HPV Infection: Present and Future of Diagnostic and Prognostic Surveys

Ruth Tachezy

Faculty of Science, Department of Genetics and Microbiology, Charles University, Prague

National Reference Laboratory for Papillomaviruses and Polyomaviruses, Public Health Institute, Ostrava

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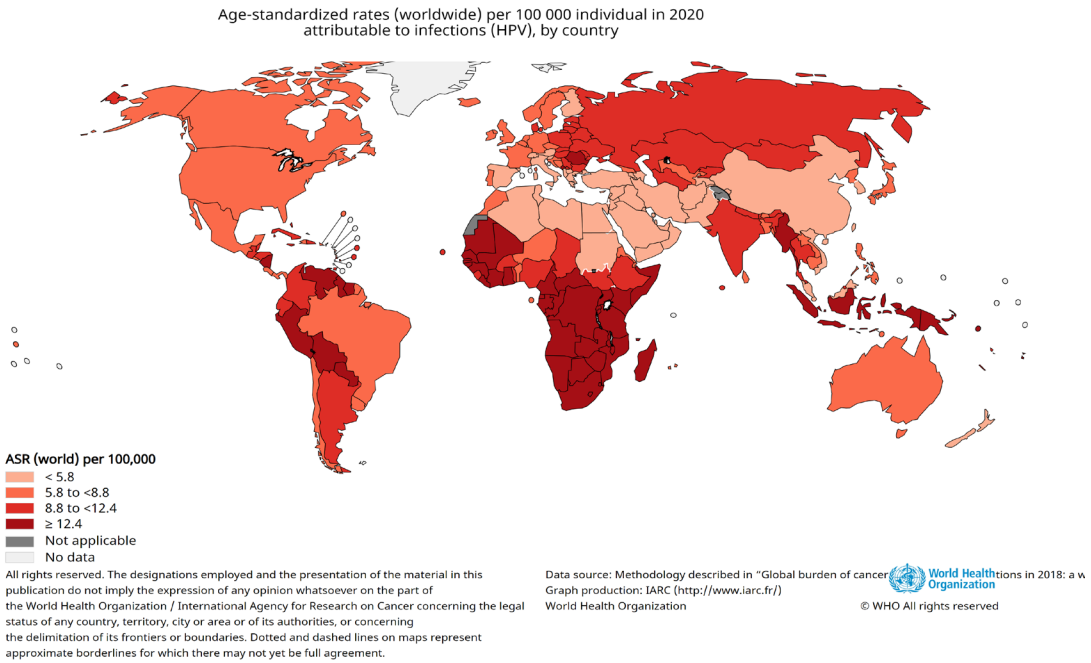
HPV-associated malignancies

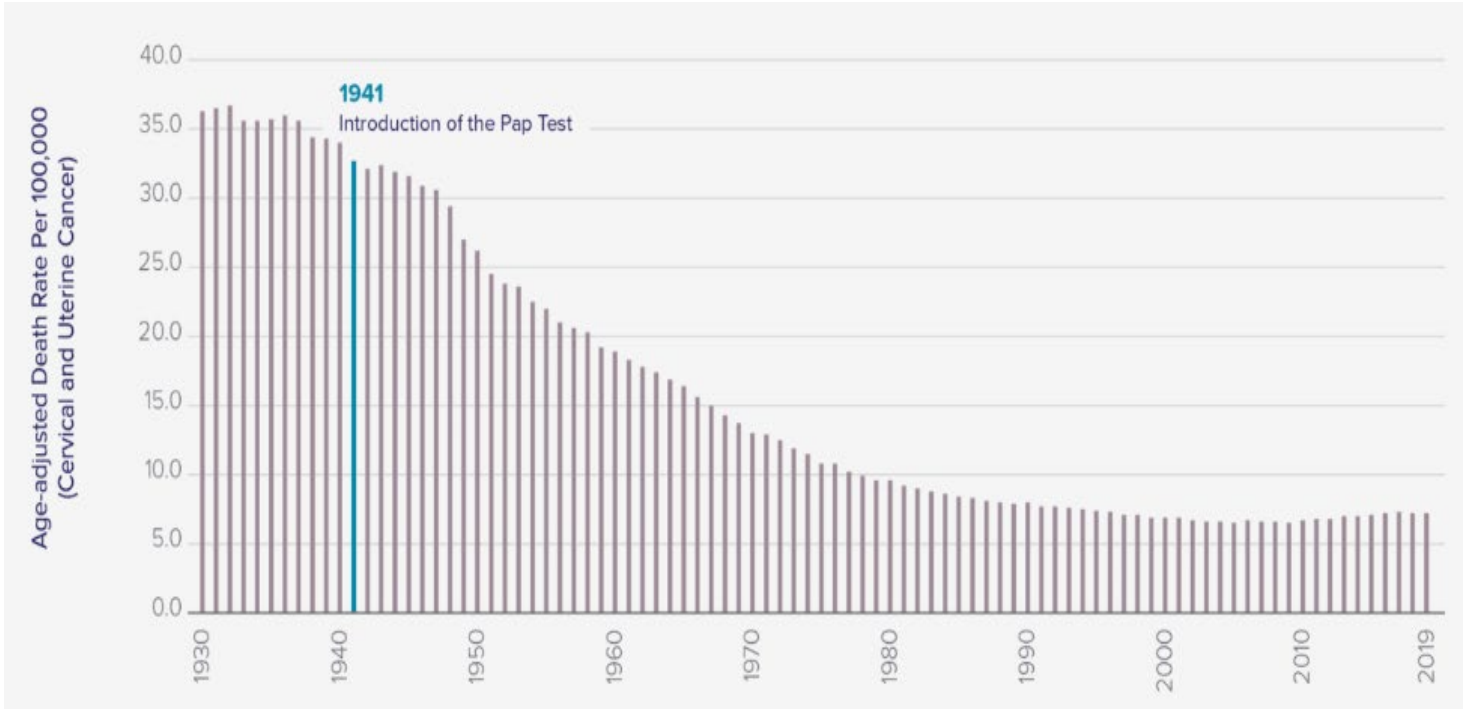
- 30% of 2.2 mil. malignancies of infectious origin
- one of 5 human carcinogens causing disease in 5 and more anatomical sites



	Number of new cases	Number of new cases attributable to infectious agents	Attributable fraction
Carcinoma			
Non-cardia gastric	820 000	730 000	89·0%
Cardia gastric	130 000	23 000	17·8%
Liver	780 000	570 000	73·4%
Cervix uteri	530 000	530 000	100·0%
Vulva	34 000	8500	24·9%
Anus	40 000	35 000	88·0%
Penis	26 000	13 000	51·0%
Vagina	15 000	12 000	78·0%
Oropharynx	96 000	29 000	30·8%
Oral cavity	200 000	8700	4·3%
Larynx	160 000	7200	4·6%
Nasopharynx	87 000	83 000	95·5%
Bladder	430 000	7000	1·6%

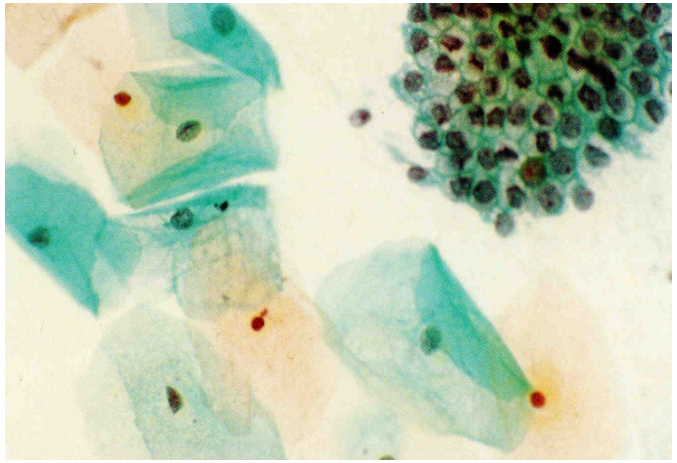
Plummer a kol., 2016; de Martel a kol., 2020

Secondary prevention = screening started in the 1940s



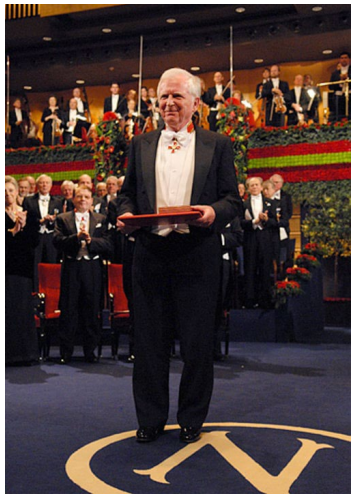
https://hologicwomenshealth.com/cervicalhealth/

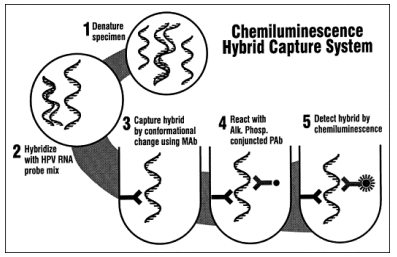
Papanicolaou Develops Pap Test, January, 1928

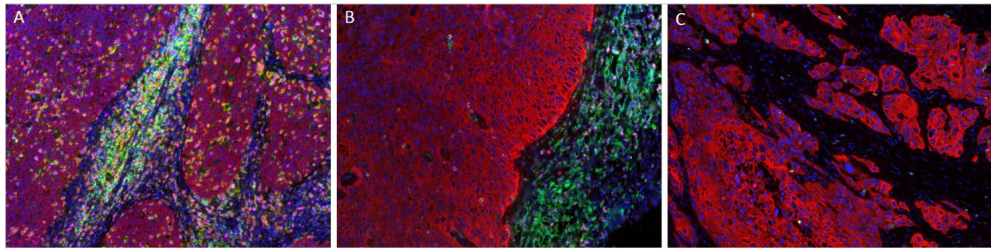


Development in the HPV field in relation to public health strategies

- HPV 16 was detected in cervical cancer **1983**
- New molecular test for HPV detection and typing FDA approved **1988,** BRL-LT, Virapap
- Triage test FDA approved **1999**, Digene, Hybrid capture II
- HPV co-testing in females >30 yrs of age guidelines
- Hybrid capture II **2003** approved by FDA for co-testing
- 2012 guidelines endorse HPV testing
- **2014** COBAS FDA approved **for primary test in screening**
- HPV association with other anogenital cancers
- Evaluation of test performance in screening
- Modification of cervical cancer screening
- Screening program for anal cancer
- HPV association with oropharyngeal cancer
- Prophylactic vaccines **(2006/2007)**
- Therapeutic vaccines and immunotherapy
- Personalized medicine

