Relevance and importance of HPV mRNA E6/E7 biomarkers in the prevention of cervical cancer

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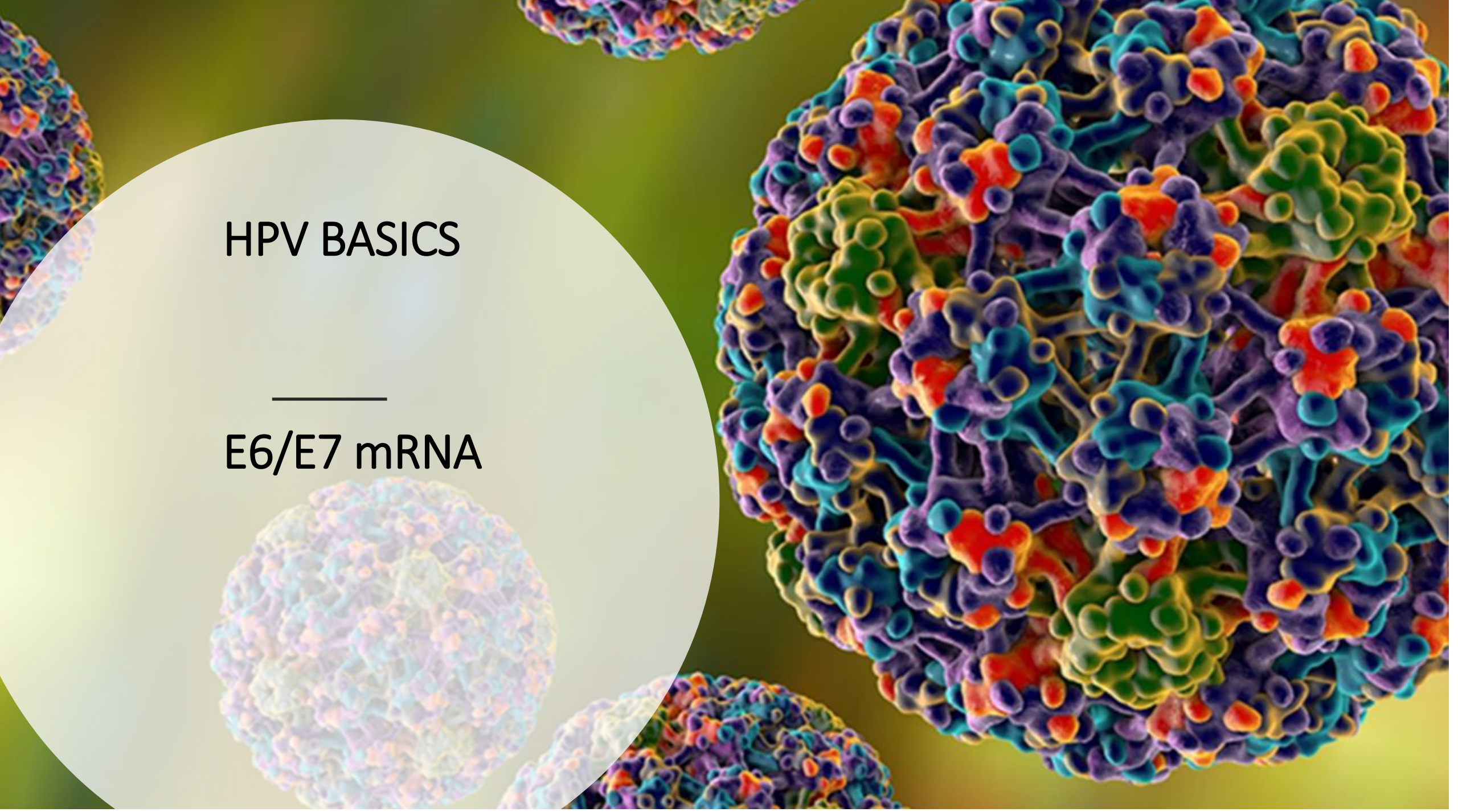
Agenda

- HPV Basics
- E6/E7 oncoproteins: the cause of CC
- Screening Goals & Strategies
- Sample Collection: Mia by XytoTest
- HPV genotyping: Why & How many
- HPV mRNA E6/E7 biomarkers in triage
- Clinical Trials: PreTect HPV-Proofer`7


HPV BASICS
E6/E7 mRNA

HPV Human papilloma virus

- Common STI (>200 genotypes)
- 80% get infected during lifetime
- 90% of the infections are harmless
- Cause 99% of all Cervical Cancers
  - Other HPV-related cancers (Anal, H&N)
- 14 types defined as high-risk (WHO)
  - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- 7 types most important in CC
  - 16, 18, 31, 33, 45, 52, 58

