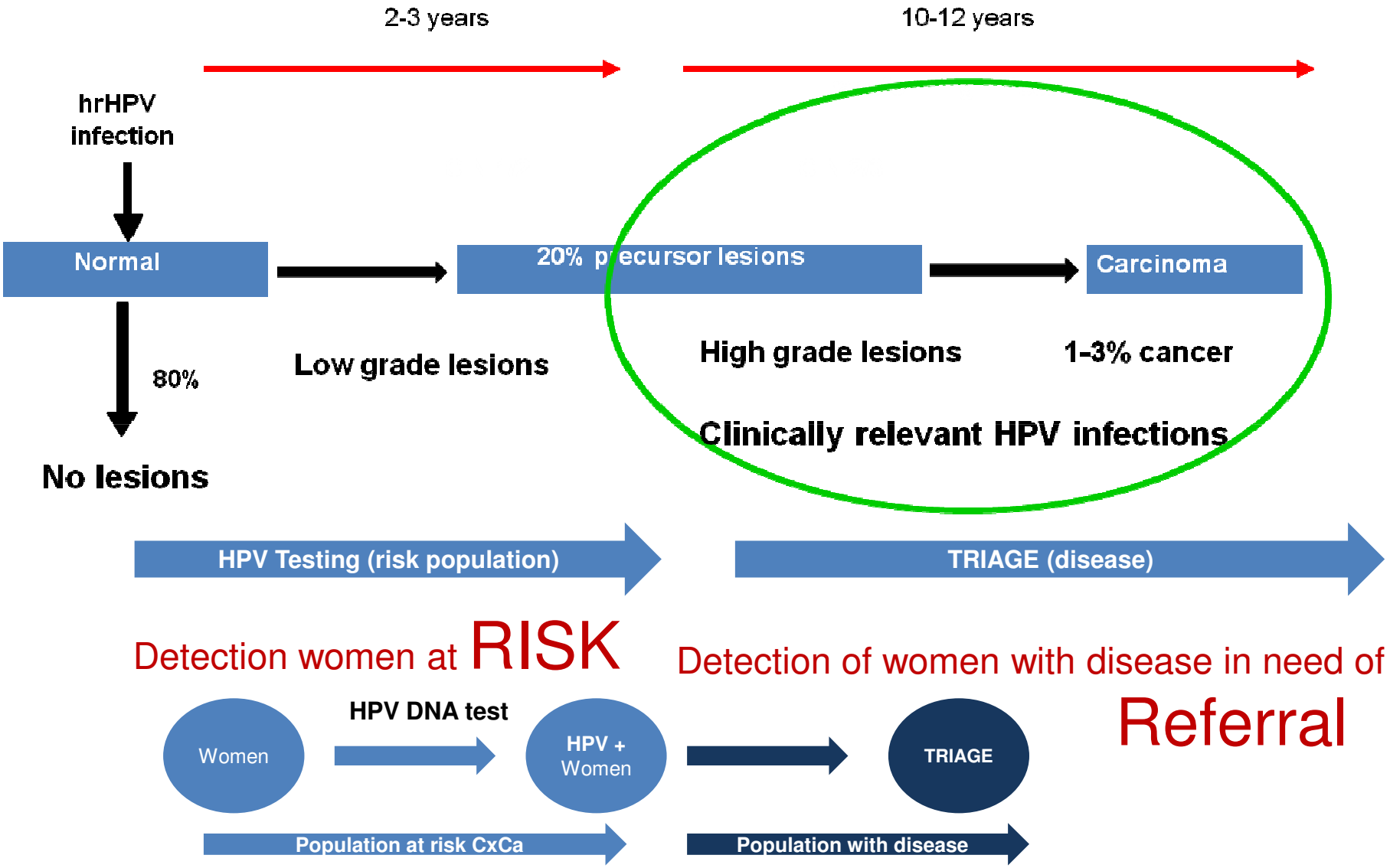




Even a clinically validated HPV test detects still both transient and clinically relevant infections

We are only interested in HPV infections associated with disease: high grade lesions and cancer

HPV testing recognizes viral infection, but we need to detect disease: triage testing necessary



Evaluation of triage tests in longitudinal studies (VUSA-Screen and POBASCAM)