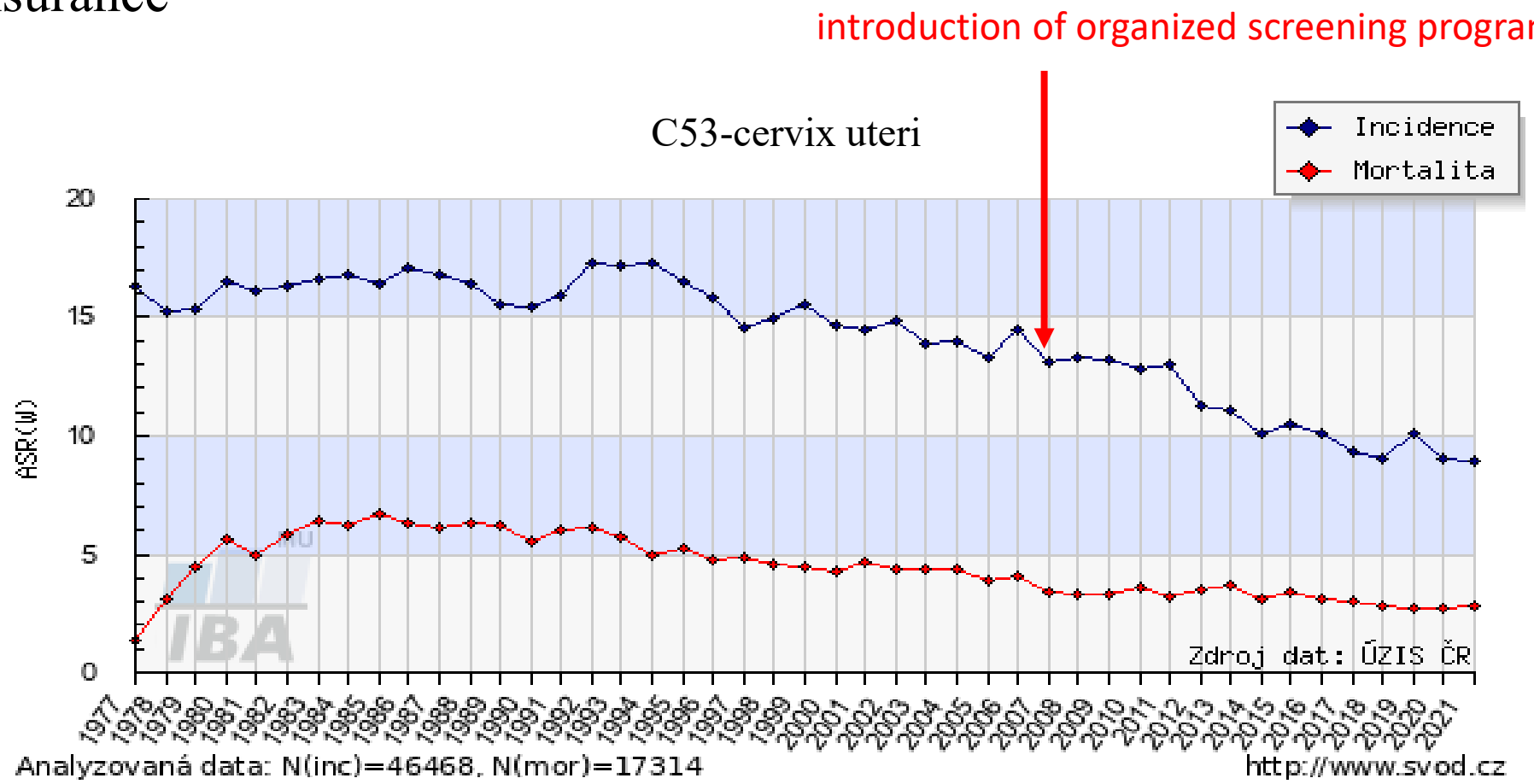






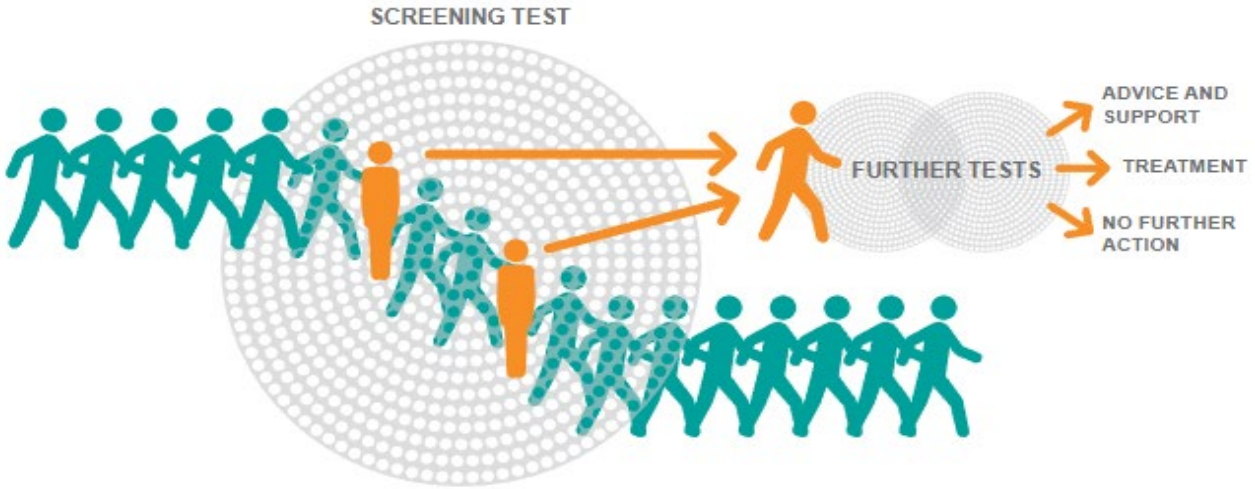
Cervical screening program in the Czech Republic

- 1966 20/1966 Coll. Act on the Care of the Health of the People
- annual regular gynaecological examinations reimbursed from compulsory health insurance



HPV as a biomarker in screening

- HPV or HR HPV positivity in the screening for cervical cancer (vaginal, vulvar)
  - Organized vs. opportunistic screening
  - Primary test
  - Variaty of triage tests
  - Self-sampling



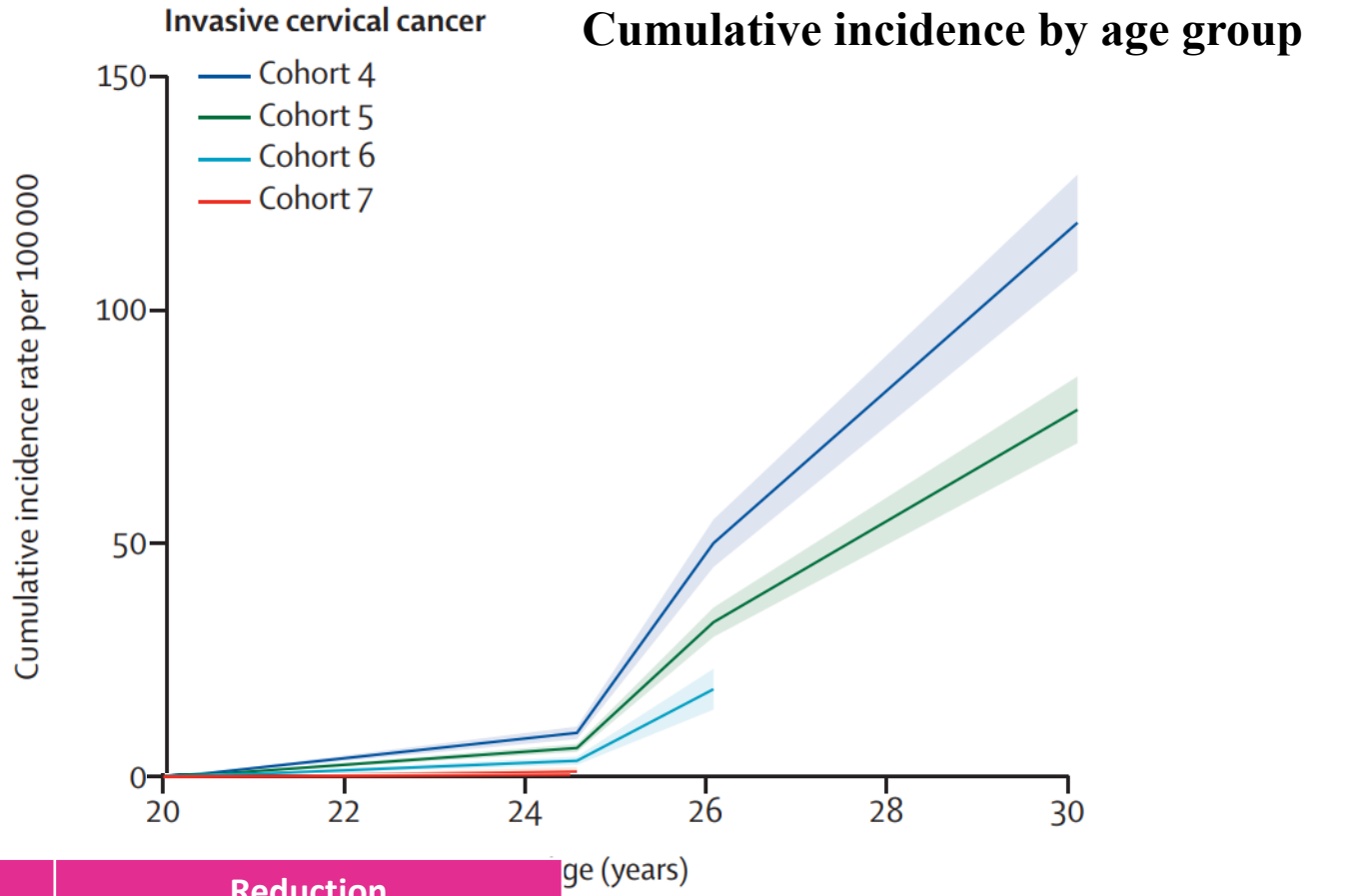
https://www.gov.uk/guidance/population-screening-explained

- HPV or HR HPV positivity in the screening of risk groups for anal screening
  - Target groups (MSM, HIV+, immunodeficient, condyloma / anal tumour in anamnesis, transplant individuals)

The efect of routine vaccination: cervical cancer

- UK, routine vaccination in 2008, girls 12-13 years and 14-18 years of age; till 2010
- till 2010 bivalent, from 2012 tetravalent vaccine
- **Women born from 1995 on**

ELIMINATION of CC



Group	Age of vaccination	Coverage	Reduction incidence
C4	non-vaccinated	0	0
C5	16-18 yrs	44.8%	34%
C6	14-16 yrs	73.2%	62%
C7	12-13 yrs	84.9%	87%

Falcaro et al., 2021

Global Strategy for the elimination of cervical cancer by 2030

- 90% of girls fully vaccinated with human papillomavirus (HPV) vaccine by the age of 15 years
- 70% of women screened with a **high-performance test** by 35 years of age and again by 45 years of age
- 90% of women identified with cervical disease receive treatment
  - Screen-and-treat approach
  - **Screen, triage and treat approach**
    - **Primary test -** HPV NAT
    - **Triage test –** genotyping, colposcopy, VIA, cytology, dual-stain cytology (Ki-67+p16, LBC)

WHO guidelines, 2021

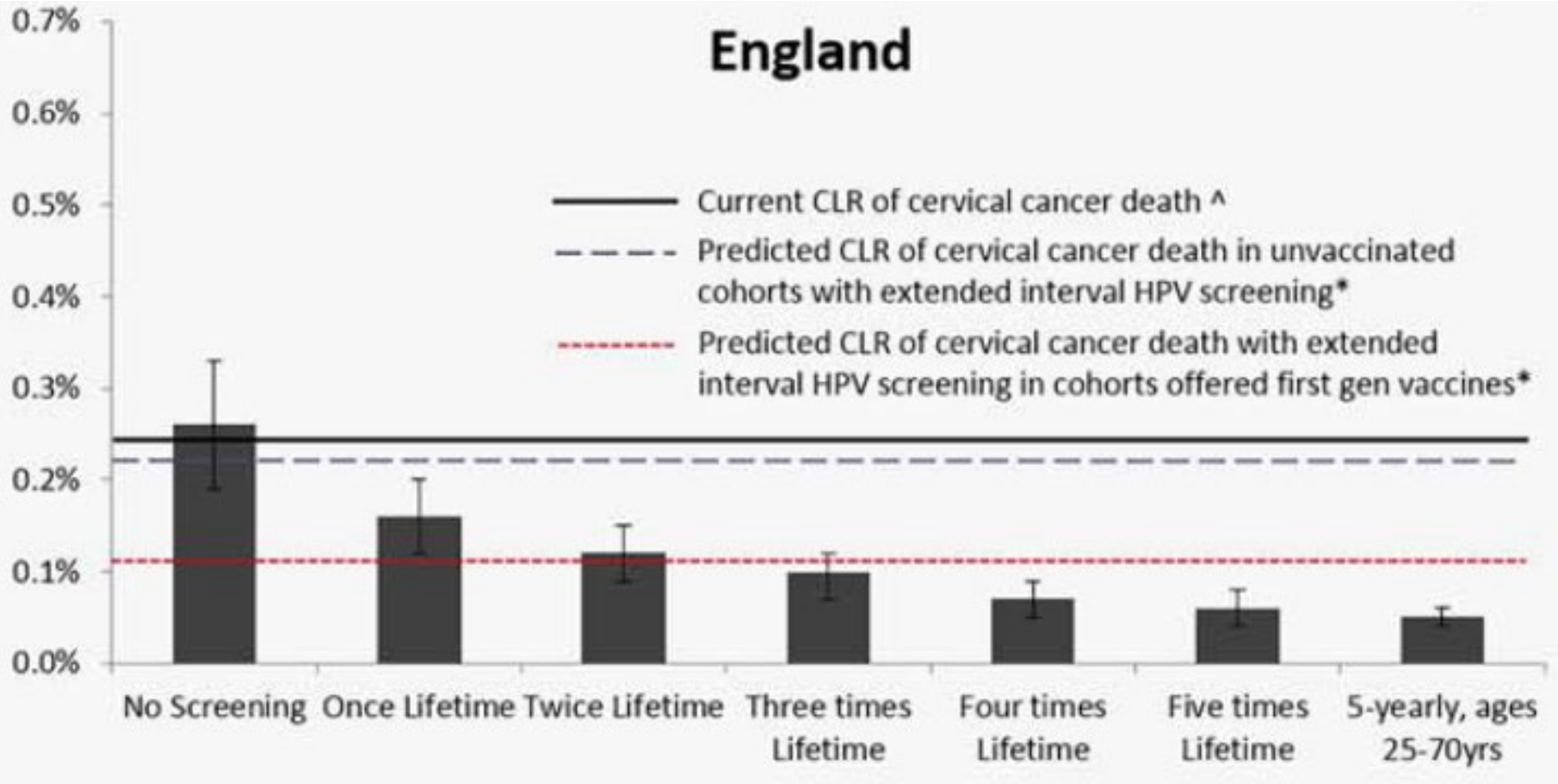
Methods used in cervical screening

Molecular	Cytologic	Visual inspection
Nucleic acid tests (NAT)[a] » HPV DNA » HPV mRNA	**Conventional Pap smear[a]**	**Visual inspection with acetic acid (VIA)[a] or with Lugol's iodine (VILI)** » naked eye » magnified by colposcope or camera
DNA methylation[b]	**Liquid-based cytology (LBC)[a]**	
Protein biomarkers[b] » HPV antibodies » oncoproteins	**Dual-stain cytology to identify p16 and Ki-67[a]**	**Automated visual evaluation of digital images[b]**

[a] Current tests
[b] Tests under evaluation (future tests).