HPV
The cause of cervical cancer

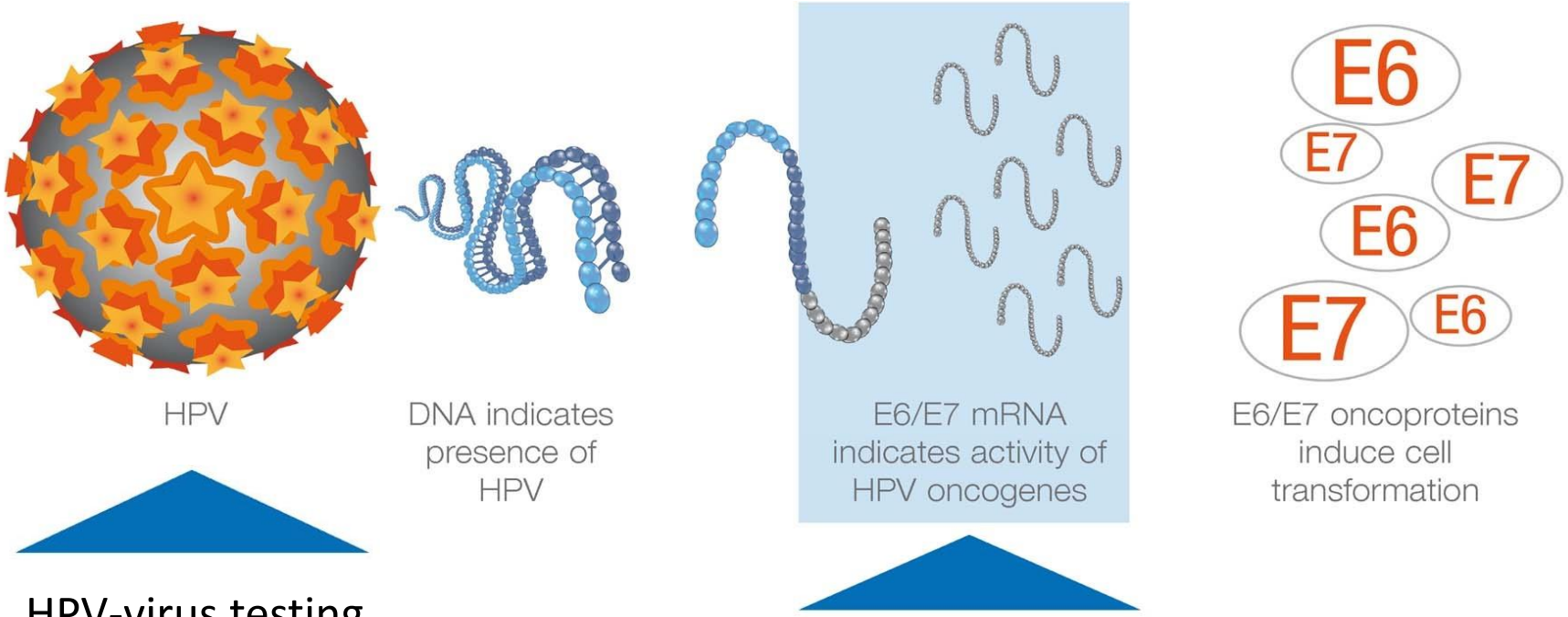
- Harald zur Hausen discovered in 1983 the link between human papilloma viruses and cervical cancer
- The real cause of cervical cancer is not the HPV infection per se
- Over-expression of E6 and E7 oncoproteins is a critical and required step for conversion to malignancy

HPV mRNA E6/E7 biomarkers

- E6/E7 mRNA are **precursors to E6/E7 oncoproteins**; directly relevant to disease progression by:
  - disturbance of cell cycle control
  - deficiency in DNA repair
  - genomic instability
  - increased risk of malignant transformation
- Detecting mRNA E6/E7 offers new opportunities
  - improve the effectiveness of screening
  - detect oncogene activity and not viral presence