- Cytology
- HPV 16/18 genotyping
- Combinations of these tests

➢ Aim to increase specificity without loosing sensitivity

Rijkaart et al Int.J Cancer 2011; Dijkstra et al CEBP 2013
Katki et al Lancet oncology 2013

Adopted triage strategies for HPV pos. women

- Presently two triage strategies have been adopted, because they are easy to implement and fullfill CIN3+ risk requirements (NPV>98%)

  A) Baseline cytology and cytology in follow-up (6 or 12 months)

  B) Baseline cytology & HPV16/18 genotyping and cytology in follow-up (6 or 12 months)

Take home message

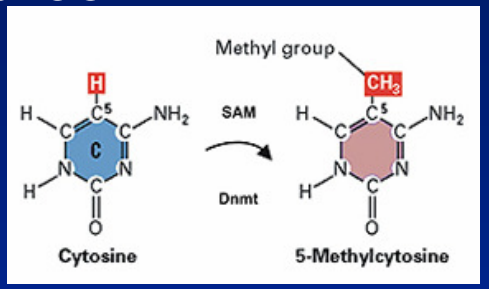
- The exact algorithm to be used for triage depends on the quality of cytology and the minimum positive predictive value for CIN3+ referral acceptable by local health decision makers (resources available)

alternative triage tests

- $p16^{INK4A}$/Ki67 dual staining
- Analysis Chromosomal alterations (eg 3q gain)
- Methylation analysis viral DNA
- Methylation analysis host cell genes

Methylation and Cancer

- Promoter methylation common event in cancer development to silence genes



- Promoter methylation of three tumor suppressor genes is functionally involved in cervical carcinogenesis
  - -CADM1
  - -MAL
  - -miR-124-2

Steenbergen et al. 2004; Overmeer et al. 2008, 2009, 2010; Wilting 2010, et al.

- Methylation levels of these genes increase with disease progression and are extremely high in CxCa

Bierkens et al. IJC 2013

Methylation assay detects cervical cancer and advanced CIN2/3 lesions

- Methylation levels increase with the severity of the lesion and duration of HPV infection

Bierkens et al. IJC 2013

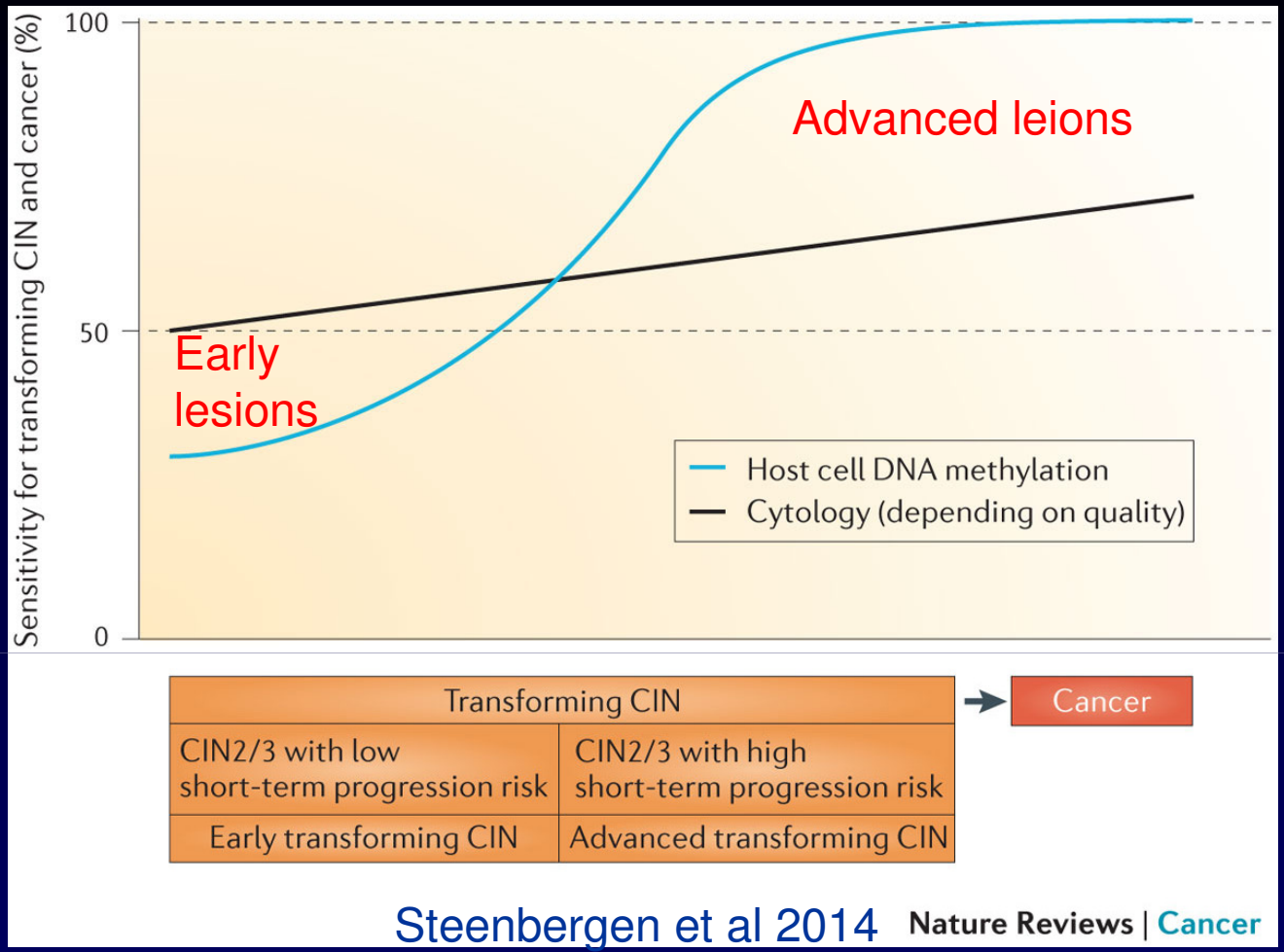
- Methylation levels are extremely high in cervical cancer: no cancers missed