WHO guidelines, 2021

Screening program in vaccinated cohorts

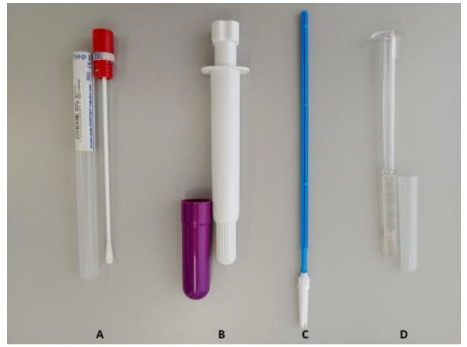


CLR = cumulative lifetime risk (CLR) of cervical cancer death

Simms et al., 2016

Biomarkers

- Purpose
  - Screening, **diagnostic, predictive, prognostic, therapeutic**
- Characteristics
  - Minimally invasive or non-invasive sampling, affordable, sensitive, specific, PPV, NPV, logistics (sampling, storage, transportation)
- Type of clinical material
  - Fixed tissue, fresh tissue, blood (serum, plasma, PBMC), saliva (stimulated, non-stimulated), lavage, cells, urine



A. Sterile viscose swab with a polystyrene stem into a sterile polypropylene tube.

B. Iune HPV sterile test cannula.

C. Viba-Brush®.

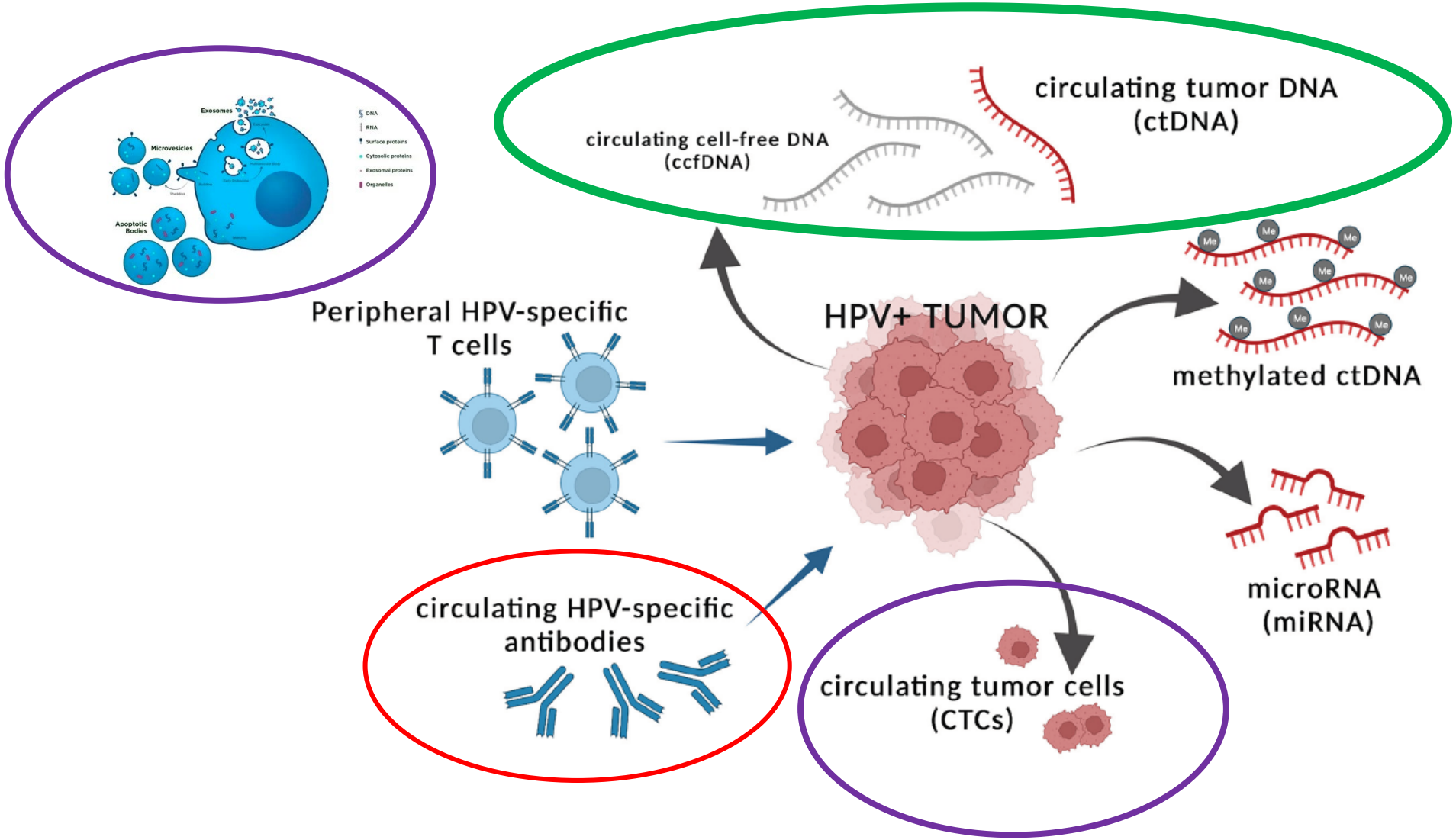
D. Mia by XytoTest®

Gibert et al., 2023

www.bdbiosciences.com

DIANA Biotechnologies

Biomarkers: tissue and body fluids



Goswami et al, 2023

BECKMAN COULTER Life Sciences

Indirect method of HPV detection - serology

- HPV VLP-antigens – antibodies type-specific, anamnestic, develop with delay after infection, low levels after natural infection, not cross-protective, unclear if protective against the same type of infection

- HPV pseudovirion approach – neutralising antibodies (Pastrana et al., 2004; Buck et al., 2005)

- **HPV E6/E7 specific antigens – level of antibodies increased when HPV-associated invasive disease present, rare presence in a healthy population, can be present many years before diagnosis**

HPV is the strongest predictive and prognostic risk factor for oropharyngeal cancer (OPC)

- Detection of HPV DNA/RNA
- Detection of HPV-specific antibodies
- Detection of indirect markers of active viral infection (8th edition of TNM classification)

Table 5. HR of death and OR of recurrence by positivity for markers of HPV infection at enrolment and follow-up

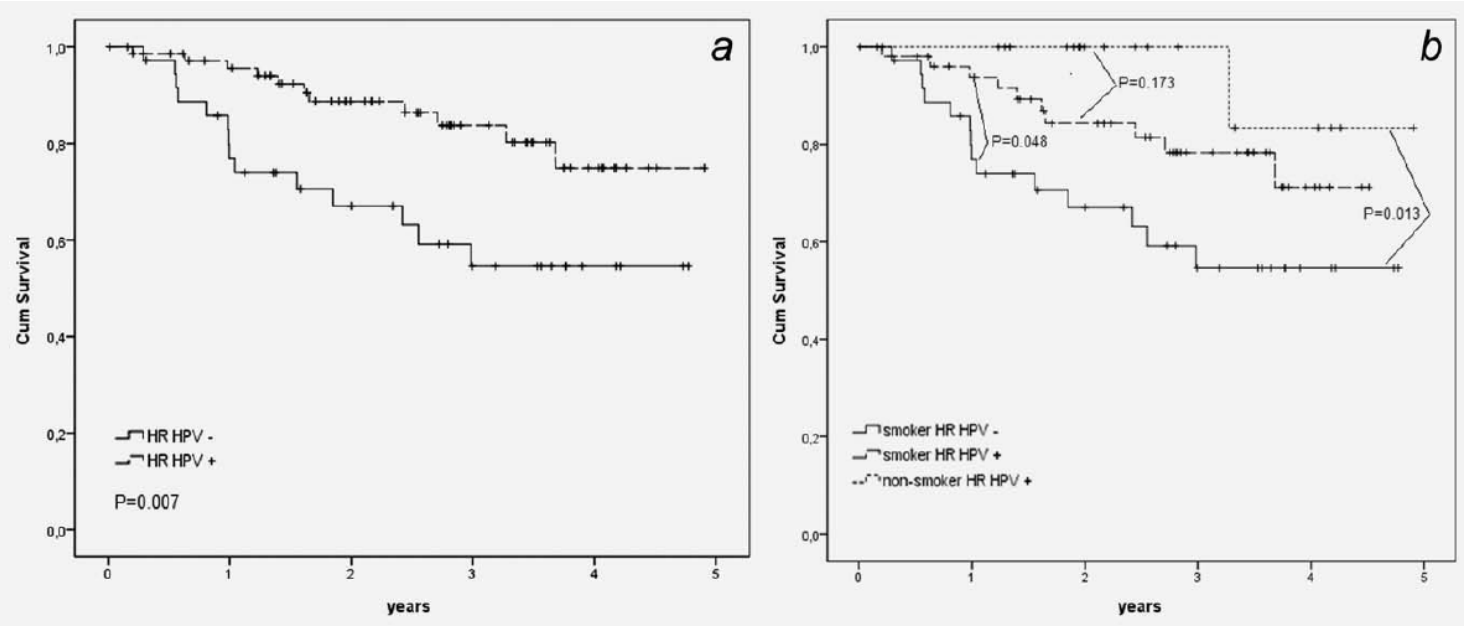
	Enrolment												Follow-up at 1 year post-treatment[1]							
	HR HPV DNA						HPV-specific antibodies						HR HPV DNA			HPV-specific antibodies				
Outcome/ marker status[2]	No (%)	Tumor tissue (95%CI)	p-Value[2]	No (%)	Oral rinse (95%CI)	p-Value[2]	No (%)	VLPs 16 (95%CI)	p-Value[2]	No (%)	HPV 16 E6 and/or E7 (95%CI)	p-value[2]	No (%)	Oral rinse (95%CI)	p-Value[2]	No (%)	VLPs 16 (95%CI)	p-Value[2]	No (%)	HPV 16 E6 and/or E7 (95%CI)	p-Value[2]
Overall Deaths																				
Negative	16 (27.6)	2.4 (1.2–4.7)	0.012	15 (21.7)	2.8 (1.3–5.9)	0.009	14 (17.5)	1.1 (0.6–2.3)	0.700	19 (27.5)	3.1 (1.4–6.7)	0.004	25 (23.6)	0.7 (0.1–3.2)	0.616	21 (30.4)	1.7 (0.5–5.2)	0.371	19 (37.3)	3.1 (0.9–1.0)	0.064
Positive	8 (9.5)			9 (12.5)			10 (16.1)			5 (6.8)			5 (41.7)			9 (18.4)			11 (16.4)		
Disease-Specific Deaths																				
Negative	15 (25.9)	3.5 (1.5–8.2)	0.003	13 (18.8)	3.1 (1.2–7.9)	0.018	10 (12.5)	1.1 (0.5–2.4)	0.876	16 (23.2)	5.2 (1.8–14.9)	0.002	19 (17.9)	1.1 (0.1–9.0)	0.964	14 (20.3)	2.2 (0.5–8.9)	0.267	14 (27.5)	3.1 (0.8–12.4)	0.240
Positive	2 (2.4)			4 (5.6)			7 (11.3)			1 (1.4)			1 (8.3)			6 (12.2)			6 (9.0)		
Recurrence																				
Negative	20 (60.6)	2.9 (1.2–7.1)	0.024	22 (31.9)	3.1 (1.2–8.4)	0.024	19 (57.6)	0.8 (0.3–2.1)	0.723	26 (78.8)	6.4 (2.1–19.0)	0.001		NA[3]			NA[3]			NA[3]	
Positive	13 (39.4)			11 (15.3)			14 (42.4)			7 (21.2)										

[1]Samples taken 1 year after the end of the treatment and outcome of patients at the longest follow-up available.
[2]Adjusted for age ≤55/>55, tobacco, alcohol consumption, tumor size, incidence, and extent of lymph node metastasis.
[3]Only two patients with recurrence were alive when last seen.