The Cause of Cervical Cancer

Different Prevention Concepts

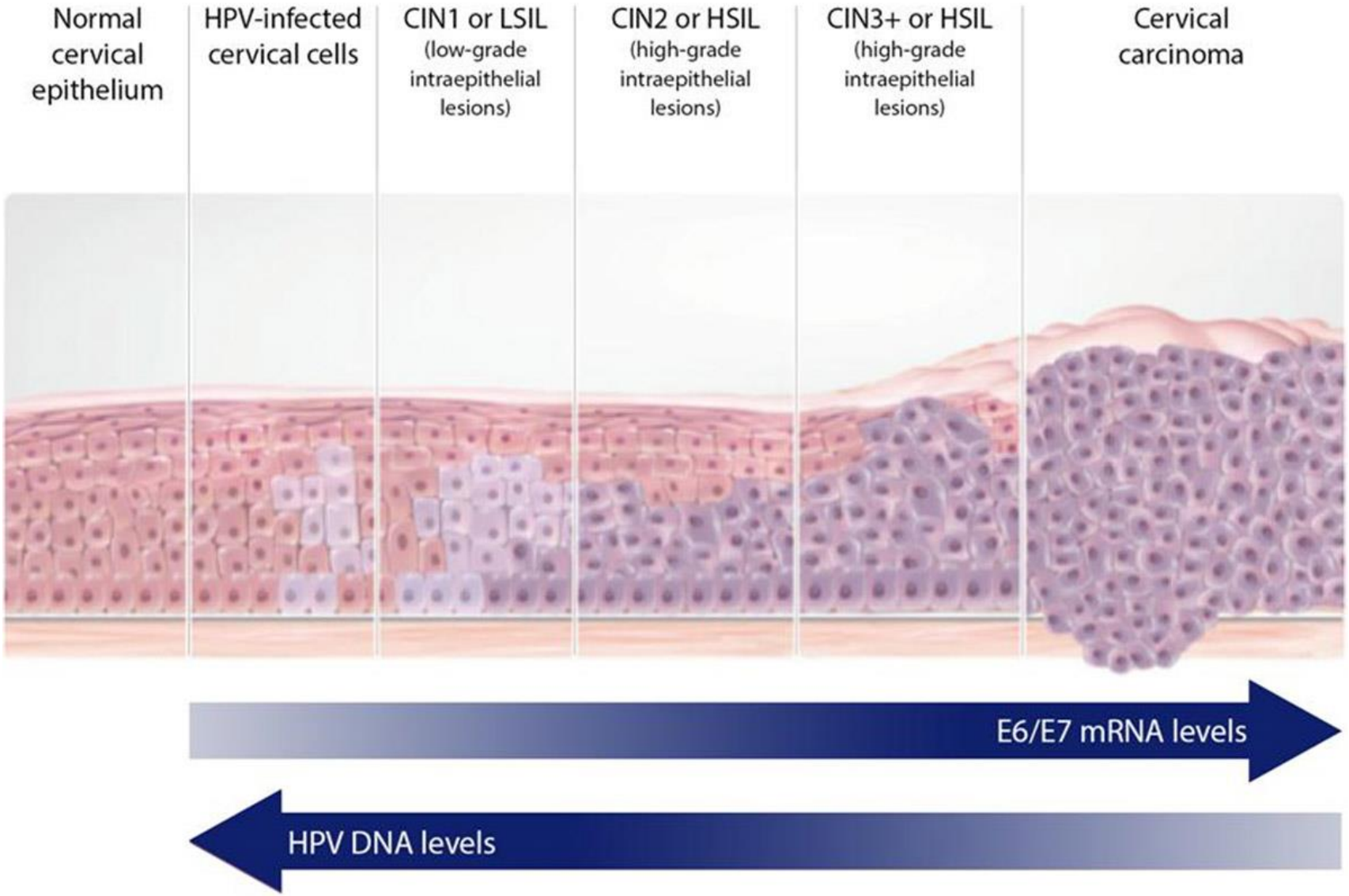


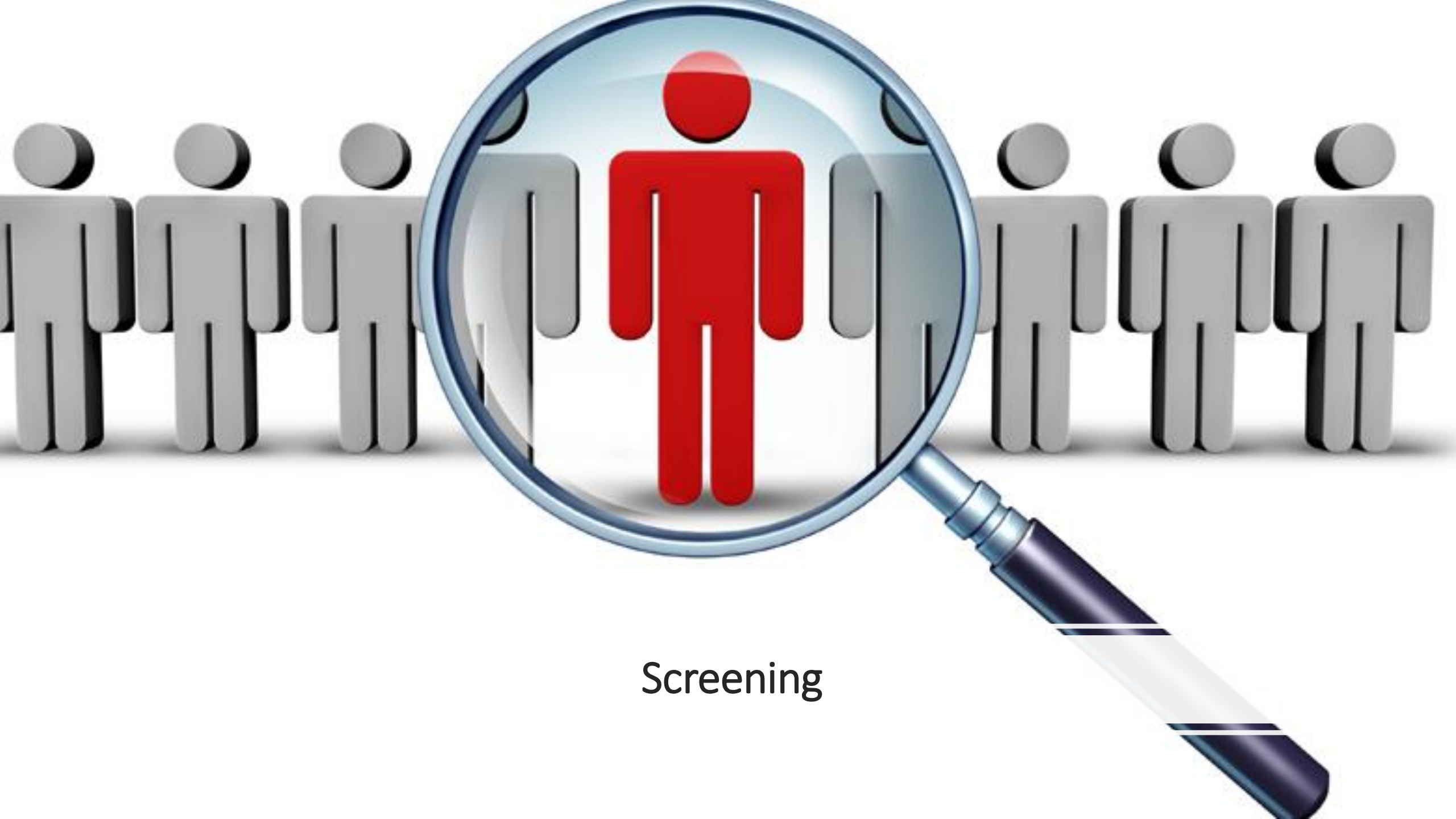
HPV-virus testing identifies a harmless condition

