



# Détection plasmatique d'ADN d'HPV par droplet digital PCR (ddPCR) chez les patients atteints d'une tumeur liée à HPV

Emmanuelle Jeannot, PhD  
Pôle de Médecine Diagnostique et Théranostique  
Institut Curie

# HPV-associated cancers

## ➤ Cervical cancer: world public health issue

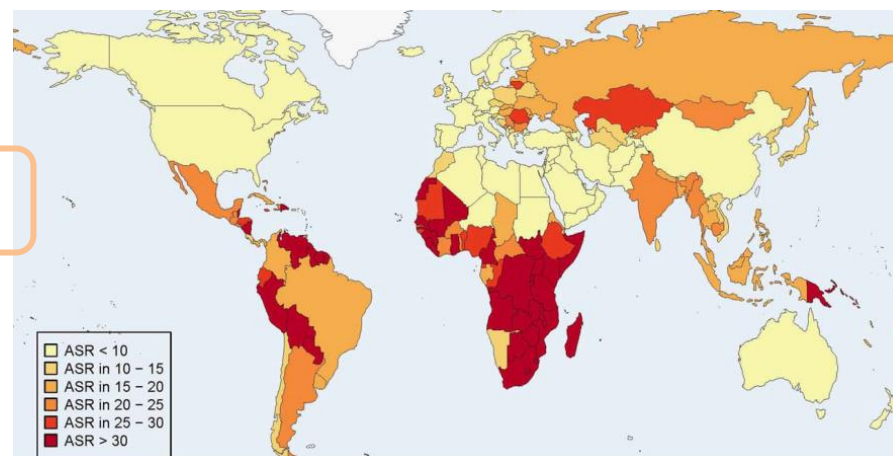
### KEY STATS.

About **527,624** new cervical cancer cases are diagnosed **annually** in **World** (estimates for 2012).

Cervical cancer **ranks\*** as the **4<sup>th</sup>** leading cause of female cancer in the **World**.

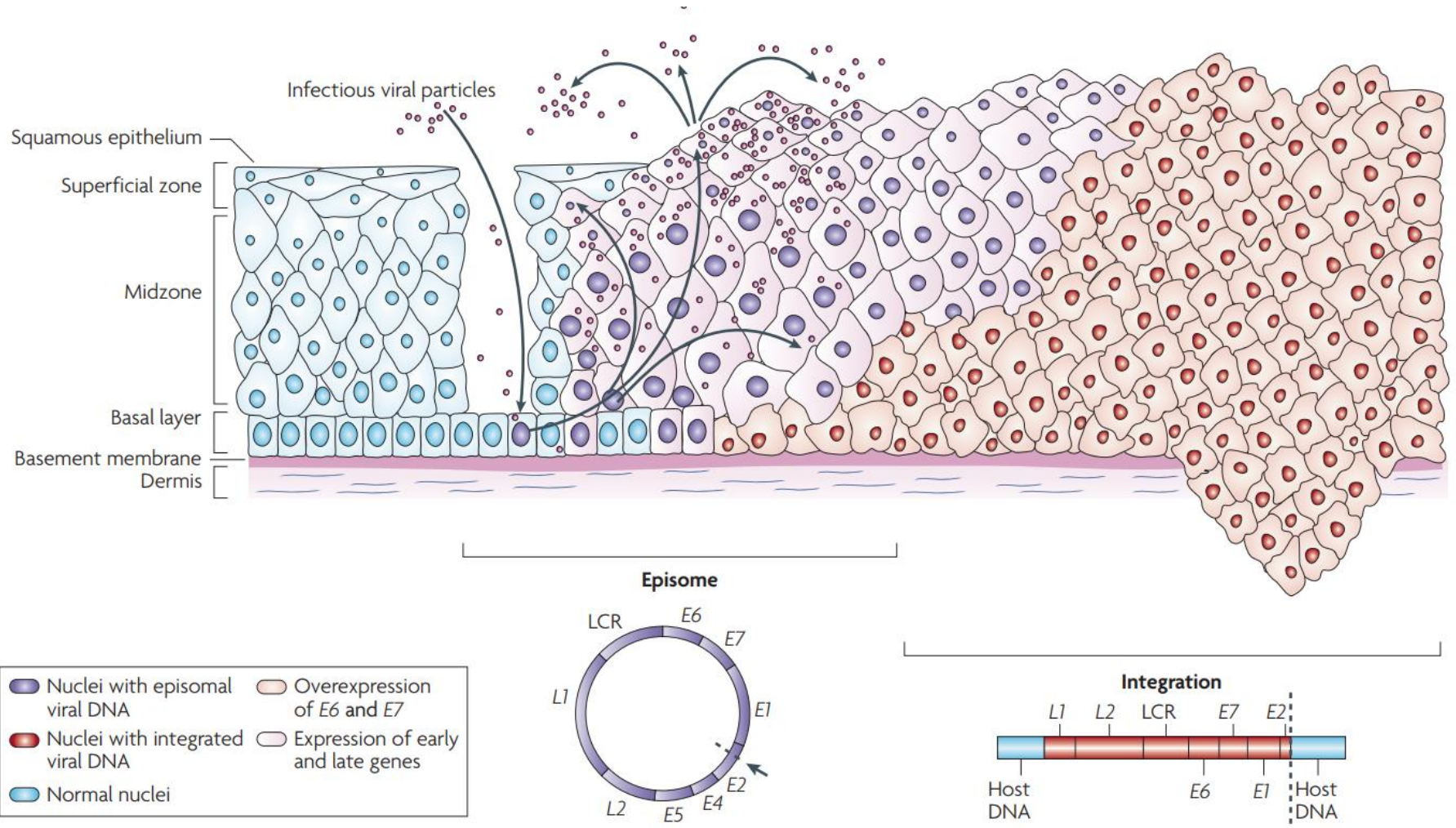
Cervical cancer is the **2<sup>nd</sup>** most common female cancer in the **women** aged **15 to 44 years** in **World**.