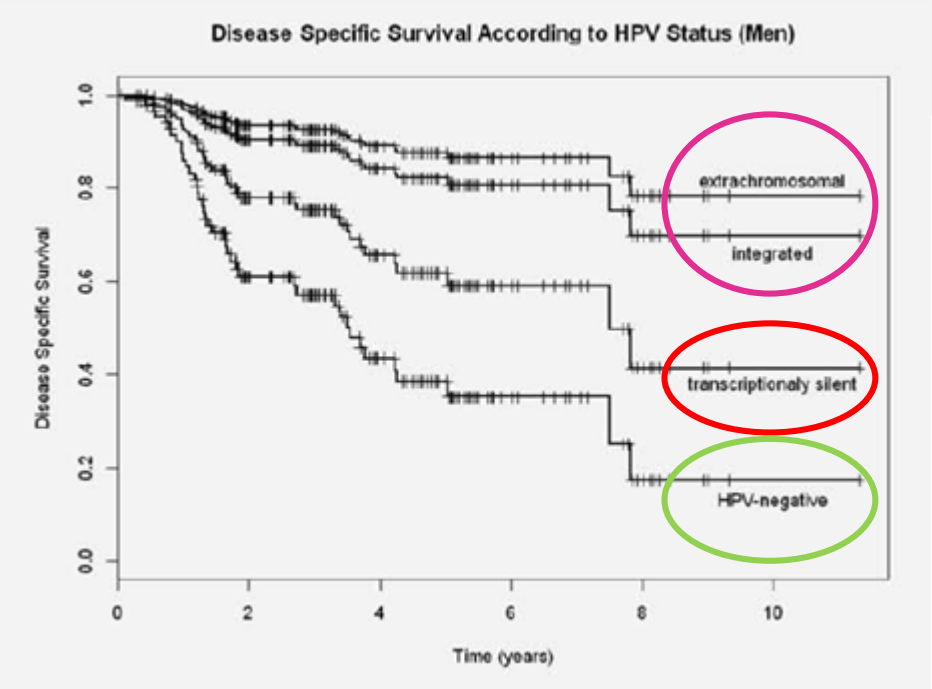
Košlabová et al, 2013

Survival of patients with OPC

- HPV
- Smoking
- Integration



Rotnáglová et al, IJC, 2011

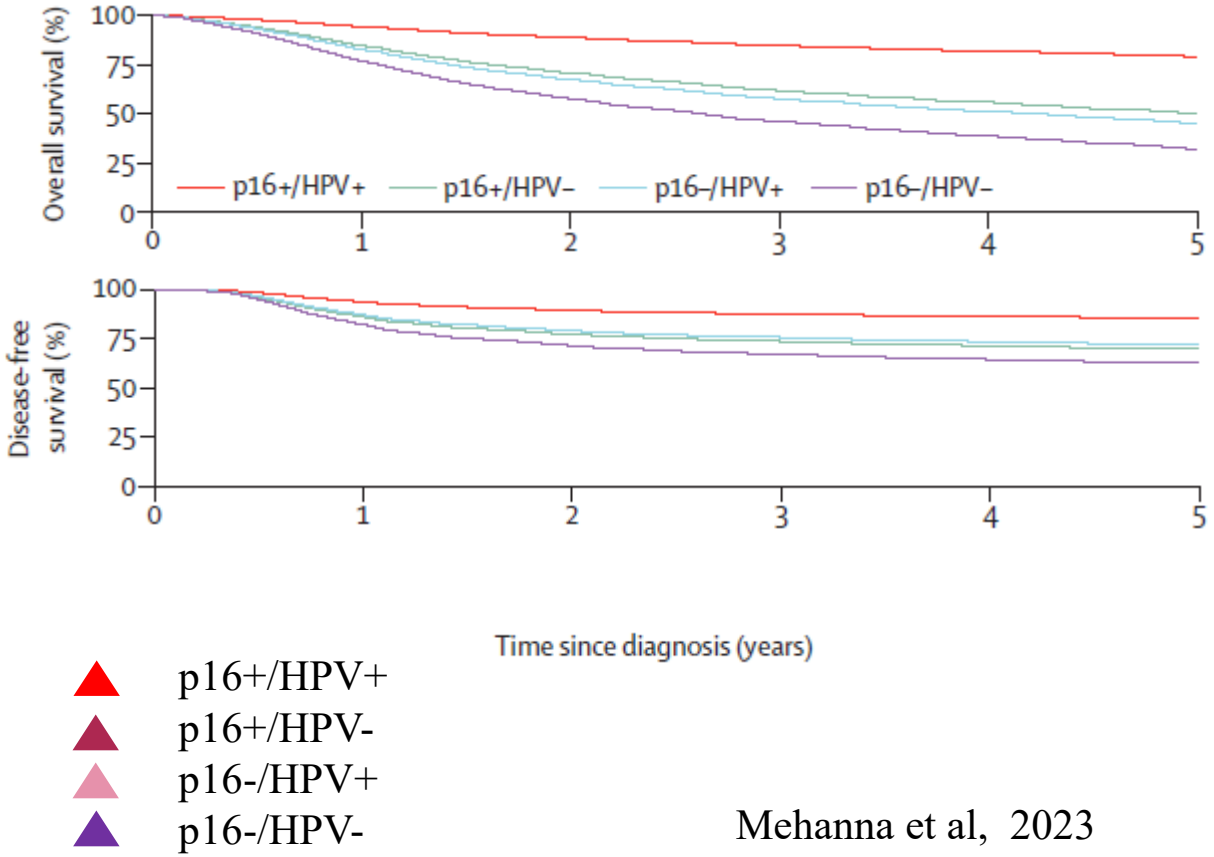


- Genotyping of HPV
- Intratype variability (SNPs)

Vojtěchová et al, IJC, 2015

De-ascalation trials for HPV-positive OPC

- Results not conclusive
- Clinical trials phase III limited or not available
- Several potential novel de-escalation strategies identified
- Important exploration and introduction of novel biomarkers

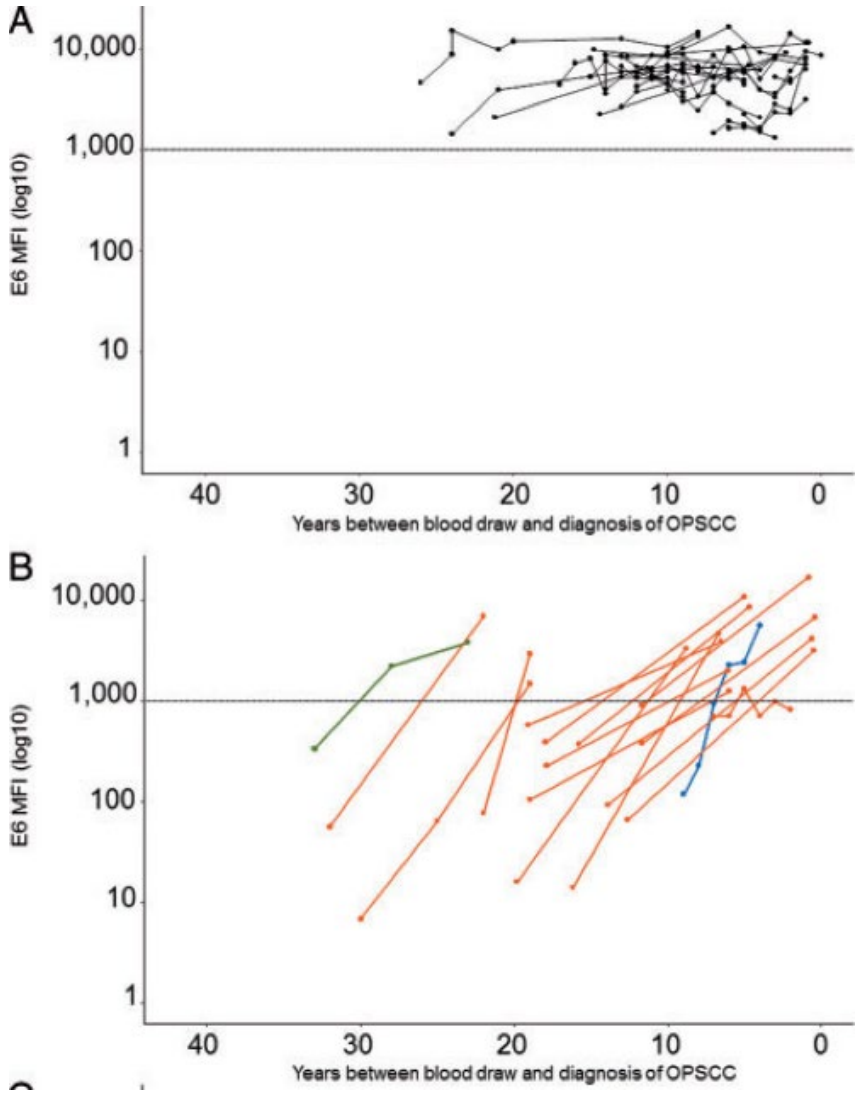


Mensour et al, 2022

Mehanna et al, 2023

HPV-specific antibodies against early viral proteins

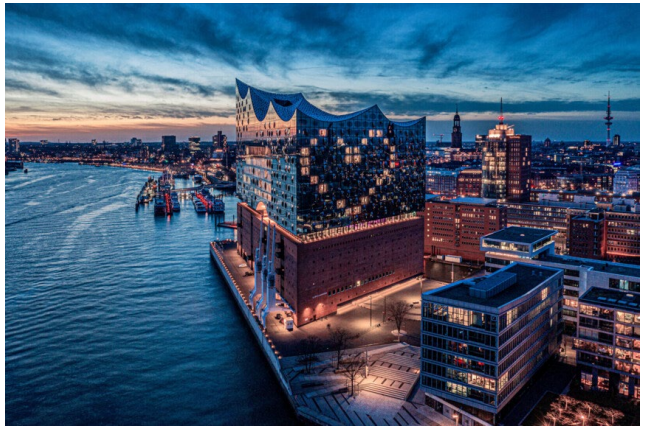
- **Low prevalence in healthy people** (HPV E6 prevalence 0.5-8%)
- **Detection of E6/7 HPV-specific antibodies up to 28 years before diagnosis**, seroprevalence correlates with time to diagnosis (35 % vs. 0.6% positive patients vs. control group)
- **Increased risk of OPC for individuals with** HPV16 E6 –specific antibodies (males 17-27%, women 4-6%; age 50-60, 10 years)



Kreimer et al., 2013; Hildesheim a spol., 2015; Kreimer a spol., 2017; Kreimer at al., 2019

Risk of OPC based on the positivity of HR HPV-specific antibodies

- **Hamburg study**, 2016, N=45,000 asymptomatic individuals
- N=4,424, age 45-74, interim analyses
- HPV16 E6 + one of E1/E2/E7 positivity
- Examination every 6 months (visual, endoscopy, ultrasonography), susp. lesion (MRI, panendoscopy, biopsy)
- N=35 (0.8%) E6 HPV +, N=11 (0.3%) high risk, N=9 examined
- N=3 OPC (3-4 years after enrolment, 1.3 years of follow-up), N=6 no disease



Maléřová et al., 2020; Waterboer et al., 2020; Busch et al., 2022

miRNA in tissues

- small 21-25 bases non-coding miRNAs, regulation of gene expression
  - 1 miRNA → regulation of multiple target mRNAs (~200)
  - 1 mRNA → target for multiple miRNAs
- Deregulation of miRNA expression in tumors
- Cancer specific profiles of deregulated miRNAs
- Reproducibility ?????
- Prognostic value ?????