E6/E7 mRNA identifies a high risk condition

HPV Development of cervical cancer

- A transient HPV-infection is not dangerous, carrying a low risk of disease
- A type-specific persistent infection over 10-15 years increases risk of high grade precancer and cancer (CIN2+)
- Risk is strongly associated with certain aggressive HPV genotypes that require closer patient management


Normal cervical epithelium
HPV-infected cervical cells
CIN1 or LSIL (low-grade intraepithelial lesions)
CIN2 or HSIL (high-grade intraepithelial lesions)
CIN3+ or HSIL (high-grade intraepithelial lesions)
Cervical carcinoma
E6/E7 mRNA levels
HPV DNA levels



Screening

Screening Goals

- Prevent cervical cancer by regular screening of asymptomatic women
- Ensure high coverage and high quality in testing
- Ensure appropriate management of abnormal test result
- Provide treatment for precancerous lesions
- Maximize benefits – Reduce harms

Trade-off between Sensitivity and Specificity

Selecting the optimal balance of sensitivity and specificity depends on the **purpose** for which the test is going to be used

- A screening test should be highly sensitive in order to **RULE OUT** those without the disease
- A confirmatory test should be highly specific in order to **RULE IN** those with the disease

Cox JT, Castle PE, Behrens CM, et al. Am J Obstet Gynecol 2012;208

"Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study"

- 9 screening strategies compared to cytology/HPV triage, being current standard
- HPV testing was more sensitive than cytology for detection of CIN2+
- Strategies that offered greater sensitivity also required more referral to colposcopy
- Strategies that depended on cytology for triage of HPV-positive women decreased this sensitivity

ATHENA (Addressing THE Need for Advanced HPV Diagnostics) N= 34,254

Strategy		Colposcopies, n	Sensitivity, %	Initial tests performed, n
Strategy 1	Cytology with reflex HPV (ASC-US Triage)	816	56.1	35,546
Strategy 2	Cytology alone	1644	57.7	34,254
Strategy 3	Cotesting with reflex for ASC-US	816	56.1	68,508
Strategy 4	Cotesting with genotyping and cytology triage: HPV 16/18 and ASCUS HPV+ threshold	1202	76.2	68,508
Strategy 5	Cotesting with genotyping and cytology triage: HPV 16/18 and LSIL threshold	1030	70.9	68,508
Strategy 6	HPV alone	2341	89.9	34,254
Strategy 7	HPV with cytology triage	596	51.9	37,126
Strategy 8	HPV with genotyping triage	580	53.4	34,254
Strategy 9	HPV with genotyping and reflex cytology: ASC-US threshold	982	72.0	36,423
Strategy 10	HPV with genotyping and cytology (LSIL cut-off) triage	810	66.7	36,423

Sensitivity for CIN3 or more severe and number of colposcopies for each screening strategy.
Cox et.al. Cervical cancer screening strategies: evaluation of results from the ATHENA HPV study. Am J Obstet Gynecol 2012.

HPV-based screening- A globally recommended public health policy

- 99.7% of all cases of cervical cancer are caused by HPV
- A paradigm shift from cytology to a more sensitive 14-type HPV DNA test in primary screening improves prevention
- Prolongs test interval for screen negatives compared to cytology (3 -> 5 yrs.)
  - The better NPV of HPV testing permits a safe extension of the screening interval, thereby reducing harms caused by screening
- Molecular testing is objective, reproduceable and allows use of self-collected samples, improving access to screening

Primary HPV-DNA challenges

- Generates a lot of screen positives (10-30%)
- Not to be used in young women < 30 years
- Most women with a positive HPV-DNA test do not have clinically significant disease (false positives)
- HPV DNA assays with 14 genotypes have a lower specificity compared with microscopic inspection of Pap smears
- Substantial increased number of biopsies without finding more severe abnormalities
- Effective Triage & Risk stratification is crucial to avoid unnecessary follow-up

Triage
A risk-based approach

- 90% of HPV infections are harmless
- To more accurately identify the women who are warranted for colposcopy by discriminating among the HPV infections
- To reduce unnecessary interventions and risk of overtreatment
- Requires a highly specific test, detecting as few false positives as possible
- “A biomarker of integration and transformation would be the ideal triage” (Zappacosta et al, Gynecol Oncol, 2012)

Triage by Cytology?

- PAP technology from 1920
- Relies on subjective skills
- Poor reproducibility
- Low sensitivity (50-60%)
- Low specificity
- Knowledge of HPV-status affects interpretation
- Difficulties detecting adenocarcinomas
- Not compatible with self-collected samples

Zappacosta et al, Gynecol Oncol (2012)

"Implementing specificity of HPV-DNA primary screening in a successful organised cervical cancer prevention programme"

Triage of DNA+ women by liquid-based cytology:

- Only a small percentage of cervical abnormalities would progress to invasive cancer
- Most cervical lesions would undergo unnecessary colposcopy or harmful treatments
- The specificity of cytological triage is still too low
- Triaging HPV-DNA positive women with Pap cytology would cause a substantially high referral rate to colposcopy, without increasing PPV