In France, 12<sup>th</sup> cause of mortality per cancer



hpvcenter.net

# Cervical carcinogenesis



Woodman et al. Nature Reviews Cancer, 2012

# HPV-associated cancers

## ➤ Cervical cancer, anus, oropharynx, vulva, penis, ...

**Table 3.** Relative contribution of HPV 16/18 or HPV6/11/16/18/31/33/45/52/58 to HPV-associated cancers by site and by sex; World, 2012

HPV-related cancer site (ICD-10 code)	Number attributable to HPV <sup>1</sup>	Relative contribution of HPV16/18 <sup>2</sup>		Relative contribution of HPV6/11/16/18/31/33/ 45/52/58 <sup>2</sup>	
		Percent	Number	Percent	Number
Cervix uteri (C53)	530,000	70.8	370,000	89.5	470,000
Anus (C21)	35,000	87.0	30,000	95.9	33,000
Vulva (C51)	8,500	72.6	6,200	87.1	7,400
Vagina (C52)	12,000	63.7	7,400	85.3	9,900
Penis (C60)	13,000	70.2	9,100	84.6	11,000
Head and neck (C01-06, C09-10, C32)	38,000	84.9	32,000	89.7	34,000
Total HPV-related sites in women	570,000	71.4	410,000	89.6	510,000
Total HPV-related sites in men	60,000	82.3	50,000	90.4	55,000
Total HPV-related sites	630,000	72.4	460,000	89.7	570,000

## ➤ 4,5% of new cases of cancers are HPV-associated cancers.

de Martel et al. *Int. J. Cancer*, 2017

# HPV-associated cancers

## ➤ Most frequent genotypes

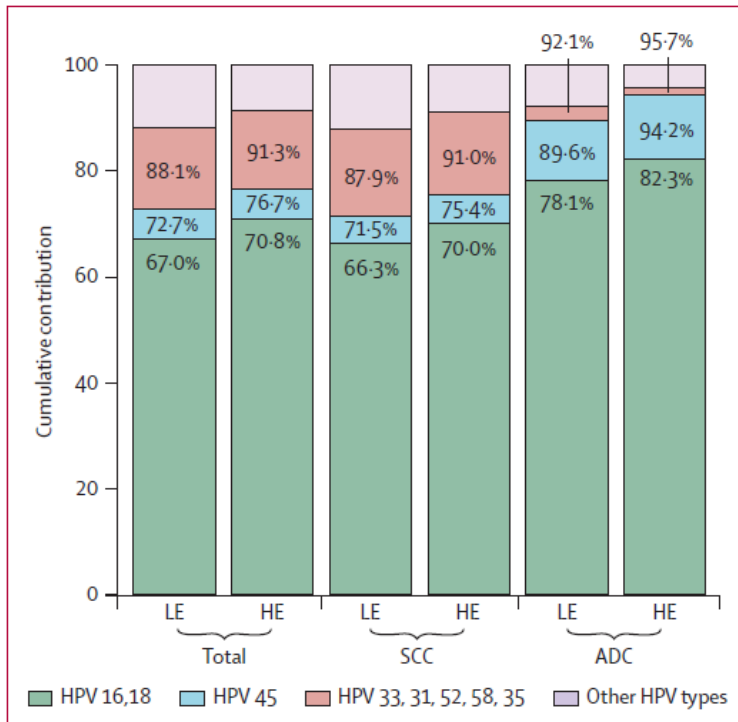


Figure 1: Cumulative relative contribution of the eight most common human papillomavirus (HPV) types as single-type and multiple-type infections by histological category of invasive cervical cancer

de Sanjose et al. *Lancet Oncol*, 2010

### Cervical cancer:

HPV 16 (61%)

HPV 18 (10%)

HPV 45 (6%)

HPV 31 et 33 (4%)

HPV 52, 58 et 35 ( 2 à 3%)

### Anal cancer:

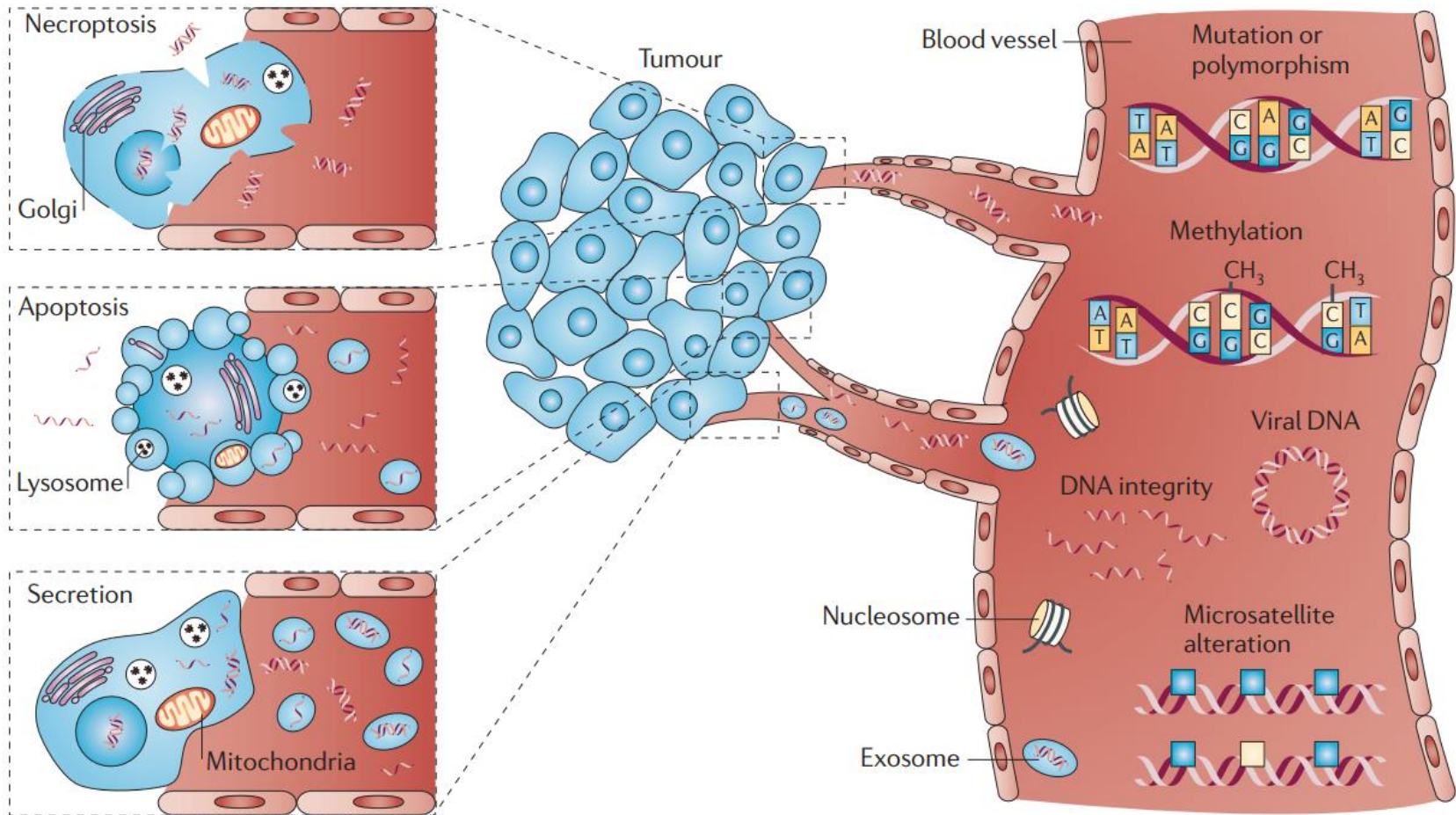
HPV 16 (90%)