Vojtechova et al. BMC Cancer (2016) 16:382
DOI 10.1186/s12885-016-2430-y

BMC Cancer

RESEARCH ARTICLE Open Access

Comparison of the miRNA profiles in HPV-positive and HPV-negative tonsillar tumors and a model system of human keratinocyte clones

Zuzana Vojtechova[1,2], Ivan Sabol[2], Martina Salakova[1,2], Jana Smahelova[1,2], Jiri Zavadil[3], Lubomir Turek[4], Marek Grega[5], Jan Klozar[6], Bohumir Prochazka[2] and Ruth Tachezy[1,2*]

PLOS ONE

biomolecules MDPI

Article

Lack of Conserved miRNA Deregulation in HPV-Induced Squamous Cell Carcinomas

Jaroslav Nunvar [1,2,*], Lucie Pagacova [1,2], Zuzana Vojtechova [1,2], Nayara Trevisan Doimo de Azevedo [3], Jana Smahelova [1,2], Martina Salakova [1,2] and Ruth Tachezy [1,2]

1 Department of Genetics and Microbiology, Faculty of Science, Charles University, 12844 Prague, Czech Republic; Lucka.pagacova@seznam.cz (L.P.); zuzana.vojtechova@natur.cuni.cz (Z.V.); jana.smahelova@natur.cuni.cz (J.S.); martina.salakova@natur.cuni.cz (M.S.); ruth.tachezy@natur.cuni.cz (R.T.)
2 BIOCEV-Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University, 25250 Vestec, Czech Republic
3 Genomics Core Facility, EMBL, 69117 Heidelberg, Germany; nayara.azevedo@embl.de
* Correspondence: jaroslav.nunvar@natur.cuni.cz

RESEARCH ARTICLE

Comparison of the miRNA expression profiles in fresh frozen and formalin-fixed paraffin-embedded tonsillar tumors

Zuzana Vojtechova[1,2], Jiri Zavadil[3], Jan Klozar[4], Marek Grega[5], Ruth Tachezy[1,2]*

1 Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic, 2 Department of Immunology, Institute of Hematology and Blood Transfusion, Prague, Czech Republic, 3 Molecular Mechanisms and Biomarkers Group, International Agency for Research on Cancer, Lyon, France, 4 Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic, 5 Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

SCIENTIFIC REPORTS

Souhrn 3

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Genome-wide miRNA profiling reinforces the importance of miR-9 in human papillomavirus associated oral and oropharyngeal head and neck cancer

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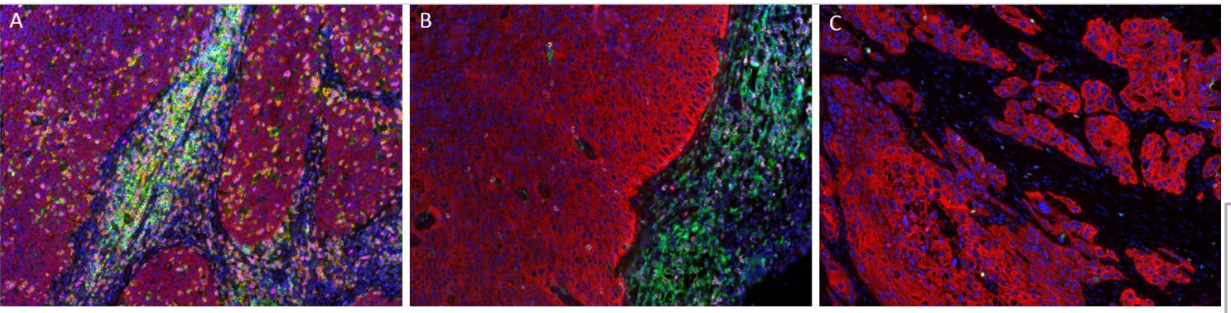
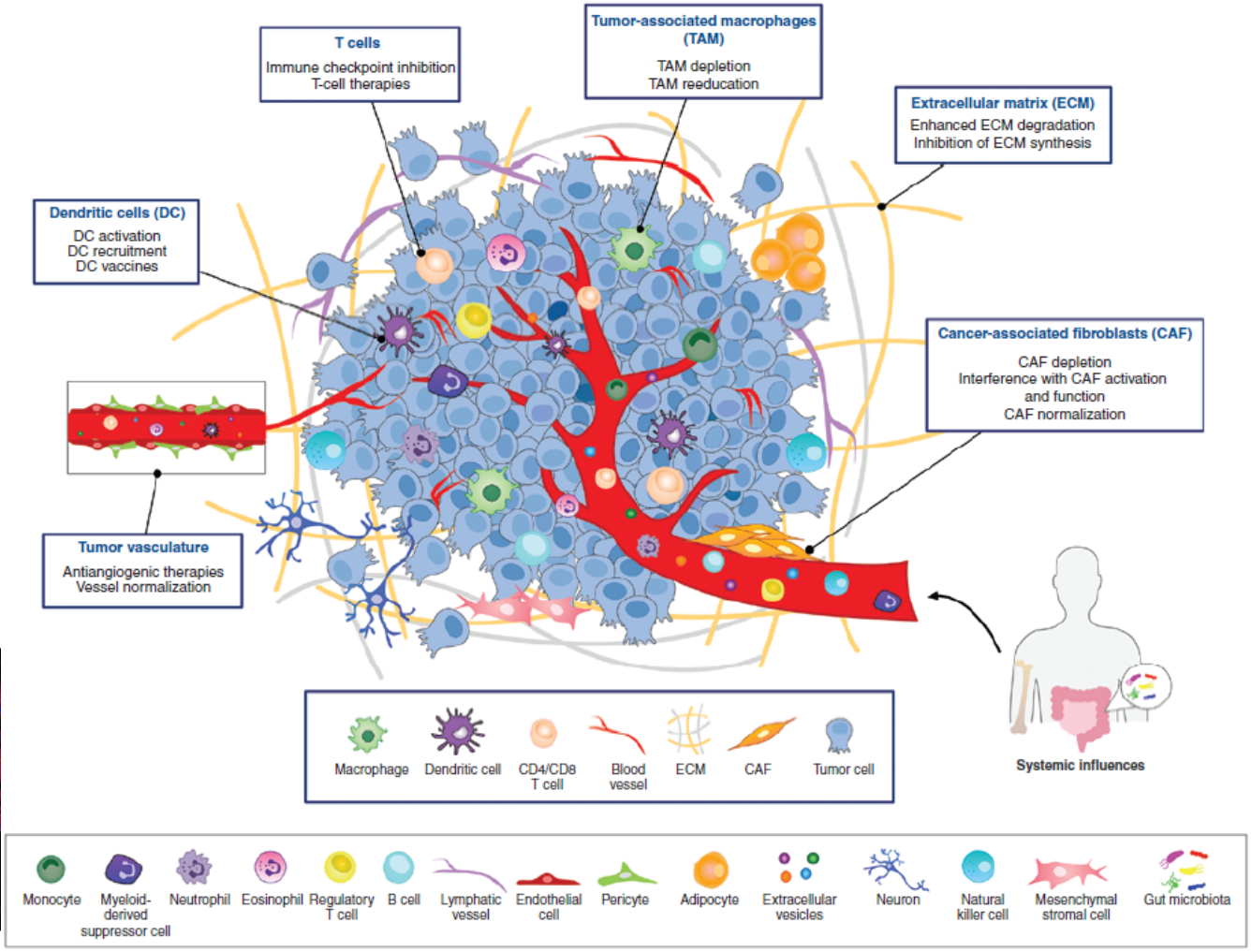
Ksenija Božinović[1], Ivan Sabol[1], Emil Dediol[2], Nina Milutin Gašperov[1], Spomenka Manojlović[2], Zuzana Vojtechova[3], Ruth Tachezy[3] & Magdalena Grce[1]

Tumour microenvironment (immunoscore)

- Complex, rich multicellular environment
- Regulation of cancer progression, influence of treatment outcome
- **Immune response - the most important determinant of survival of cancer patients**



Immune hot Immune excluded Immune cold



Bejarano et al., 2021

Different infiltration of immune cells in tumours of different aetiology, hypoxia markers

Cell populations more prevalent in particular tumor type/compartment			
HPV$^+$		**HPV$^-$**	
Parenchyma	***Stroma***	***Parenchyma***	***Stroma***
CD3$^+$ T lymphocytes [1]	CD3$^+$ T lymphocytes [1]	ARG1$^+$ cells [2]	ARG1$^+$ cells [2]
CD4$^+$ T lymphocytes [1]	CD4$^+$ T lymphocytes[1]	VEGFA$^+$ cells [3]	CD68$^+$ARG1$^+$ M2 [2]
CD8$^+$ T lymphocytes [1]	CD8$^+$ T lymphocytes [1]		
PD-1$^+$CD4$^+$ T lymphocytes [1]	PD-1$^+$CD4$^+$ T lymphocytes [1]		CTLA4$^+$CD4$^+$ T lymphocytes [1]
PD-1$^+$CD8$^+$ T lymphocytes [1]	PD-1$^+$CD8$^+$ T lymphocytes [1]		ICOS$^+$ Treg [1]
ICOS$^-$ Treg [1]			
GLUT1$^+$ cells [3]	GLUT1$^+$ cells [3]		
MMP13$^+$ cells [3]	MMP13$^+$ cells [3]		
HIF-1α$^+$ cells [3]			
ASPH$^+$ cells [3]			

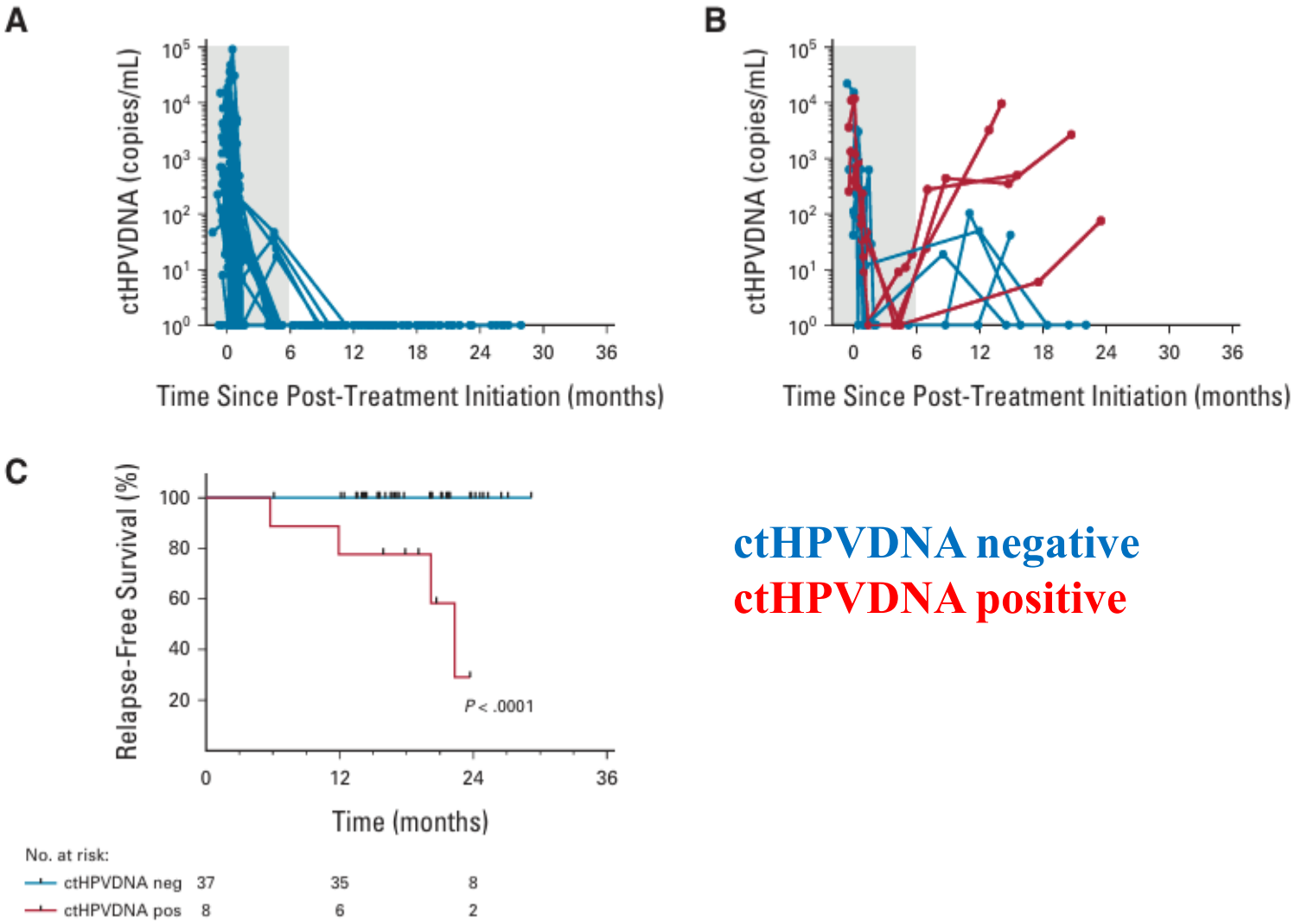
[1] Pokryvkova et al. 2022 [2] Pokryvkova et al. 2021 [3] Smahelova et al. 2024