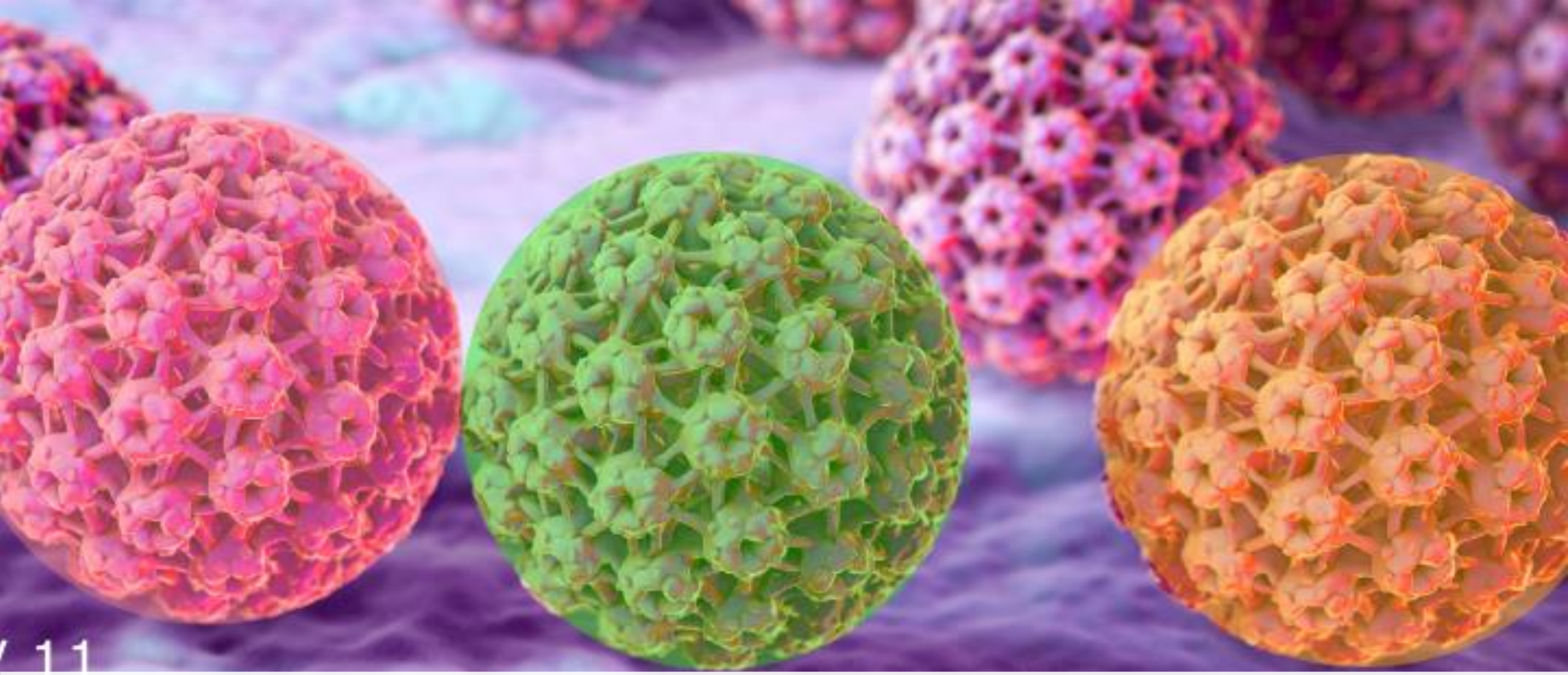
How to maximize benefits & reduce harms

- Regular screens
- Easy access to sample collection
- High quality tests in timely manner
- High sensitivity to improve prevention
- Accurate management of test positives by highly specific tests
- Reduce unnecessary interventions and overtreatment

Sample collection matters

- Sample-taking without speculum*
  - Reduce barriers/anxiety
  - Non-invasive procedure
- High cellularity for molecular testing
- Quality (no blood/ lubricants interfering)

*Aranda Flores CE, Gomez Gutierrez G, Ortiz Leon JM, Cruz Rodriguez D, Sørbye SW. Self-collected versus clinician-collected cervical samples for the detection of HPV infections by 14-type DNA and 7-type mRNA tests. BMC Infect Dis. 2021 May 31;21(1):504.



Why genotype?

Why knowledge of genotype is important

- Risk of cervical cancer strongly varies by genotypes
- Persistence tracking is important for follow-up of patients
- Implications for clinical management
  - Better selection of women who need immediate colposcopy/biopsy
  - Reduction of unnecessary colposcopy

How many HPV-types to screen for?

- Only a few HPV genotypes are highly associated with cervical cancer and require the most aggressive management, whereas others carry a lower risk of disease
- HPV 16 and 18 cause 70% of all cases of cervical cancer
- 7 HPV-types (16, 18, 31, 33, 45, 52 and 58) cause 90% of all cases of cervical cancer
- The same 7 genotypes are covered by the 9-valent HPV vaccine; documented to enable the highest level of protection possible

"The 9vHPV vaccine could potentially provide broader coverage and prevent 90% of cervical cancer cases worldwide"