HPV 18 (3%)

### Oropharynx cancer:

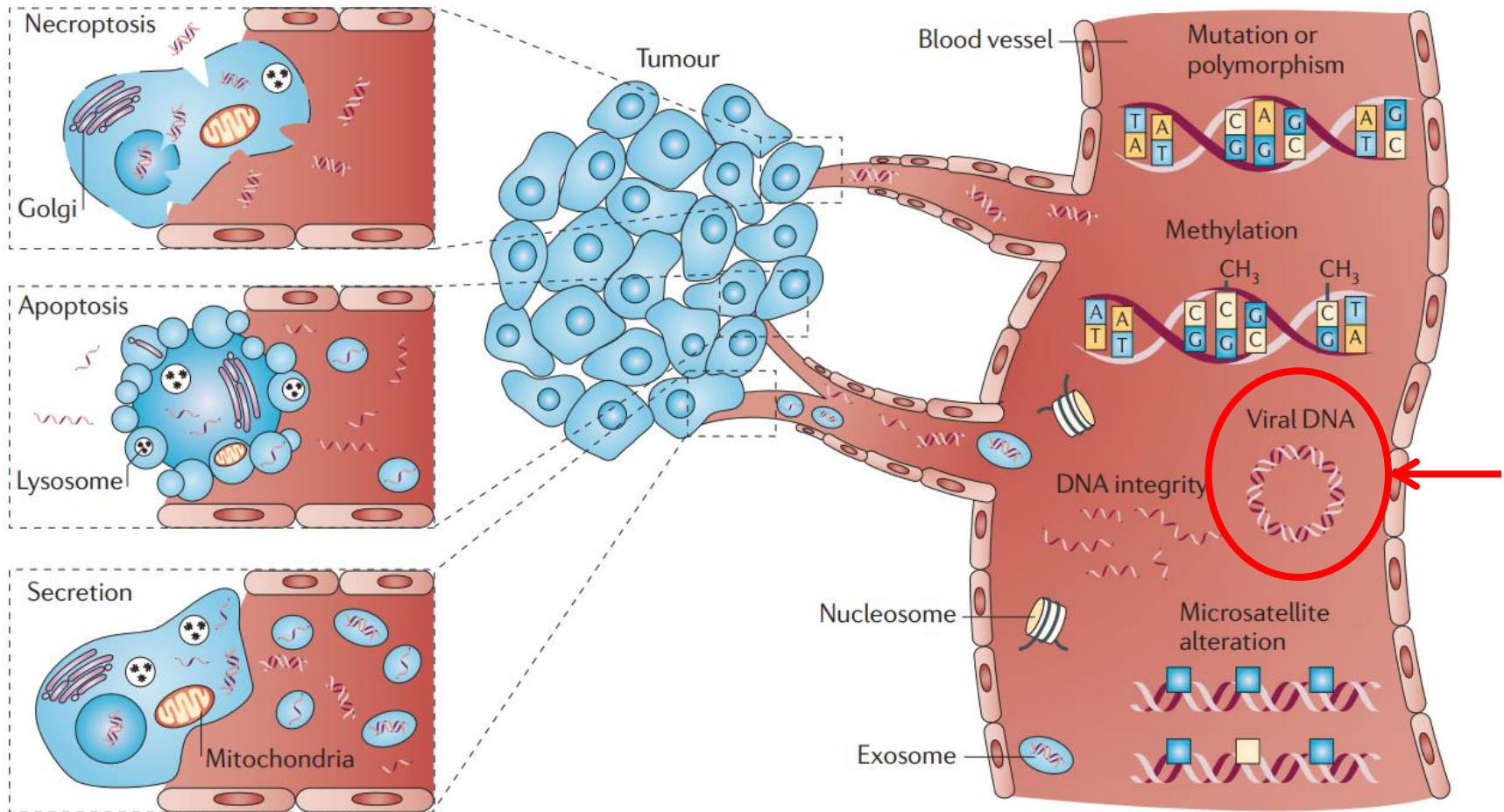
HPV 16 (90%)

# Circulating tumour DNA (ctDNA)



Schwarzenbach et al. Nature Reviews Cancer, 2011

# Circulating tumour DNA (ctDNA)



Schwarzenbach et al. Nature Reviews Cancer, 2011

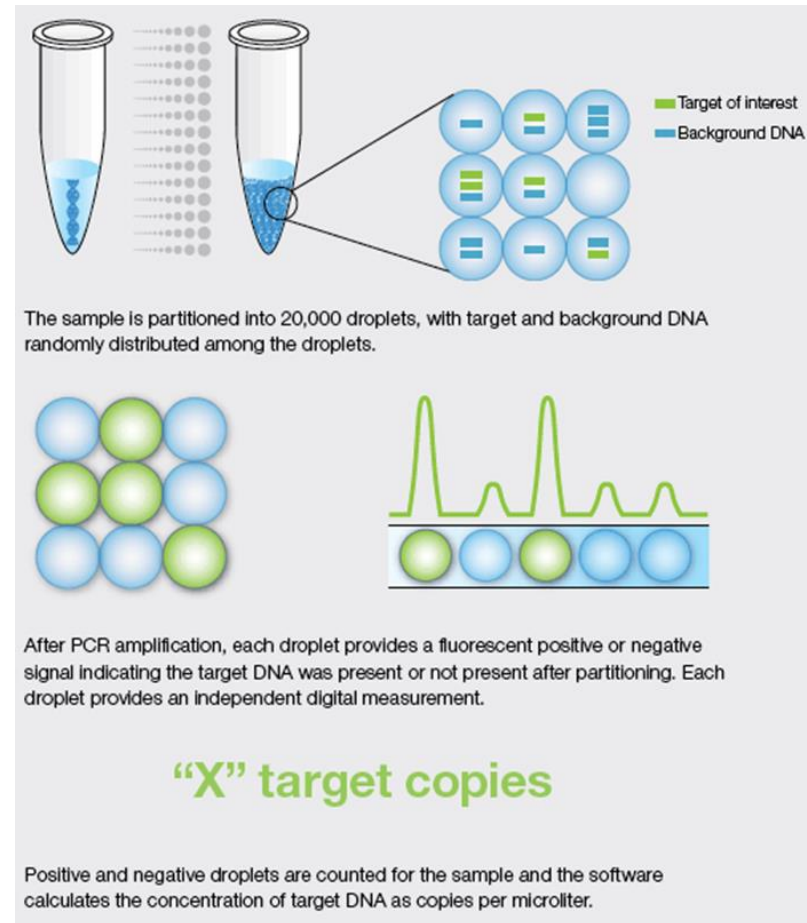
# Advantages of HPV model

- **Limitations of the ctDNA detection.**
  - Identification of a specific alteration in the primary tumour.
  - Low quantity of ctDNA.
  - Heterogeneity of tumour tissues.
  - Choice of a useful marker for treatment and follow-up.
- **Characteristics of HPV-related tumours:**
  - Circulating HPV DNA, viral load from 1 to >100 copies/cell.
  - HPV16: very frequent genotype (60 to 90% of HPV-related tumours).
  - Stability of the marker (HPV and cell/virus junction identified in the metastasis).