Circulating (cell free=cf) DNA/RNA

- Released e.g. during necrosis, apoptosis or autophagy
- Length < 200 bp
- Including circulating tumour DNA (ctDNA)
- HPV-associated cancers - cfHPVDNA
- Detection of viral oncogenes
- Detection of mutations of somatic genes
- Number of the genome changes (CNA=copy number abnormalities)
- Methods:
  - qPCR
  - ddPCR
  - NGS

Prognostic value of ctHPVDNA analyzed by ddPCR

- N=45, 19.2 months follow-up (FU)
- N=8 positive for ctHPVDNA at FU
- N=4 patients with recurrent disease, all patients positive at least on two occasions during FU



ctHPVDNA negative
ctHPVDNA positive

Chera et al, 2020

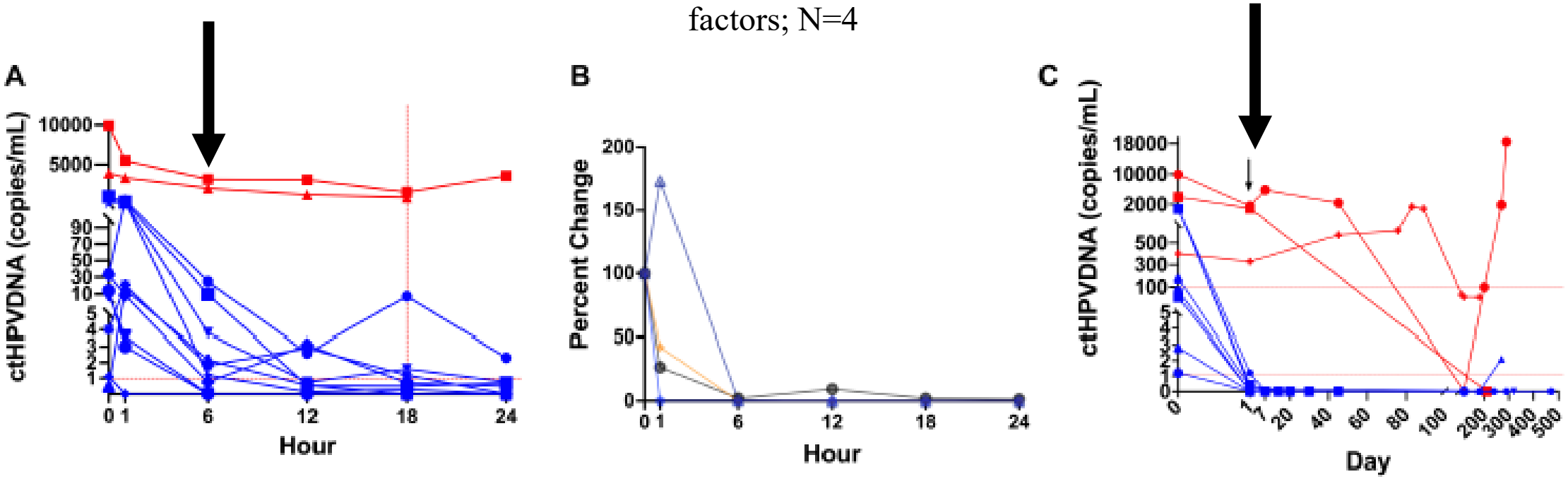
Prognostic value of ctHPVDNA analyzed by ddPCR

- N=33, surgery treatment, N=12 multiple sampling within 24 h
- Pacients with and without risk factors of recurrent disease (distant metastasis, positive margins, extranodal spread, nodal positivity……)

Risk of persistent macroscopic disease +/-; N=12

Patients without clinical or pathological risk factors; N=4

Pacient risk +/-; N=33

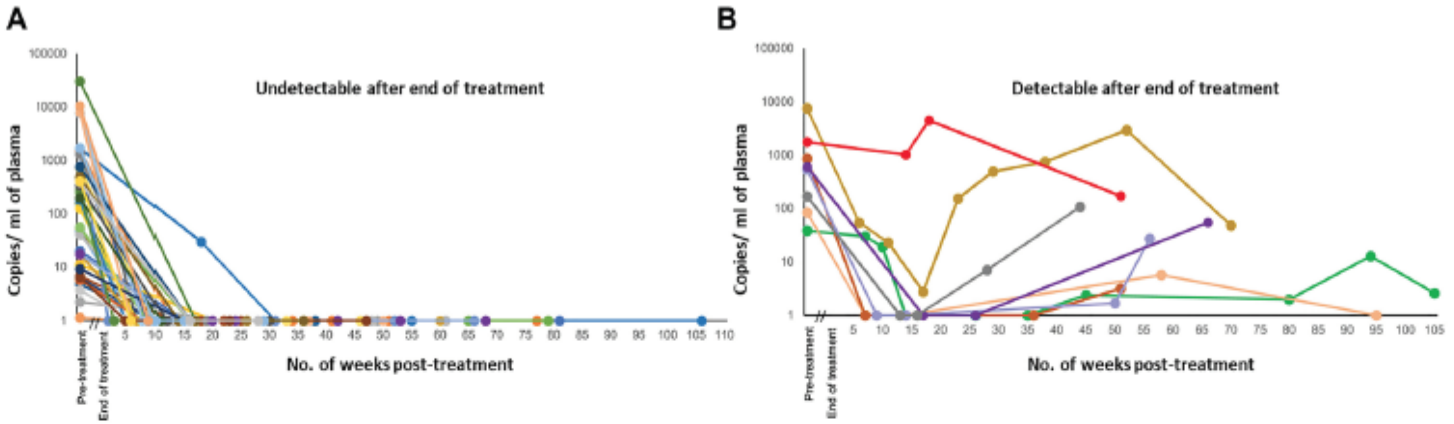


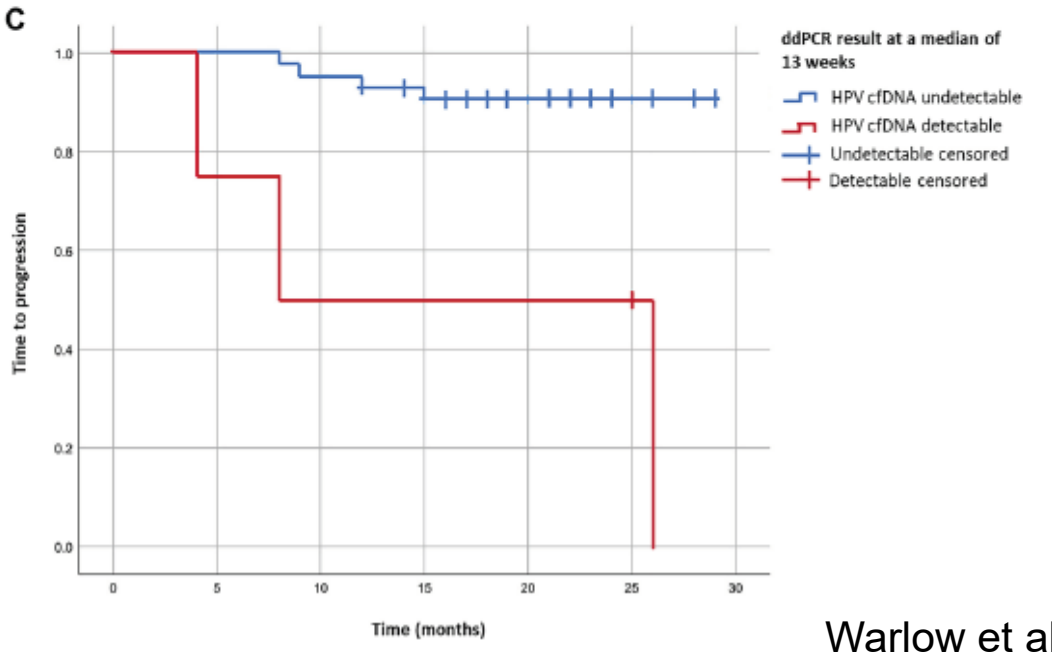
O´Boyle et al, 2022

Treatment response according to the number of ctHPVDNA load

- **Group 1**
- N=48 ctHPVDNA before the treatment
- N=40 no ctHPVDNA after the treatment (2-22 weeks)
- N=8 ctHPVDNA positive after the treatment
- N=2 persistent ctHPVDNA – regional or distant progression
- N=5 decrease or increase of ctHPVDNA level, progresive and metastatic disease, in other cases the increase of ctHPVDNA preceded or correlated with recurrence detection