HPV vaccines

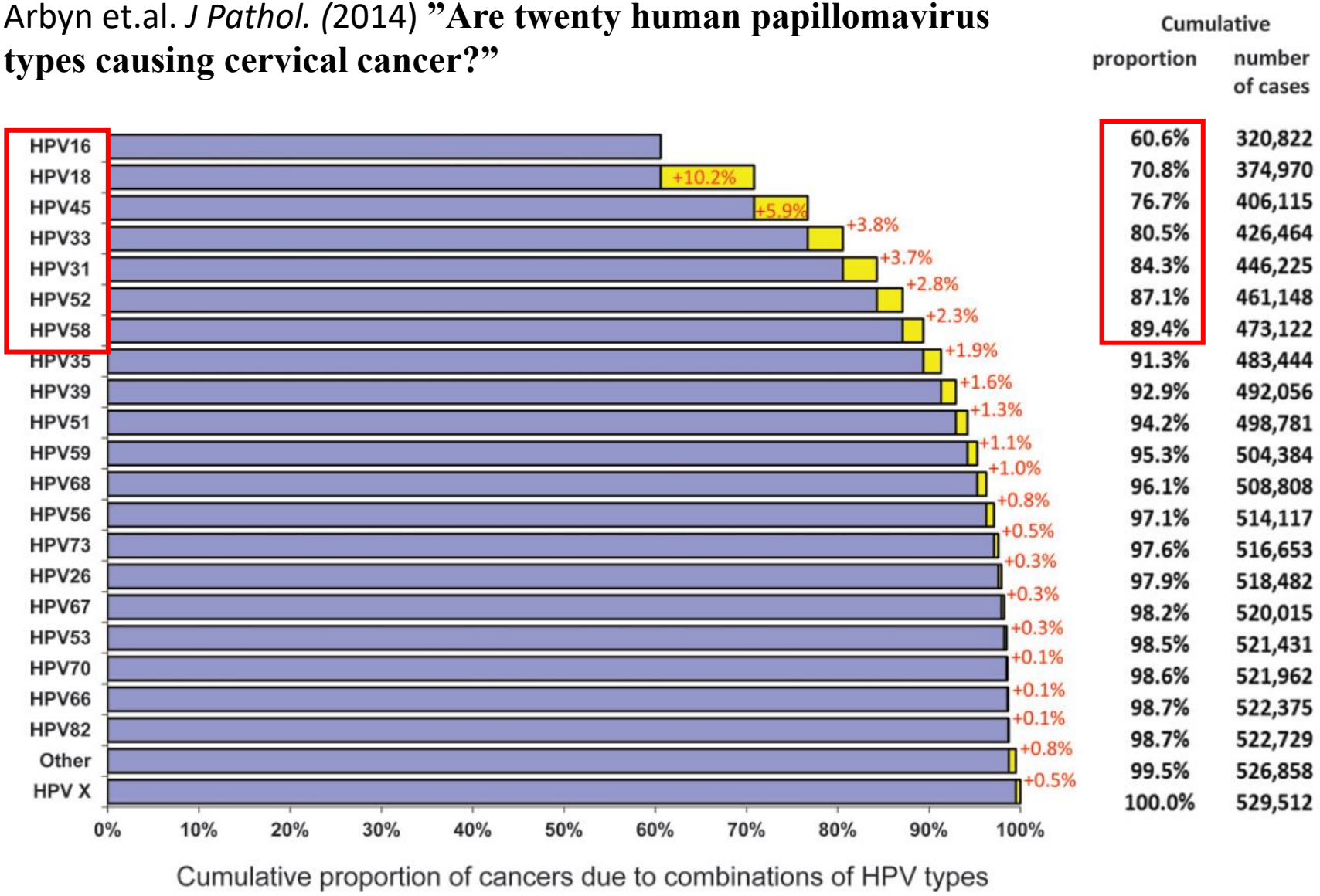
Bivalent	Cervarix ®	16 18
Quadrivalent	Qardasil ®	16 18 6 11
9-valent	Qardasil ® 9	16 18 6 11 31 33 45 52 58

Numerous studies show a large reduction on genital warts and vaccine-related HPVs in females[1-4]

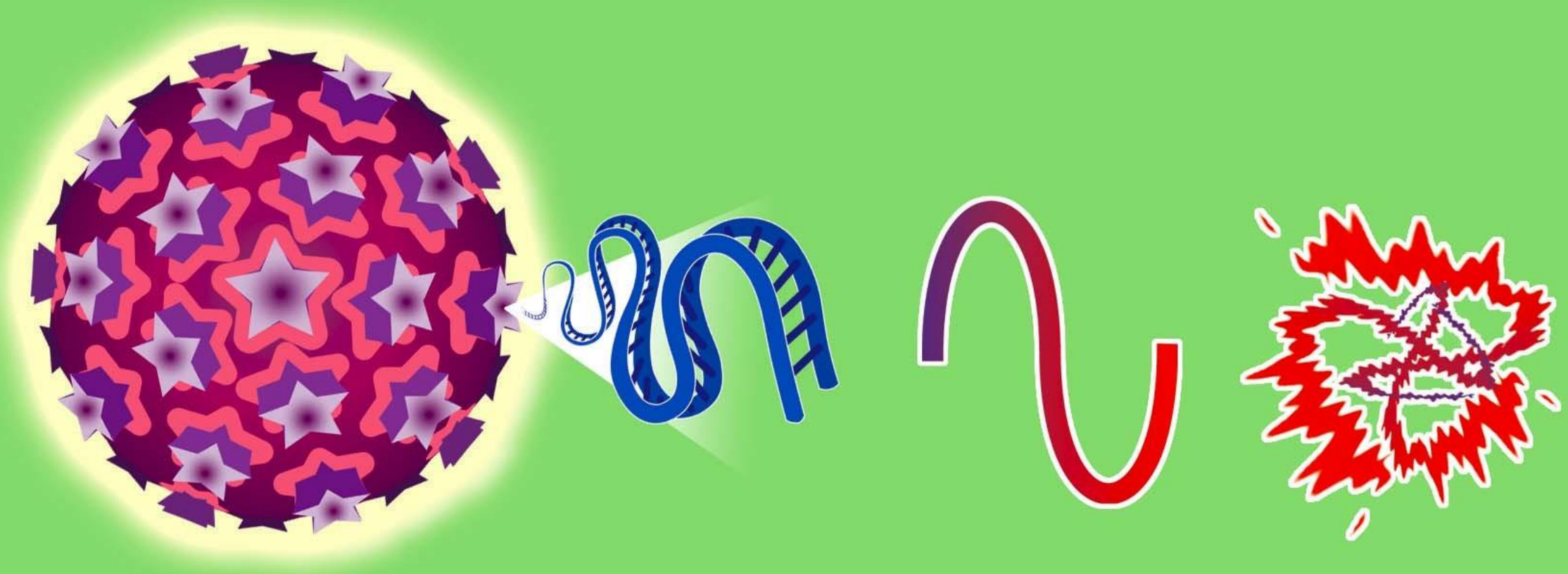
[1] *Chow EP et al. Lancet Inf Dis. 2015; 15: 1314-1323*
[2] *Chow EP et al. Sex Trans Inf. 2015; 91: 214-219*
[3] *Ali H et al. BMJ. 2013: 346: f2032.*
[4] *Tabrizi SN et al. Lancet Inf Dis. 2014; 14: 958-66*