De Strooper JCP 2014

- Cin2/3 lesions detected by methylation are complementary to Lesions detected by cytology or HPV 16/18 genotyping

Verhoef Gyn.Oncology2014

Methylation markers: CADM1/Mal and MAL/miR



Methylation levels increase with the severity of the lesion and duration of HPV infection

Methylation levels are extremely high in cervical cancer: no cancers missed

[1]Soutter, Int J Cancer 2006; [2]Kocken, Lancet Oncol 2011; [3]Overmeer, J Pathol 2008; [4]Overmeer, J Pathol 2009; [5]Bierkens, Int J Cancer 2013; [6] Steenbergen, NRC 2014..

Methylation marker of TSGs involved in cervical carcinogenesis

Concept supported by data:

Cytology:

Detects prevalent lesions (early and advanced) with reduced sensitivity for CIN3 and CxCa (sensitivity~65% at best)

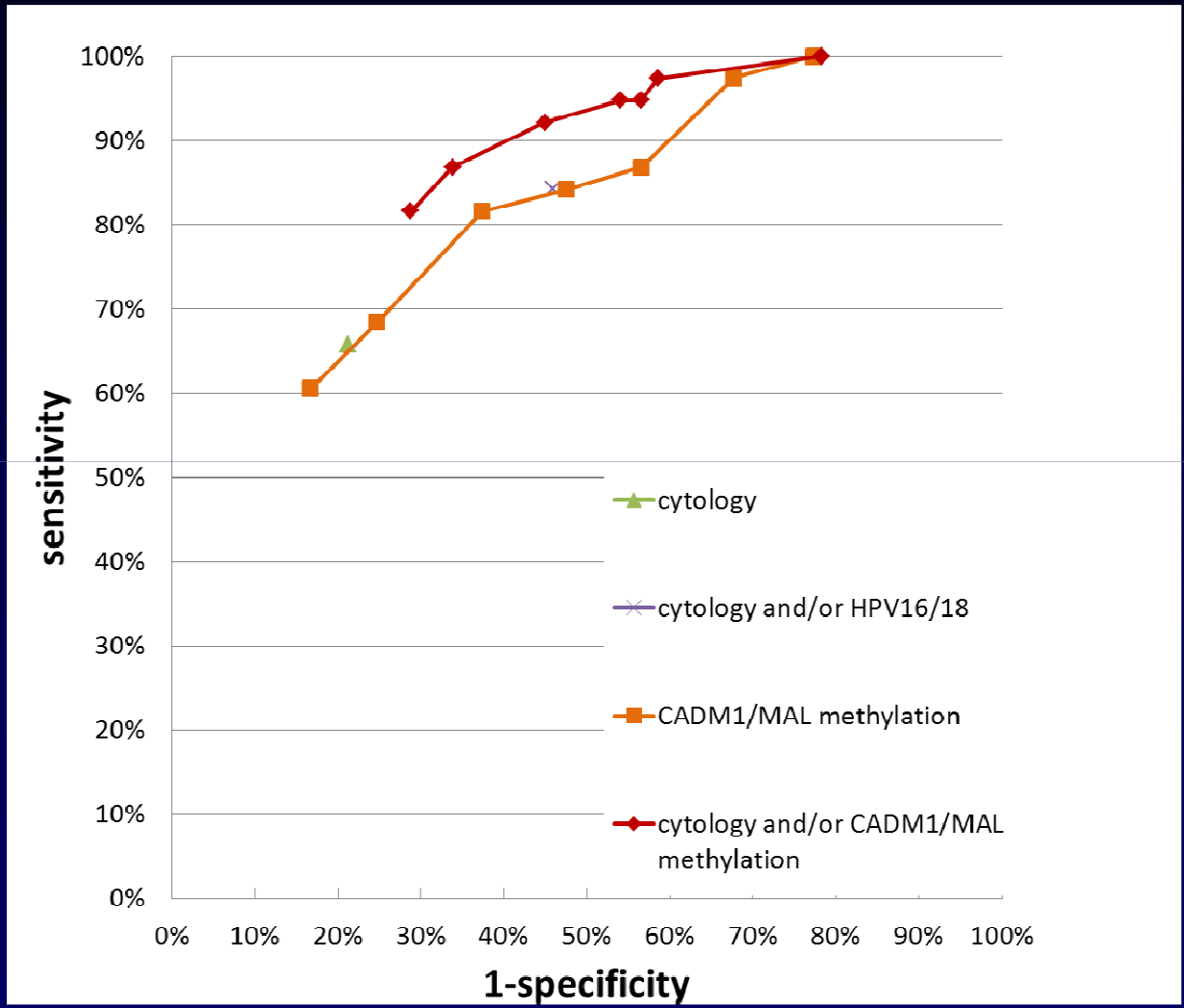
Methylation marker panel:

Detects advanced CIN lesions with high sensitivity: carcinoma proof (n=144)

➢ *Methylation marker analysis (cut-off 70% specificity for CN3) and cytology are complementary in detection CIN3+ in hrHPV pos women : high sensitivity (~90%), low referral rate (~50%)*

Bierkens et al Int.J.Cancer 2013; Hesselink et al : submitted

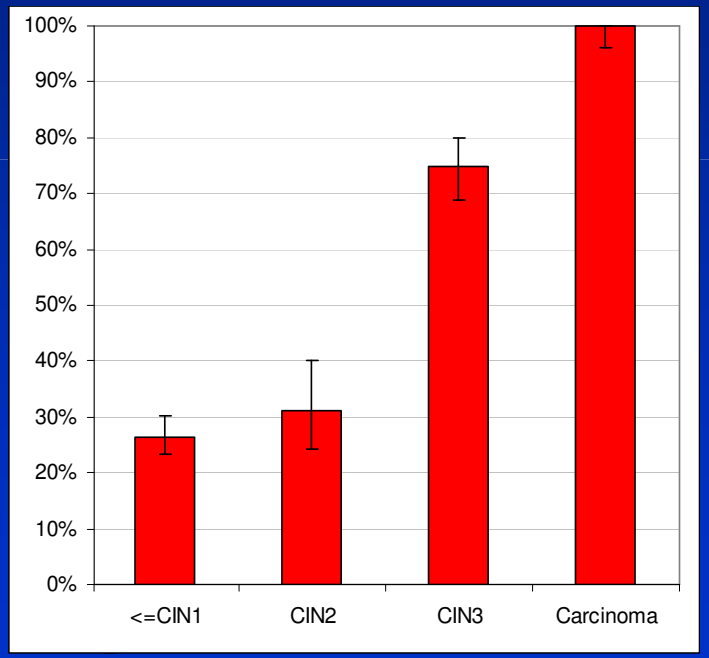
CADM1/MAL methylation analysis and cytology combined (CIN3+ outcome, physician taken scrapes)