- **Group 2**
- N=8 no cfHPVDNA before and after treatment

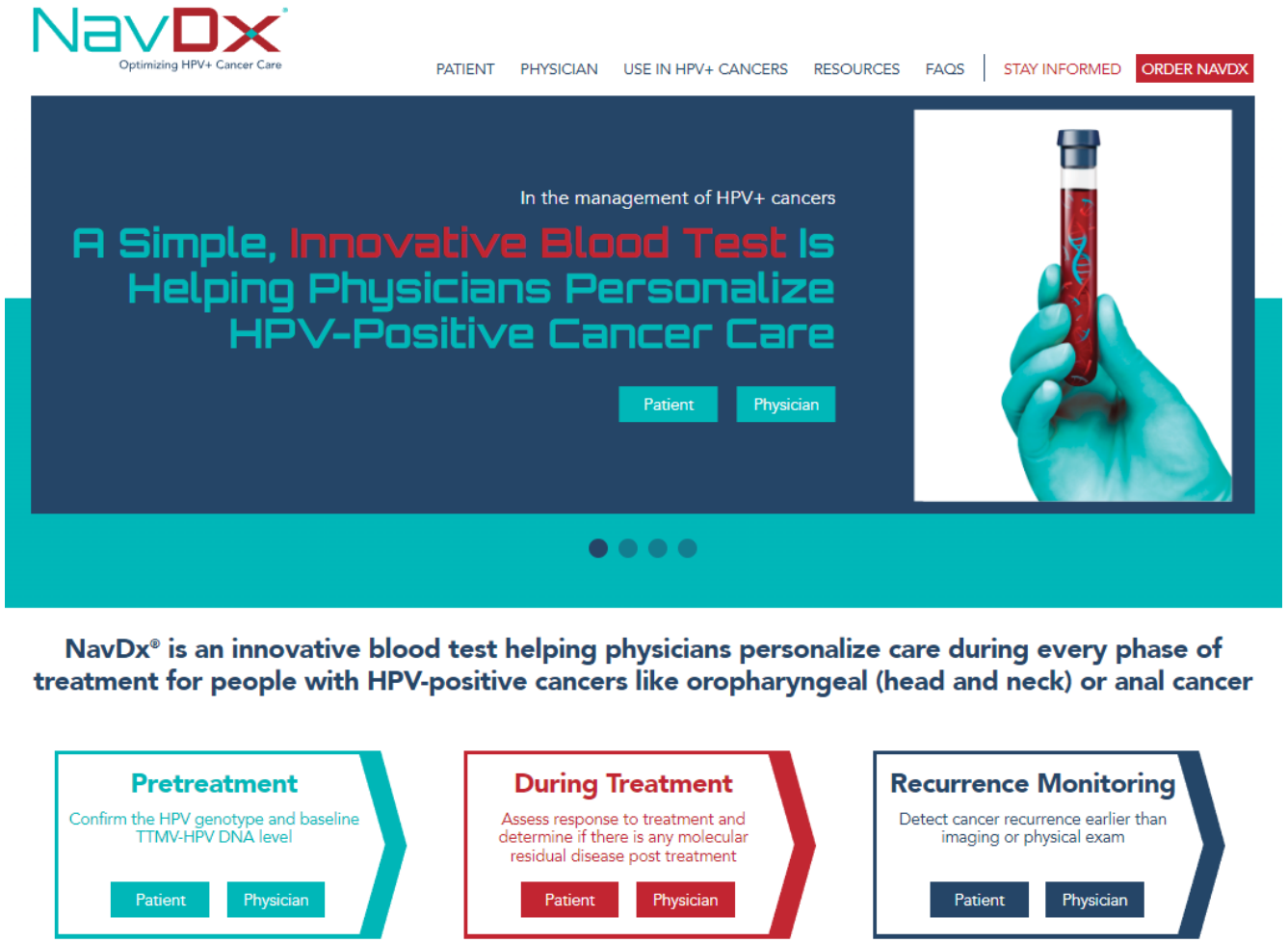




Warlow et al., 2022

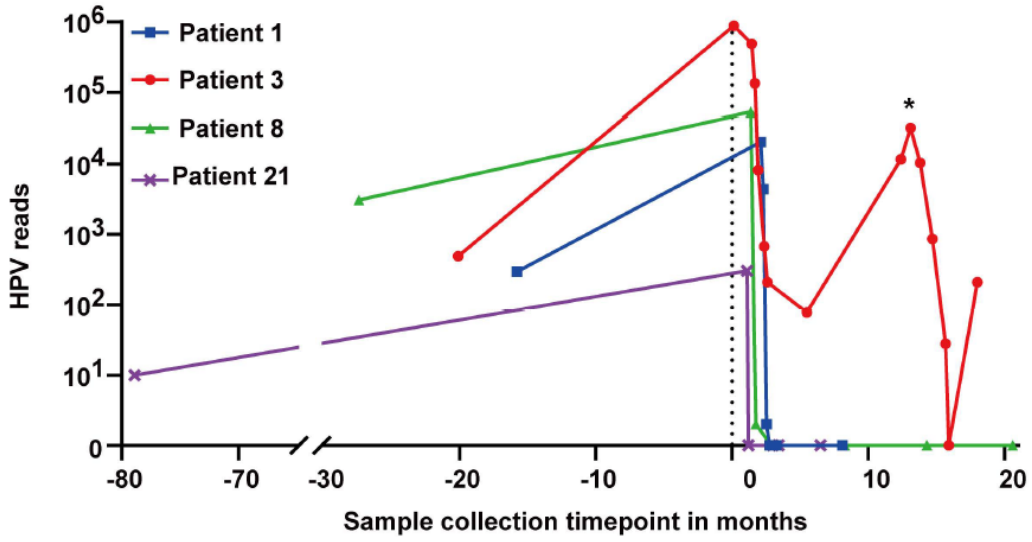
Detection of ctHPVDNA by ddPCR

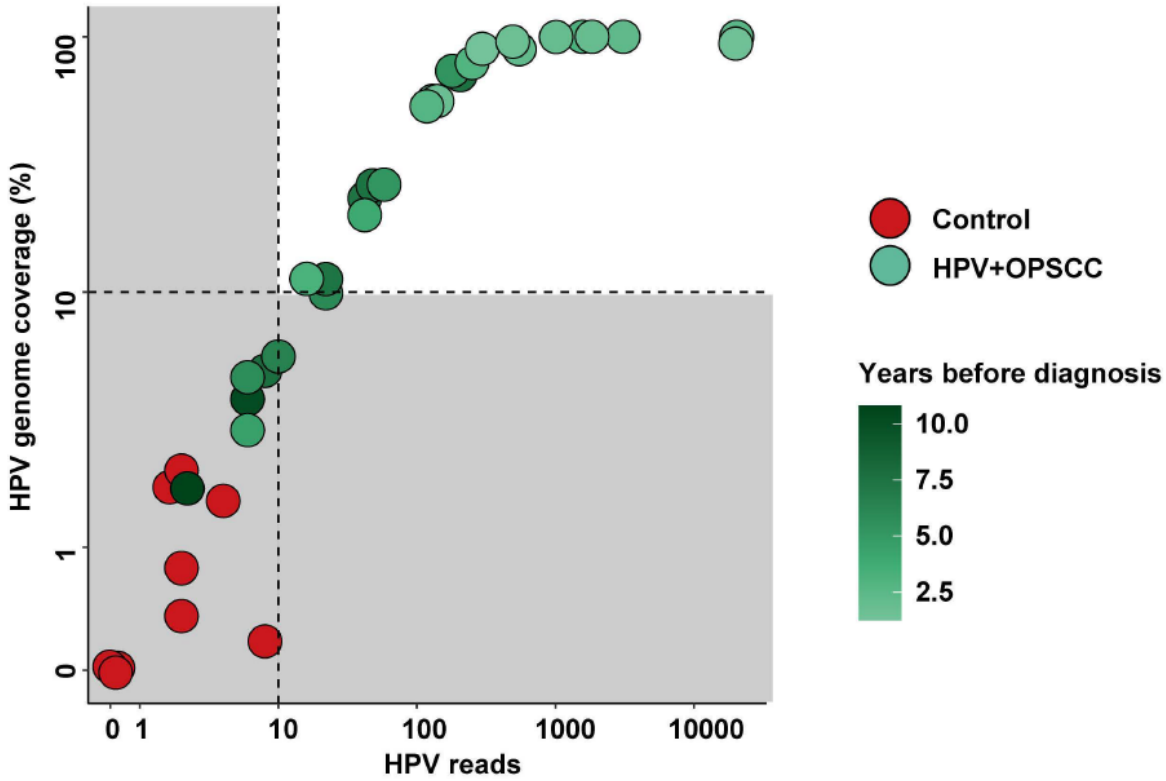
- **Detection of cfDNA in the peripheral blood**
- ddPCR
- 95% PPV, NPV
- Diagnosis of HPV-associated cancer
- FU of treatment response, prognosis



Detection of ctHPVDNA by NGS

- HPV-DeepSeek=HPV WGS
- 98.7% sensitivity and specificity N=153 patients and N=153 controls
- N=28, sampling 1.3 – 10.8 years before diagnosis (the earliest positivity 7.8 years before diagnosis)
- 100% sensitivity 4 years before diagnosis
- „machine learning“ – 100% sensitivity 10 years

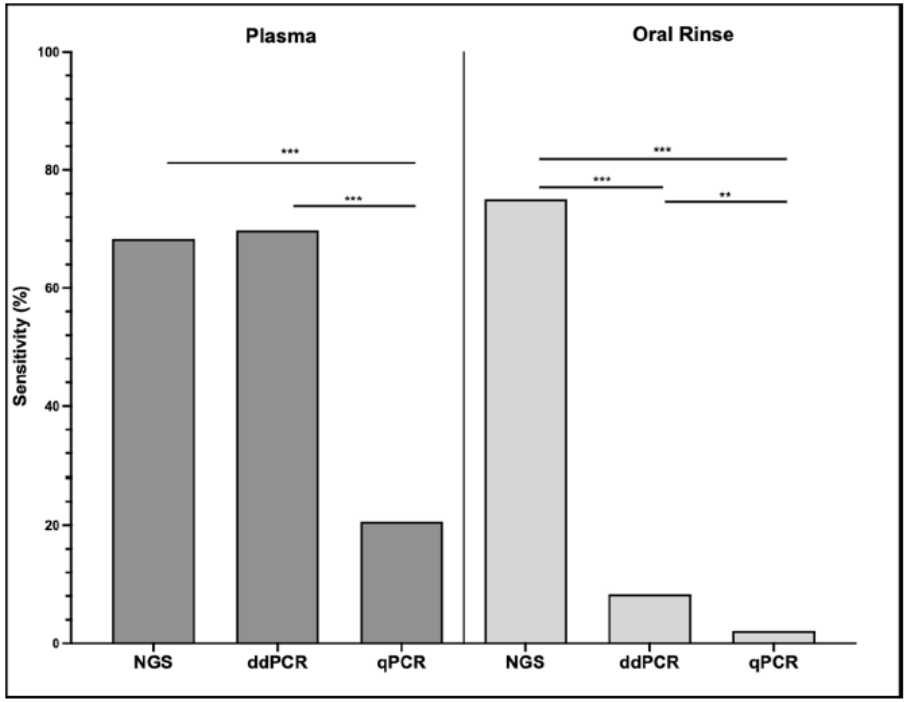




Das et al, 2024

Parameters of ctHPVDNA detection

- Sensitivity, specificity, NPV, and PPV dependent on:
  - Method (qPCR, ddPCR, NGS)
  - Type of clinical material
    - Plasma
    - Lavage
    - Saliva
  - Storage and transport
  - Modification of the detection method (SyberGreen vs. probe; number of genes screened, WGS)

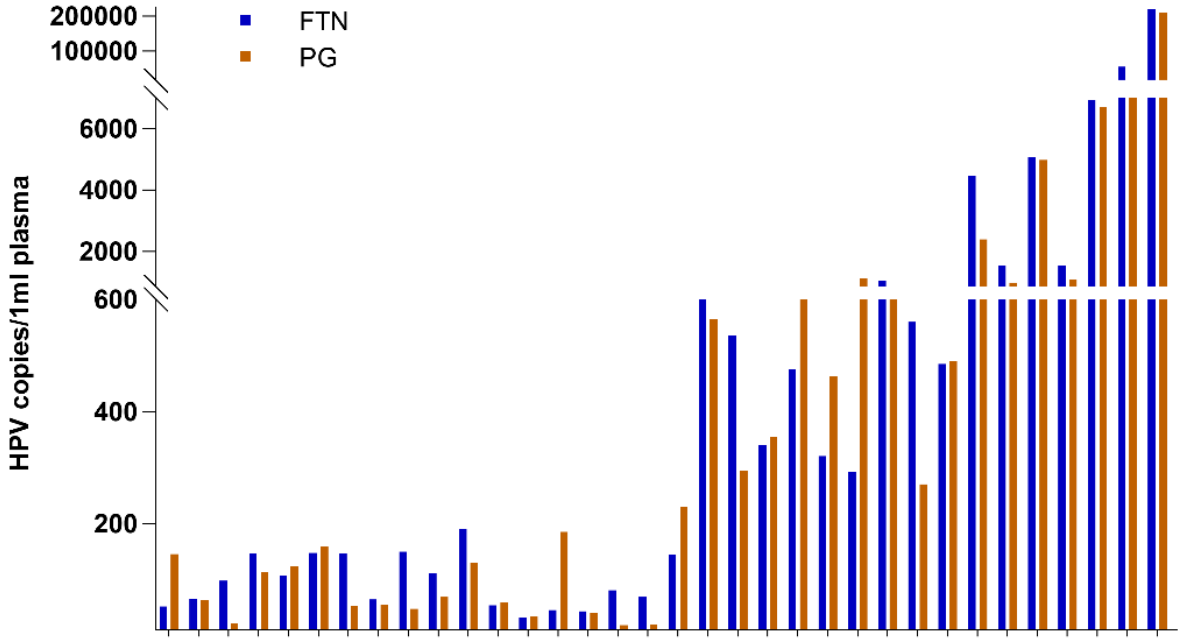


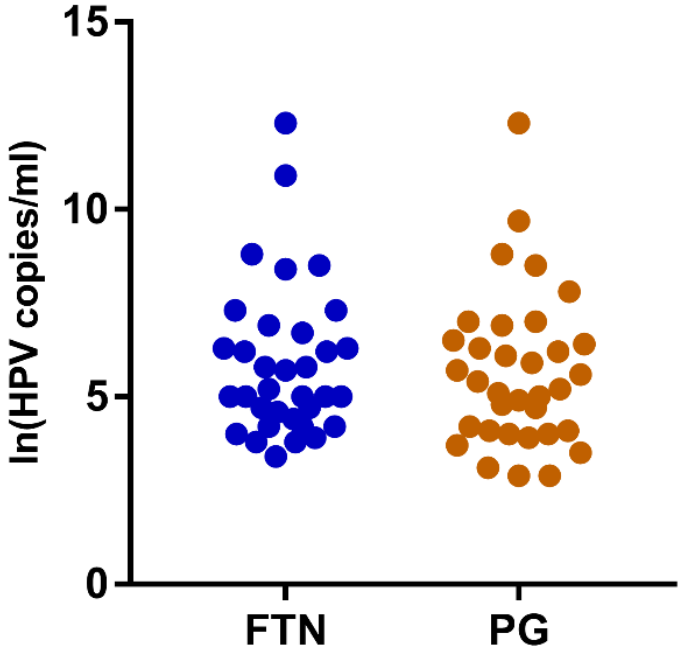
Mattox et al, 2022

Two methods of plasma collection

- centrifugation of venous whole blood immediately after collection → plasma storage -80 °C (**FTN**)
- collection of blood in PAXgene Blood cfDNA tubes (**PG**)

analyses of cfDNA isolated from 55 paired samples by ddPCR (FTN + PG)