*CERVARIX es propiedad de Glaxosmithkline Biologicals, S.A.
**GARDASIL y GARDASIL 9 son propiedad de MSD VACCINS

Arbyn et.al. *J Pathol. (*2014) **"Are twenty human papillomavirus types causing cervical cancer?"**



Cumulative proportion	Cumulative number of cases
60.6%	320,822
70.8%	374,970
76.7%	406,115
80.5%	426,464
84.3%	446,225
87.1%	461,148
89.4%	473,122
91.3%	483,444
92.9%	492,056
94.2%	498,781
95.3%	504,384
96.1%	508,808
97.1%	514,117
97.6%	516,653
97.9%	518,482
98.2%	520,015
98.5%	521,431
98.6%	521,962
98.7%	522,375
98.7%	522,729
99.5%	526,858
100.0%	529,512



HPV mRNA E6/E7 biomarkers

Biomarkers E6/E7 mRNA triage

- Detects HPV mRNA E6/E7; precursors of the oncoproteins known to disturb normal cell cycle control (oncogene activity)
- Genotypes the 7 most prevalent HPV-types causing cervical cancer (HPV 16, 18, 31, 33, 45, 52 and 58)
- Holds a high clinical specificity and positive predictive value (PPV) for CIN2+
- Holds low positivity rate in general population (only 1/3 of HPV-DNA positives)
- Identifies the women at increased risk for future abnormalities; warranted for immediate colposcopy and biopsy

Follow-up of triage positives

- Only about 1/3 of the women carrying an HPV-DNA infection express mRNA from the 7 genotypes and should be referred for immediate colposcopy

Follow-up of triage negative women

- The remaining 2/3 can be followed up with a new HPV DNA test after 12-24 months where only women with a persistent positive HPV DNA test needs colposcopy and biopsy
- In women with a positive HPV DNA test, 50% have a negative test after 12-24 months

Why not use a 14-type HPV mRNA test?

- Aptima HPV test performs similar to a 14-type HPV-DNA test
- No genotyping
- No sample integrity control
- High positivity rate
- Not suitable as triage of DNA-positives

PreTect HPV-Proofer`7

Clinical Trials
University Hospital North Norway

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"7-type E6/E7 mRNA-test in triage of HPV DNA+ women 34-69 years old, attending primary screening in Troms and Finnmark 2019-2021

Unpublished data, UNN, Norway

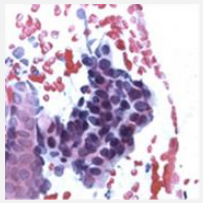
16,729 women enrolled 2019-2021

5.0% HPV DNA+ (836/16,729)