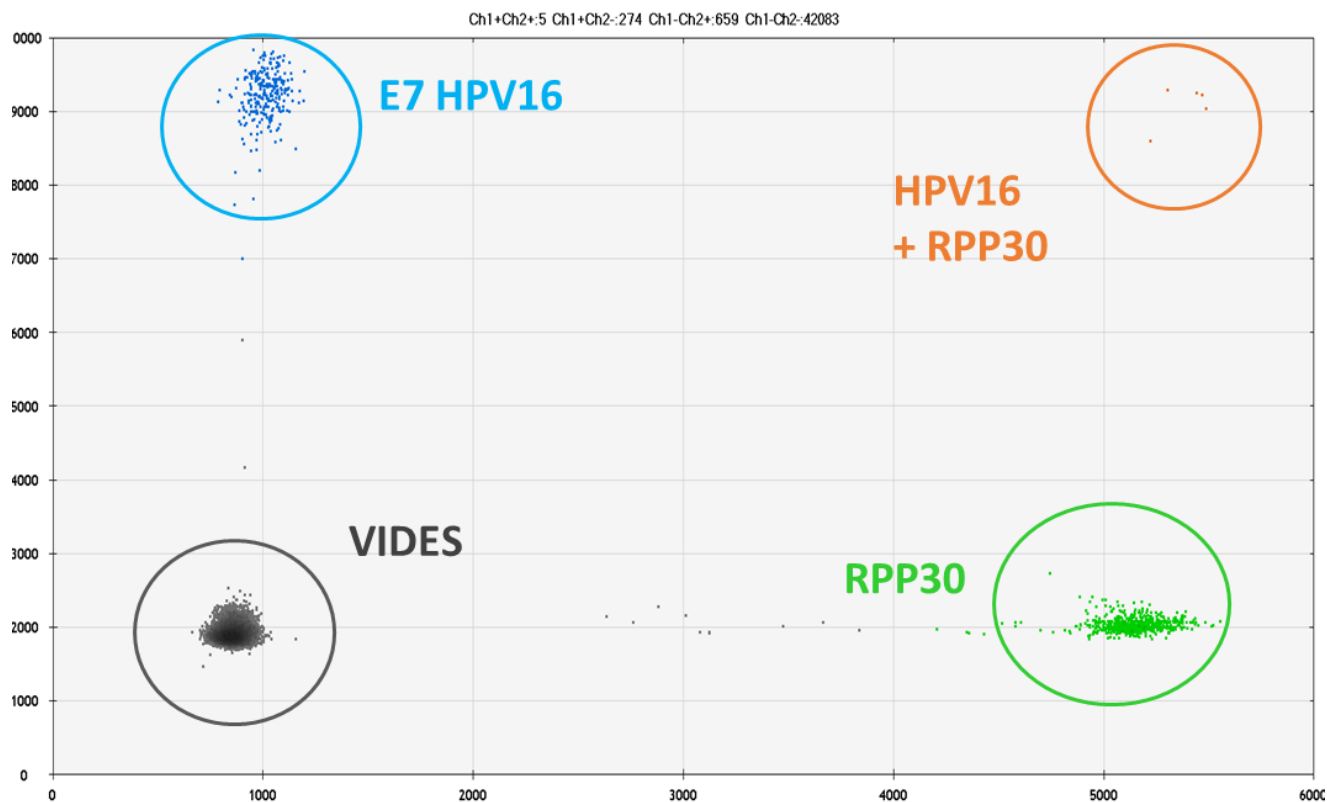


# ddPCR: droplet digital PCR, PCR of 3<sup>rd</sup> generation, Bio-Rad

- Partitioning in water-in-oil droplets
- PCR reaction with Taqman probes
- Analysis in flux cytometry
- Quantification in copie/ $\mu$ L



# ddPCR: droplet digital PCR, PCR of 3<sup>rd</sup> generation, Bio-Rad



*ddPCR for HPV16*

# ctDNA HPV : a new biomarker ?

- Detection by digital PCR (ddPCR) for HPV16 and HPV18
- HPV ctDNA in HPV-related cancers

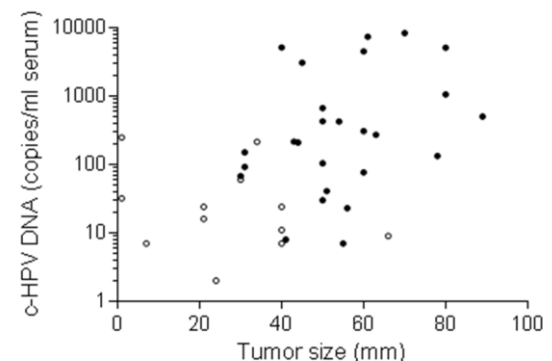
Detection of HPV ctDNA at baseline in 61/70 (87%) of the patients.

*Jeannot et al., 2016*

Table 1. Detection rate of c-HPV DNA using ddPCR and qPCR in patients diagnosed with HPV-associated invasive carcinoma

Tumour localization	Number of cases	Positive cases		$p^*$
		ddPCR N (%)	qPCR N (%)	
Uterine cervix	47	39 (83)	28 (60)	0.02
Anal canal	15	14 (93)	12 (80)	0.60
Head and Neck <sup>†</sup>	8	8 (100)	8 (100)	1.00
Total	70	61 (87)	48 (69)	0.01

- Positive correlation between the tumour size and the level of HPV ctDNA (cervical cancer, n=38).