PreCursor-M kit

PreCursor-M kit (CE/IVD certified):
quantitative multiplex methylation-specific PCR for CADM1, MAL, and miR-124-2 *Snellenberg et al., 2012*

Diagnosis	N=	methylation positivity in cervical scrapes
<=CIN1	209	26%
CIN2	32	31%
CIN3	60	75%
Carcinoma	67	100%



In cervical scrapes the PreCursor-M kit detects all carcinomas

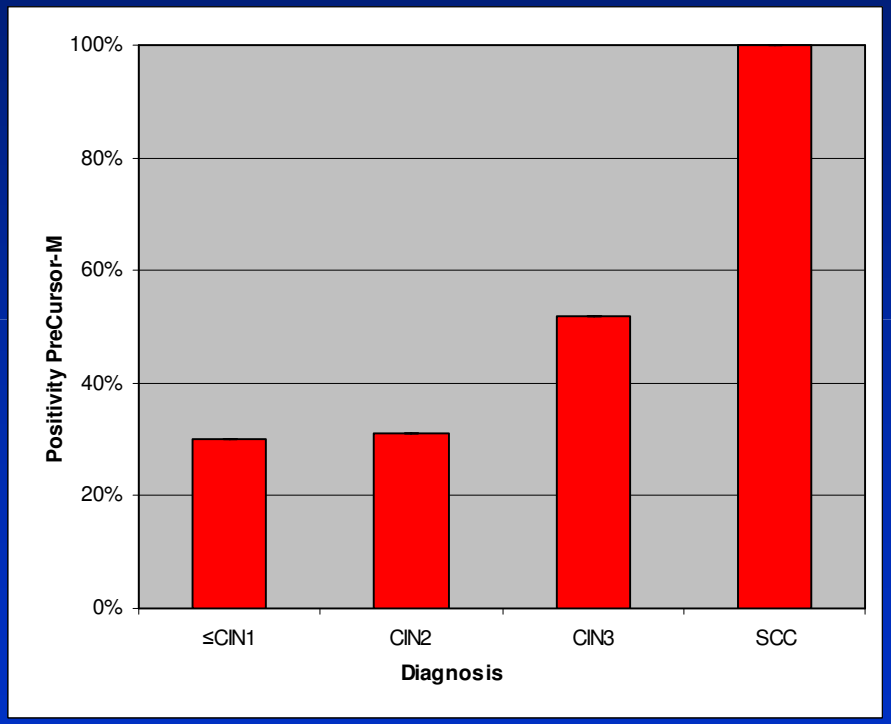
Summary on physician taken smears

• CADM1 and MAL (miR124-2) methylation analysis is an alternative or complementary triage tool to cytology for HPV positive women

•Sensitivity particularly high for advanced CIN2/3 and cervical cancer in need of treatment

Performance PreCursor-M kit in lavage self-samples

Positivity PreCursor-M kit



Also in lavage self-samples the PreCursor-M kit detects all carcinomas

Take home messages

- Direct triage of HPV-positive women by PreCursor-M test makes objective and full molecular cervical screening possible

Present International situation cervical screening by HPV

Primary HPV Screening will be implemented in

The Netherlands: Jan 2016

- Women 30-60 years, 30,35,40, 50,60y. Triage with cytology at baseline and 6 months.

-If HPV screen pos and triage test neg at 40,50, or 60y: repeat testing after 5 years

-Non-responder women are offered opt-in for HPV self-sampling

www.gr.nl;

Australia: advice medical services advisory committee 4/04/2014:

-Start primary HPV screening

-Women: 25-69 years, 5 years interval, Triage by cytology and HPV 16/18 genotyping at baseline and cytology at 12 month

www.msac.gov.au

Italy: 5 regions start HPV screening in 2015

women 25-65 y, 5 years interval, Triage by cytology and HPV 16/18 genotyping

Nordic countries: are considering or doing implementation pilot studies

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