triaged by Cytology and mRNA E6/E7

55.0% (460/836): Cytology+

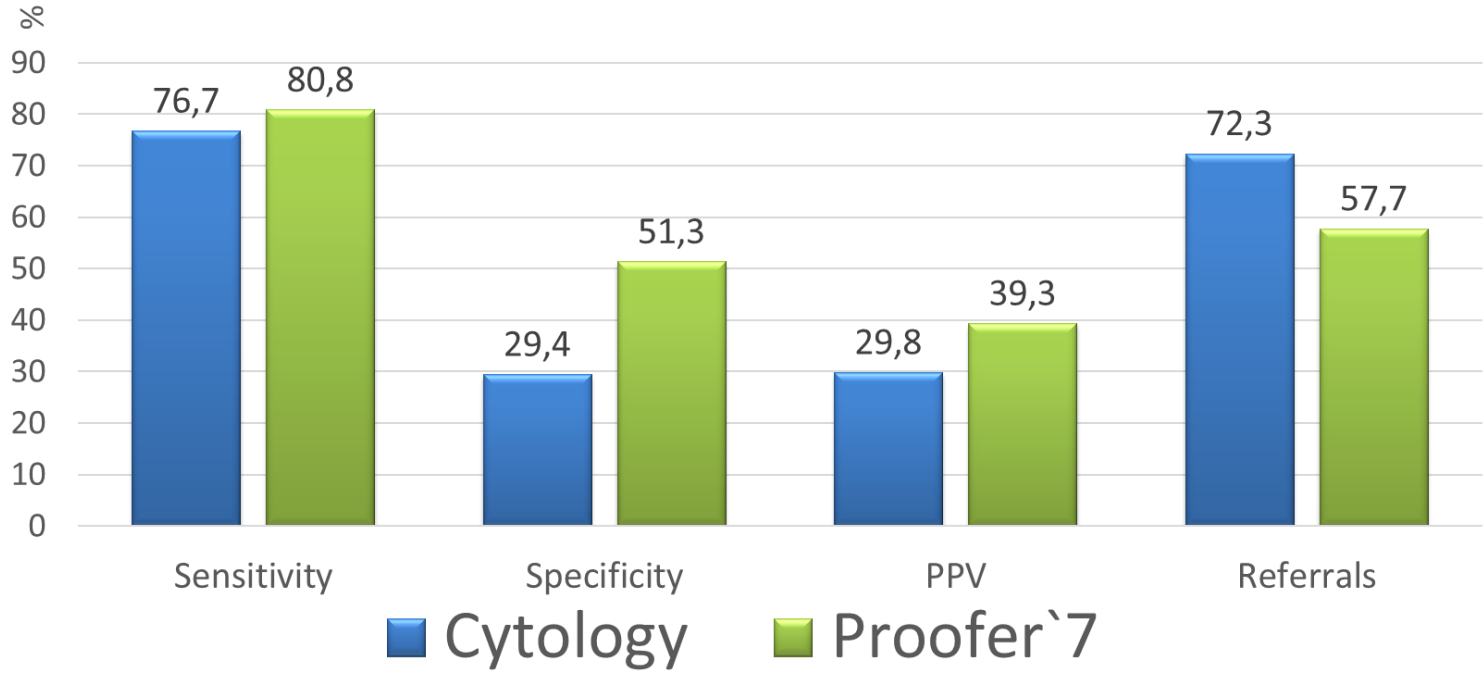
36.5% (305/836): E6/E7 mRNA`7+



31.1% (260/836) biopsy

8.7% (73/836) CIN2+

Cytology versus 7-type HPV mRNA test for the detection of CIN2+

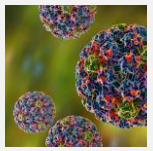


Unpublished data University Hospital North Norway 2019-2021

- The 7-type HPV mRNA test was more specific than cervical cytology in triage of HPV-DNA positive women
- A low positivity rate of the triage test can be translated into a low referral rate to colposcopy which is very appealing in a triage setting
- The use of Mia by XytoTest may increase the coverage of the cervical cancer screening programme, thus reducing the number of cervical cancers

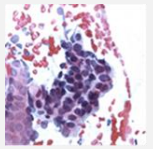
Take home messages

HPV DNA test provides high sensitivity and improved prevention



7 HPV-types are crucial:
HPV 16, 18, 31, 33, 45, 52 and 58 cause 90% of cervical cancer

Triage of HPV DNA positives
Risk stratification is required for accurate patient management- mRNA E6/E7 balance benefits/harms



A low positivity rate for mRNA translates into a low referral rate for colposcopy and reduces over-treatment

References

Aranda Flores CE, Gomez Gutierrez G, Ortiz Leon JM, Cruz Rodriguez D, Sørbye SW. Self-collected versus clinician-collected cervical samples for the detection of HPV infections by 14-type DNA and 7-type mRNA tests. BMC Infect Dis. 2021 May 31;21(1):504.

Arbyn et.al. J Pathol. (2014) ”Are twenty human papillomavirus types causing cervical cancer?”

Baasland et al.(2019) PLoS ONE 14(1): e0210997 “Clinical performance of Anyplex II HPV28 by human papillomavirus type and viral load in a referral population”

Bonde et al. Journal of Lower Genital Tract Disease • Volume 24, Number 1, January 2020 «Clinical Utility of Human Papillomavirus Genotyping in Cervical Cancer Screening: A Systematic Review”

Cox JT, Castle PE, Behrens CM, et al. Am J Obstet Gynecol 2012;208 “Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study

Cox et.al. Cervical cancer screening strategies: evaluation of results from the ATHENA HPV study. Am J Obstet Gynecol 2012

Harald Zur Hausen (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342-350. 10.1038/nrc798 [doi].

Sundstrøm et al. 2020 The Journal of Infectious Diseases® “How Many Human Papillomavirus Types Do We Need to Screen For?»

Sorbye SW, Fismen S, Gutteberg TJ, Mortensen ES, Skjeldestad FE (2014) HPV mRNA is more specific than HPV DNA in triage of women with minor cervical lesions. PLoS One 9: e112934. 10.1371/journal.pone.0112934 [doi];PONE-D-14-28018 [pii].

Sorbye SW, Suhrke P, Reva BW, Berland J, Maurseth RJ, Al-Shibli K (2017) Accuracy of cervical cytology: comparison of diagnoses of 100 Pap smears read by four pathologists at three hospitals in Norway. BMC Clin Pathol 17: 18. 10.1186/s12907-017-0058-8 [doi];58 [pii].

Zappacosta et al, Gynecol Oncol (2012) “Implementing specificity of HPV-DNA primary screening in a successful organised cervical cancer prevention programme“

If there is one thing, I would like you to remember from today's presentation, it's that Prevention is Possible!

MUCHAS GRACIAS!