# Potential applications in liquid biopsy

- **Prediction and early detection of relapse.**
- **Monitoring of the efficacy of treatment.**

# Prognostic Impact of HPV ctDNA : clinical validity (1)

## Clinical Cancer Research

### Prognostic Impact of Residual HPV ctDNA Detection after Chemoradiotherapy for Anal Squamous Cell Carcinoma

Luc Cabel, Emmanuelle Jeannot, Ivan Bieche, Sophie Vacher, Celine Callens, Louis Bazire, Adeline Morel, Alice Bernard-Tessier, Walid Chemlali, Anne Schnitzler, Astrid Lièvre, Joelle Otz, Mathieu Minsat, Anne Vincent-Salomon, Jean-Yves Pierga, Bruno Buecher, Pascale Mariani, Charlotte Proudhon, François-Clément Bidard, and Wulfran Cacheux

### 33 patients with localized anal cancer (SCCA) :

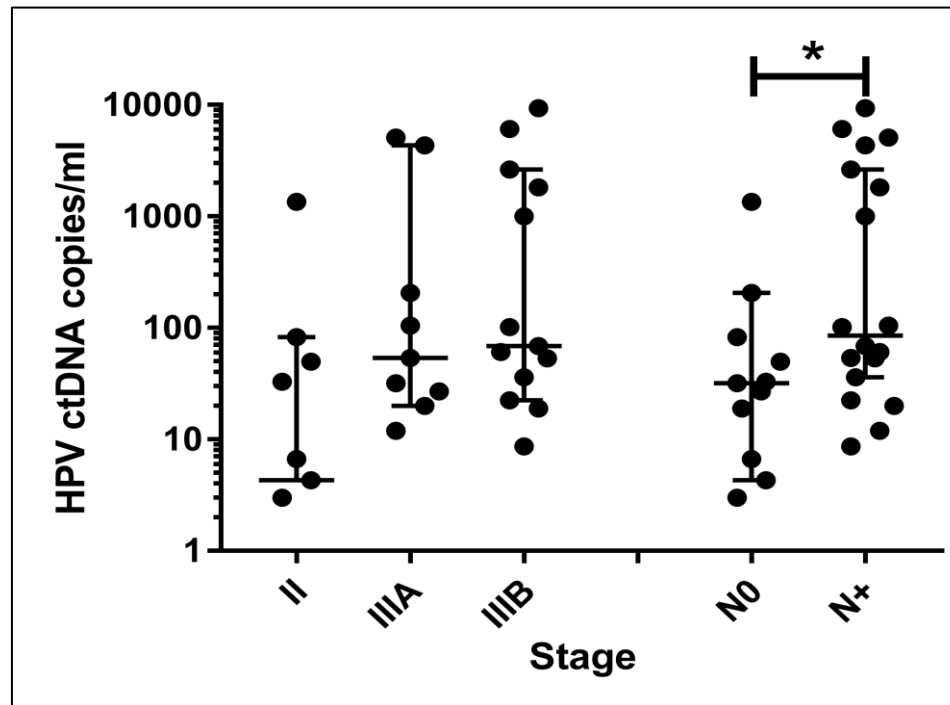
- Stage II and III treated by radiochemotherapy (RCC)
- HPV-16 or -18
- serum/plasma sample: before and after RCC (<30 days)

Characteristics	Pre-CRT samples	Post-CRT samples
	N=33 patients	N=18 patients
	N (%)	N (%)
<b>Tumor stage</b>		
Stage II	11 (33%)	4 (22%)
Stage IIIA	9 (27%)	7 (39%)
Stage IIIB	13 (40%)	7 (39%)
<b>HPV genotype</b>		
HPV 16	31 (95%)	17 (94%)
HPV 18	2 (5%)	1 (6%)

# HPV ctDNA level at diagnosis of localized SCCA

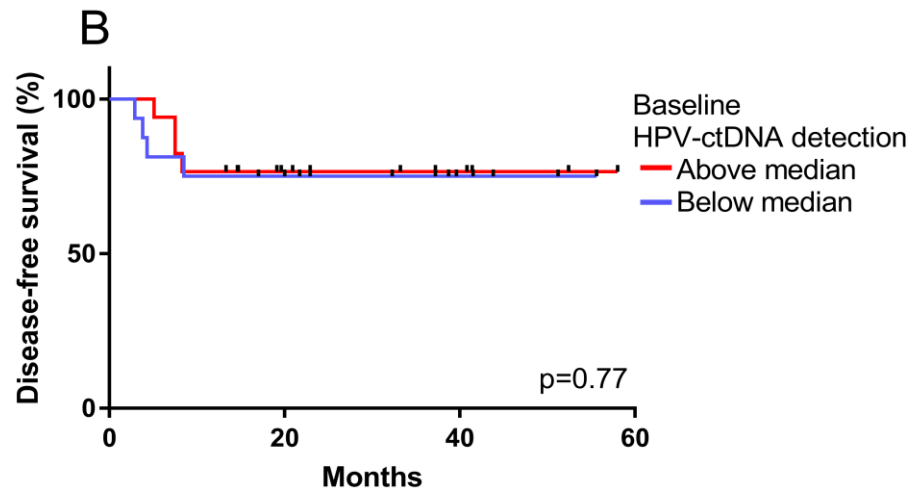
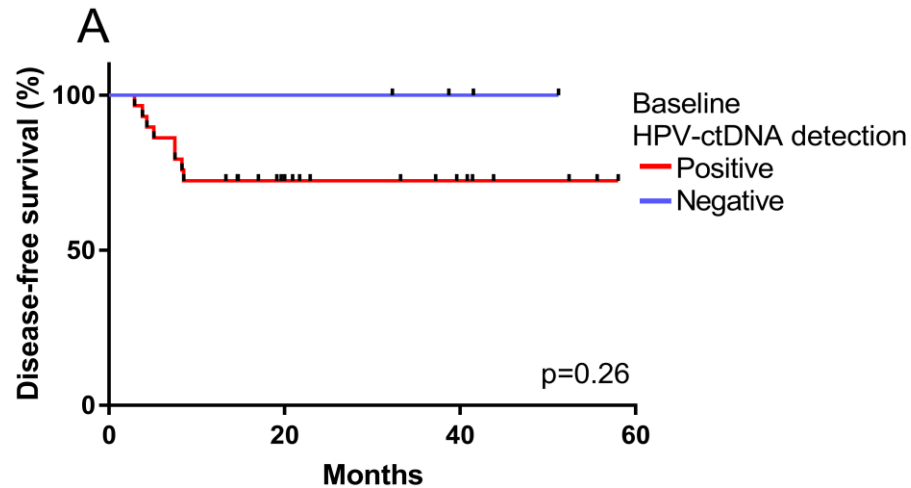
## Baseline detection in 88% of the patients :

- 36 copies/mL in serum/plasma
- HPV-16 or -18
- Level related to the stage and to the lymph node status



Cabel et al., 2018 Clin. Cancer Res.