→ comparable results

Vojtechova et al., preliminary data

Table 2: Clinical trials incorporating ctHPV-DNA assessment as of April 19, 2023 in the United States

Identifier	Clinical trial name	Patient cohort	Treatment agents
NCT04564989	Prospective Observational Study to Validate Circulating HPVDNA and Prognostic Genomic Biomarkers in HPV-associated OPSCC	HPV+ OPSCC	Curative-intent treatment
NCT05541016	Blood-Based Biomarkers to Inform Treatment and Radiation Therapy Decisions for HPV Associated Oropharyngeal Squamous Cell Head and Neck Cancers - DART 2.0	HPV+ OPSCC	Standard of care surgery; diffusing alpha-emitter radiation therapy + docetaxel; intensity-modulated radiation therapy +/− cisplatin
NCT04900623	Risk-adapted Therapy in HPV+ Oropharyngeal Cancer Using Circulating Tumor (ct)HPV DNA Profile - The ReACT Study	HPV+ OPSCC	Radiation therapy; cisplatin, carboplatin, or paclitaxel
NCT04965792	Post-treatment Surveillance in HPV+ Oropharyngeal SCC	HPV+ OPSCC	Curative-intent treatment
NCT05606133	Circulating Human Papilloma Virus (HPV) DNA for the Screening and Surveillance of Gynecologic Cancers	HPV+ cervical dysplasia and cancer	Curative-intent treatment
NCT04857528	Detecting HPV DNA in Anal and Cervical Cancers	HPV+ cervical and anal cancers	Radiation therapy
NCT05307939	A Study on Using Cell-Free Tumor DNA (ctDNA) Testing to Decide When to Start Routine Treatment in People With Human Papilloma Virus (HPV)-Associated Oropharynx Cancer (OPC)	HPV+ OPSCC	Surveillance; adjuvant radiation therapy; cisplatin or carboplatin

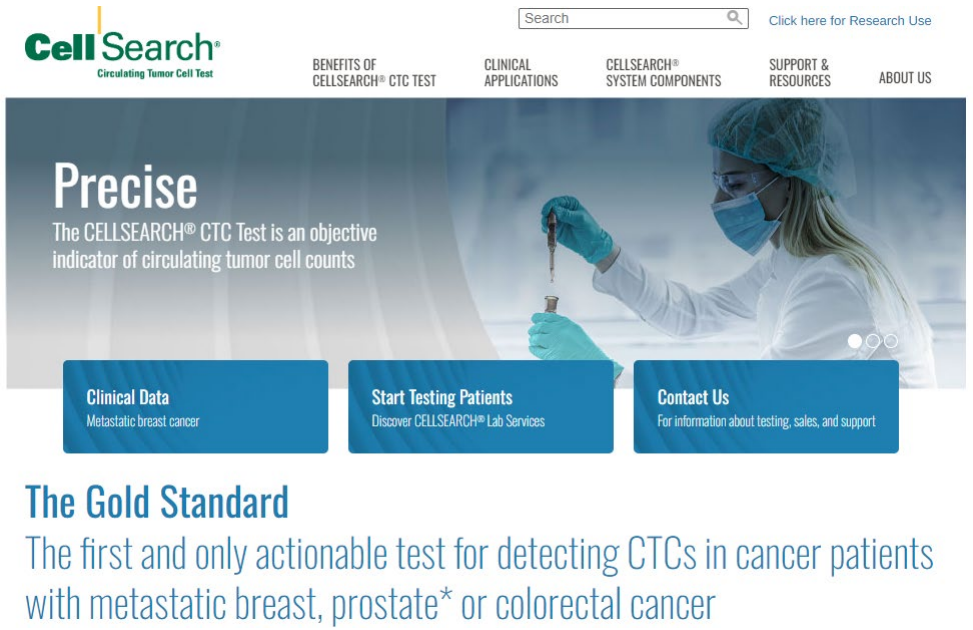
Circulating tumour cells, cfDNA methylation, miRNA

- CellSearch method (isolation of tumour cells of epithelial origin from blood CD45-, EpCAM+, cytokeratin 8, 18 a 19+), metastatic breast cancer, prostate and colorectal cancer, monitoring of progression

- Methylation of 5 genes in cf(ct)DNA saliva – HR=8.3

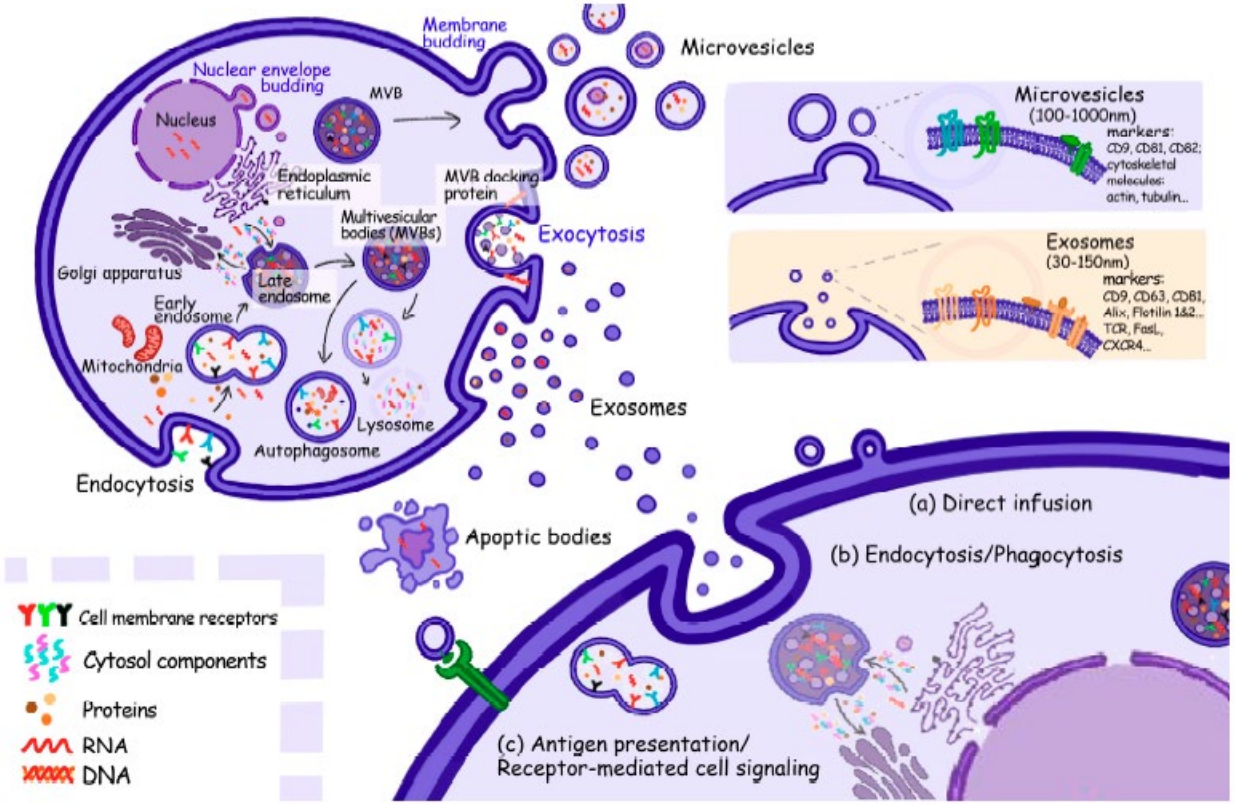
  Rapado-Gonzales et al., 2021

- miRNAs in plasma and saliva, panel of markers



Extracellular vesicles

- Vesicles enveloped with membrane, more stable content in comparison to cell-free circulating DNA/RNA
- Based on the biogenesis, size and surface markers:
  - Mikrovesicles (budding), 20-5000 nm
  - Exosomes (endocytosis, endosom, multivesicular bodies), 30-150 nm
  - Apoptotic bodies
- Different cargo (ExoCarta)(RNA, DNA, proteins, lipids)
- Cell communication, creation of tumour niche - metastasis
- Immunosupression, vascularization
- Compatible with host, stable in circulation, easy transport through membranes and barriers
- Biomarkers, transportation of molecules for treatment



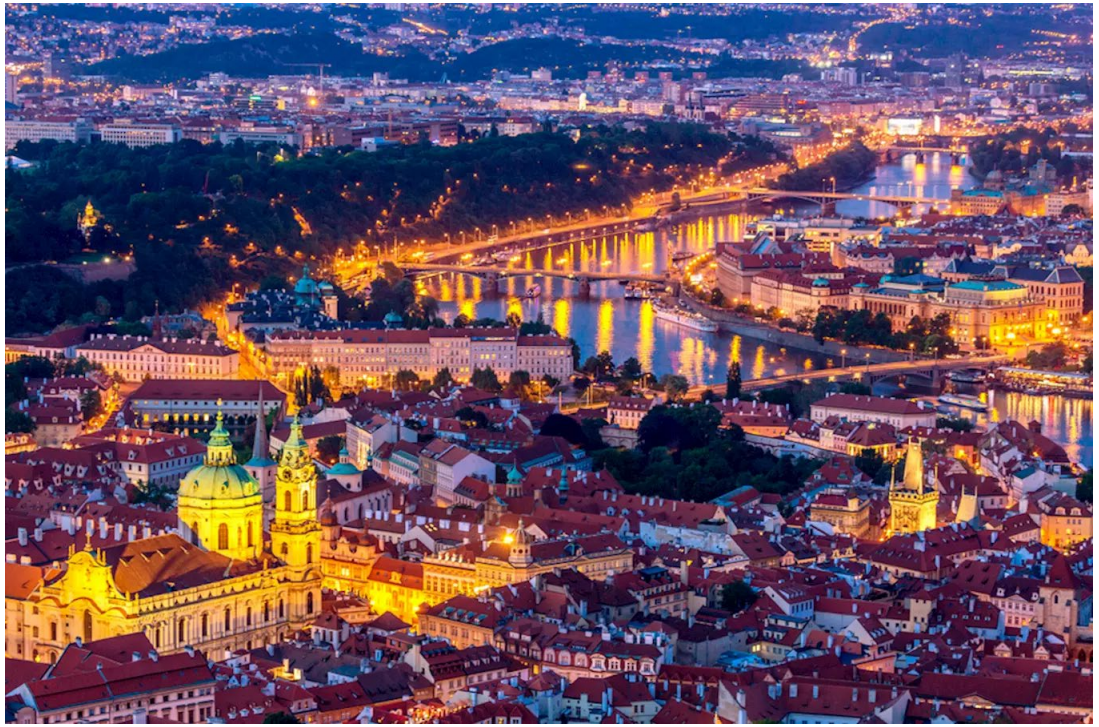
Summary I

- Discovery of HPV etiology of cervical cancer allowed improving the screening algorithms
- Discoveries of the causal relationship between HPV and part of cancers of the anatomical location led to the introduction of new management procedures for high-risk groups of patients
- Besides the detection of HPV DNA and RNA, the detection of HPV-specific antibodies and indirect markers of active HPV infection is introduced or are being evaluated

Summary II

- To specify aetiology of OPC the most precise is the detection of HPV mRNA or the combination of p16 and HPV DNA detection
- HPV-specific antibodies against early antigens can be detected many years before the diagnosis and their prevalence in the asymptomatic population is very low, they are the potential biomarker for screening
- The exploration of other targets, both viral, non-viral, in the bodily fluids, can lead to the specification of biomarkers or their groups for individualization of patients‘ treatment to improve survival and quality of life
- From the newly studied markers, the detection and monitoring of the dynamics of ctHPVDNA level seems the most promising
- All new methods need very precise standardization before introducing to routine clinical practice
- With more sensitive, specific, and sophisticated methods for biomarkers detection it is more and more difficult, since the results of these techniques are influenced by many parameters

Thank you for your attention !!!!!!!!!!!!!!!!



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