# No significant impact of HPV ctDNA detection at baseline

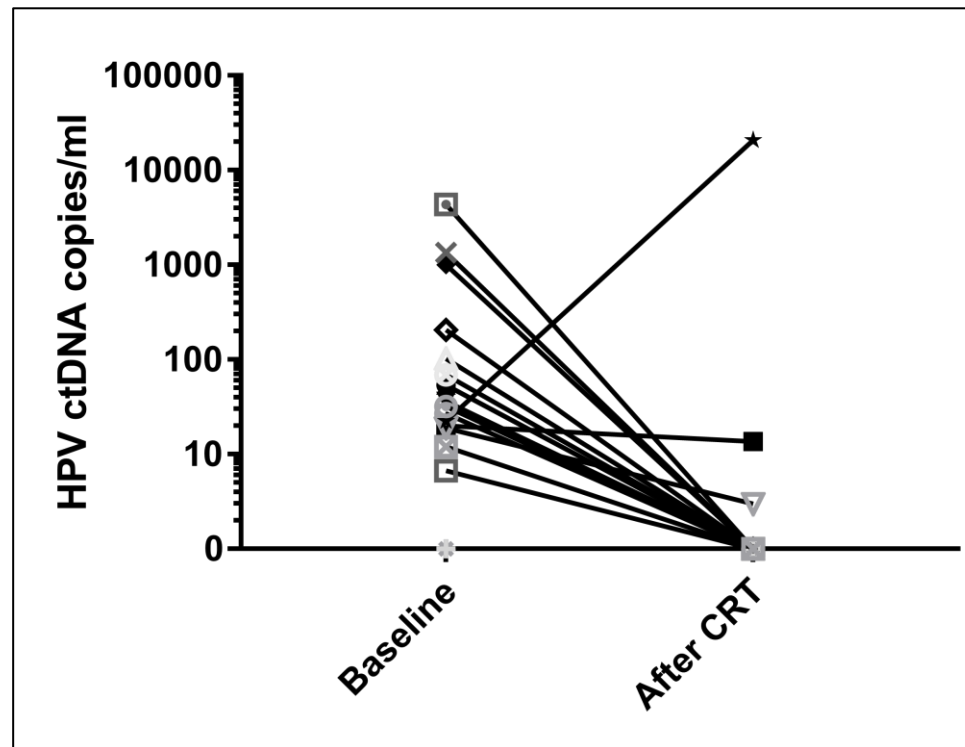


Cabel et al., 2018 Clin. Cancer Res.

# HPV ctDNA level changes during therapy

After RCC, detection in 17% of the patients :

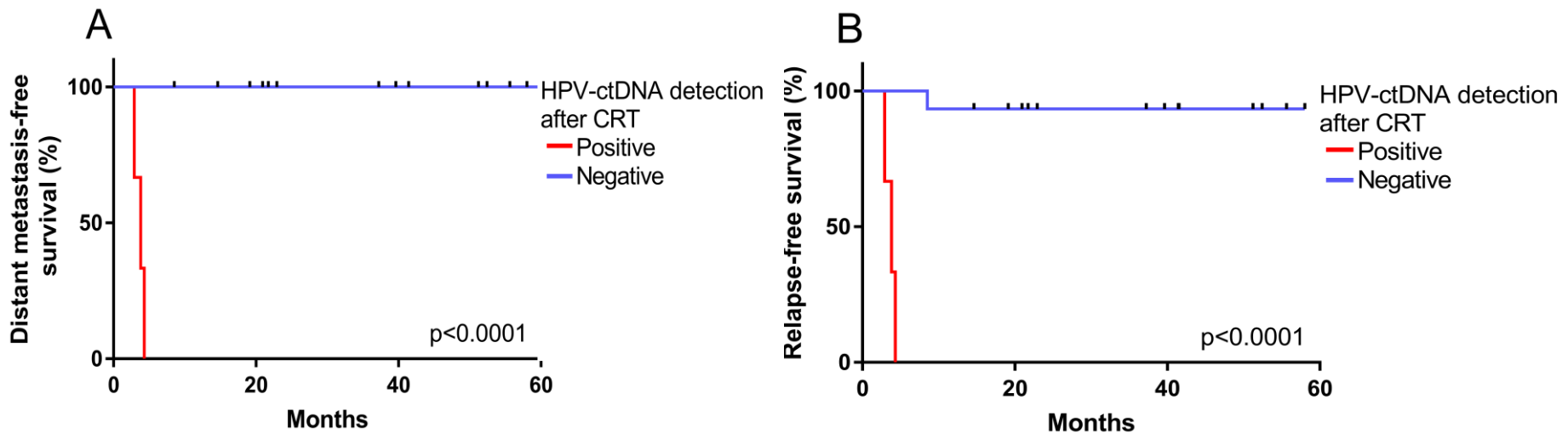
- Analysis of samples before/after treatment for 18 patients



Cabel et al., 2018 Clin. Cancer Res.



# Strong prognostic impact of HPV ctDNA detection at the end of treatment



1 patient HPV ctDNA negative locally relapsed at 8.5 months  
HPV ctDNA positive at relapse: 12 copies/mL

# Prognostic impact of HPV ctDNA : clinical validity (2)

**Clinical validity of HPV circulating tumor DNA in advanced anal carcinoma: an ancillary study to the Epitopes-HPV02 trial.**

## **59 patients with an advanced SCCA :**

- Metastatic or localized relapse without surgery
- HPV-16
- Serum sample: before and after chemotherapy

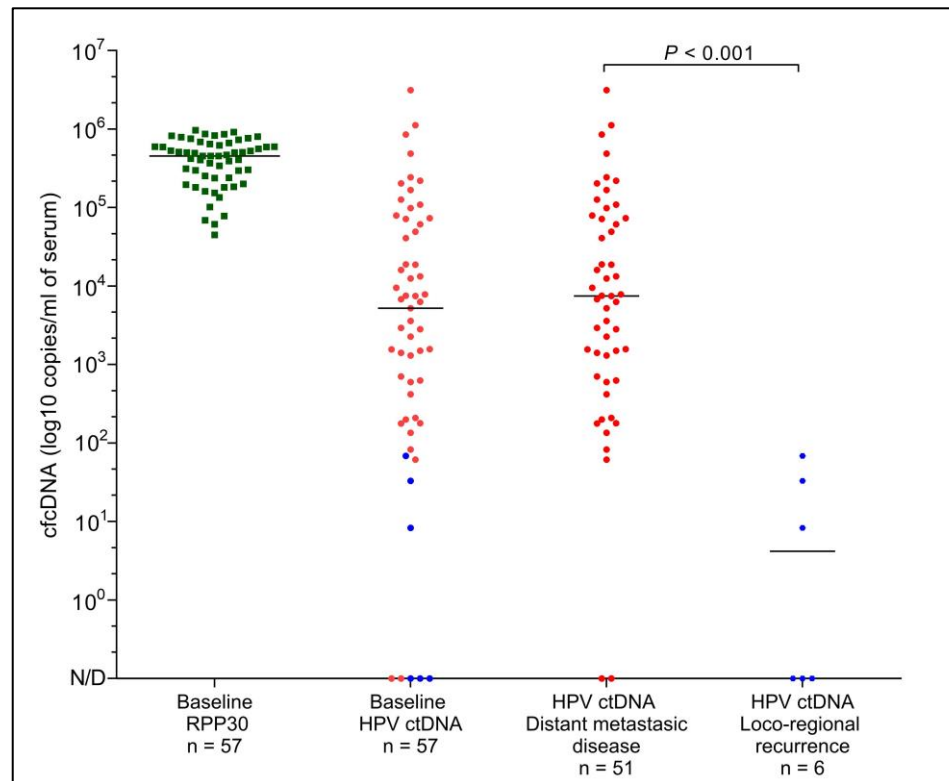
<b>Characteristics</b>	<b>All patients n=59 n (%)</b>	<b>Patients with paired samples n=42 n (%)</b>
<b>Age</b>		
Median (range)	60 (38-78)	59 (38-78)
<b>Gender</b>		
Female	49 (83%)	34 (81%)
Male	10 (17%)	8 (19%)
<b>Loco-regional recurrence</b>	6 (10%)	3 (7%)
<b>Metastatic disease</b>	<b>53 (90%)</b>	<b>39 (93%)</b>
Metachronous	38 (64%)	27 (64%)
Synchronous	15 (26%)	12 (29%)

Bernard-Tessier et al., accepted in Clin. Cancer Res.

# HPV ctDNA level at diagnosis of advanced SCCA

## Baseline detection in 91% of the patients :

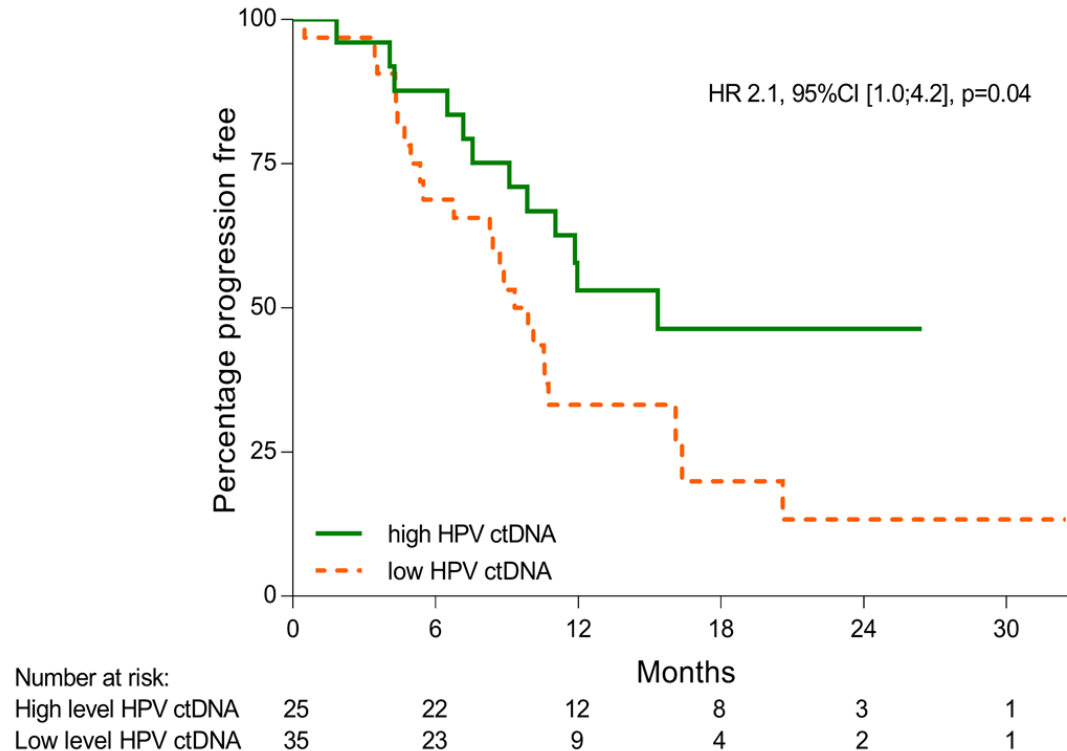
- Median of 7 148 copies/mL (8 – 3 147 000)
- level correlated to metastatic stage



Bernard-Tessier et al., accepted in Clin. Cancer Res.

# Prognostic impact of HPV ctDNA detection at baseline

- No impact of the HPV ctDNA on PFS (positive versus negative,  $p=0.91$ )
- Baseline level  $<2940$  copies/ml was associated with better PFS

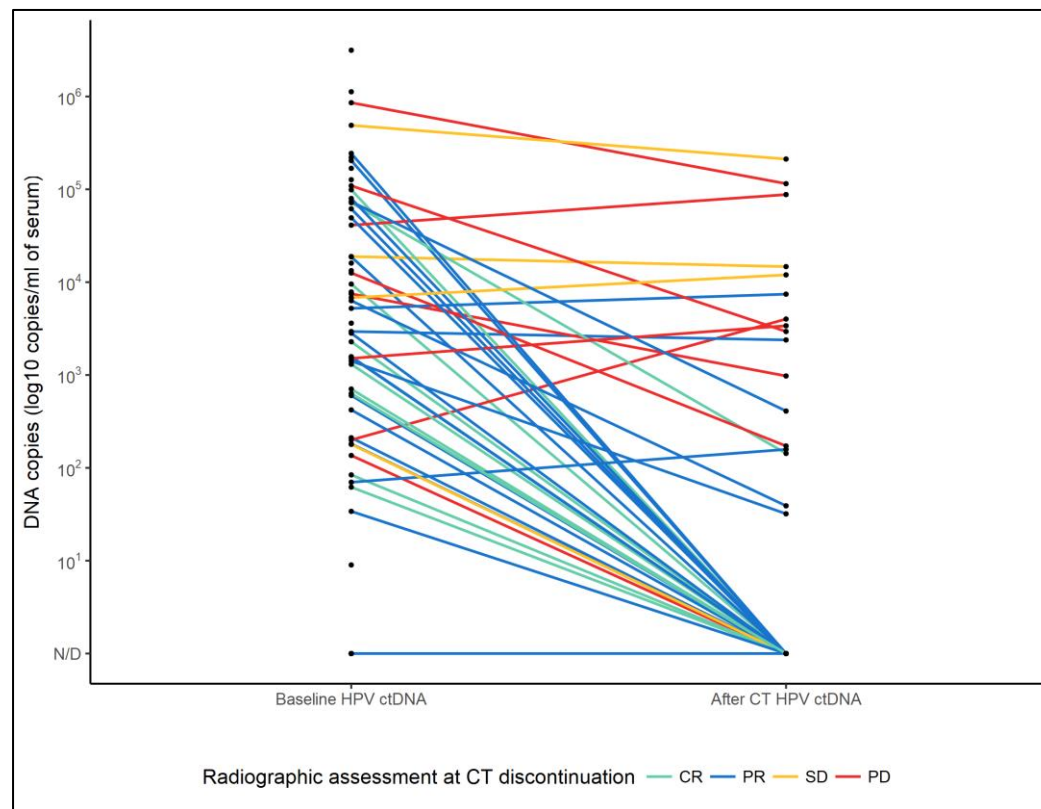


Bernard-Tessier et al., accepted in Clin. Cancer Res.

# HPV ctDNA level changes during therapy

## Detection after CT in 39% of the patients :

- Serum sample before/after in 44 patients
- Median 2 662 copies/mL (31-211 950)



Bernard-Tessier et al., accepted in Clin. Cancer Res.

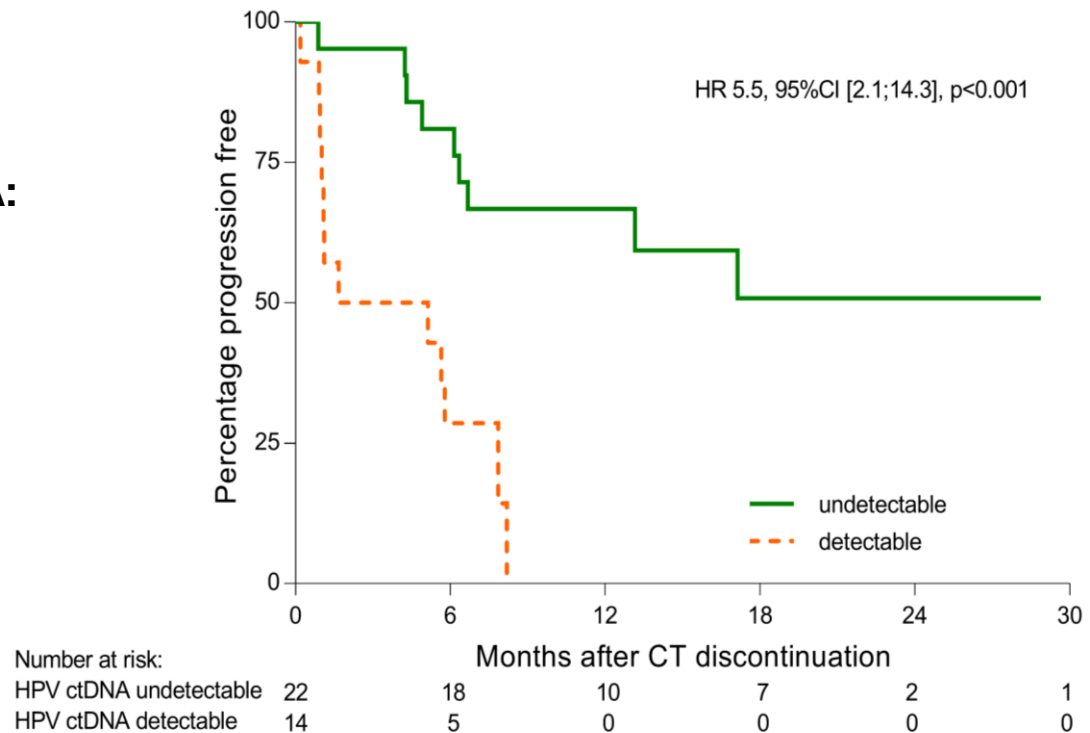
# Strong prognostic impact of HPV ctDNA detection after CT

## Detection after CT in 39% of the patients :

- Serum sample before/after in 44 patients
- Median 2 662 copies/mL (31-211 950)

**Undetectable HPV ctDNA:**  
Median PFS not reached


**Detectable ctDNA HPV:**  
Median PFS = 3.4 months

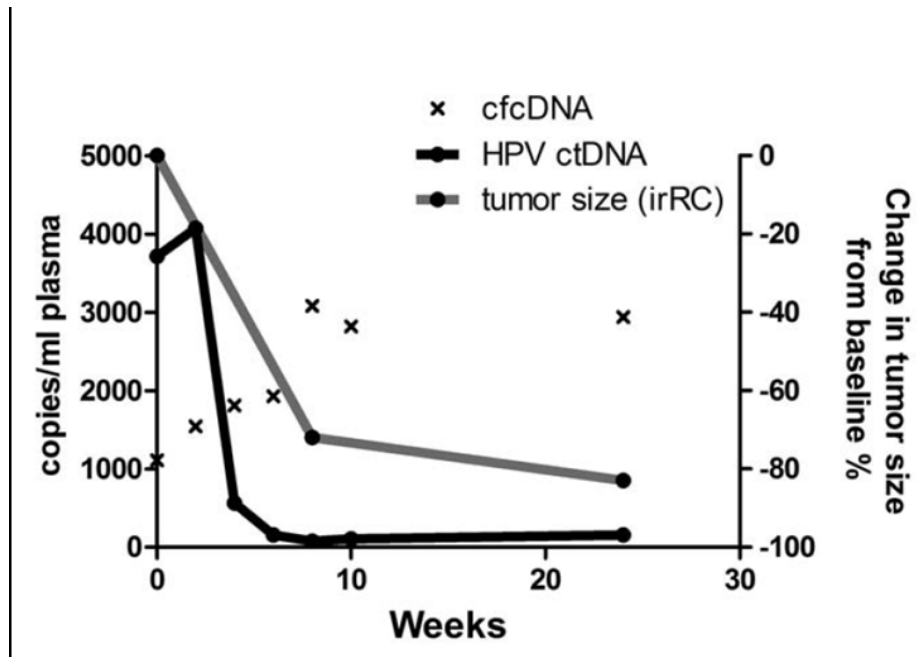


Bernard-Tessier et al., accepted in Clin. Cancer Res.

# Monitoring of the efficacy of immunotherapy

## HPV circulating tumor DNA to monitor the efficacy of anti-PD-1 therapy in metastatic squamous cell carcinoma of the anal canal: A case report

Luc Cabel <sup>1</sup>, François-Clément Bidard<sup>1,2</sup>, Vincent Servois<sup>3</sup>, Wulfran Cacheux<sup>1</sup>, Pascale Mariani<sup>4</sup>, Emanuela Romano<sup>1,5</sup>, Mathieu Minsat<sup>6</sup>, Ivan Bieche<sup>7,8</sup>, Fereshteh Farkhondeh<sup>9</sup>, Emmanuelle Jeannot<sup>7</sup> and Bruno Buecher<sup>1</sup>



**Significant early response of the level of HPV ctDNA at 4 weeks.**

Cabel et al., 2017 Int. J. Cancer.

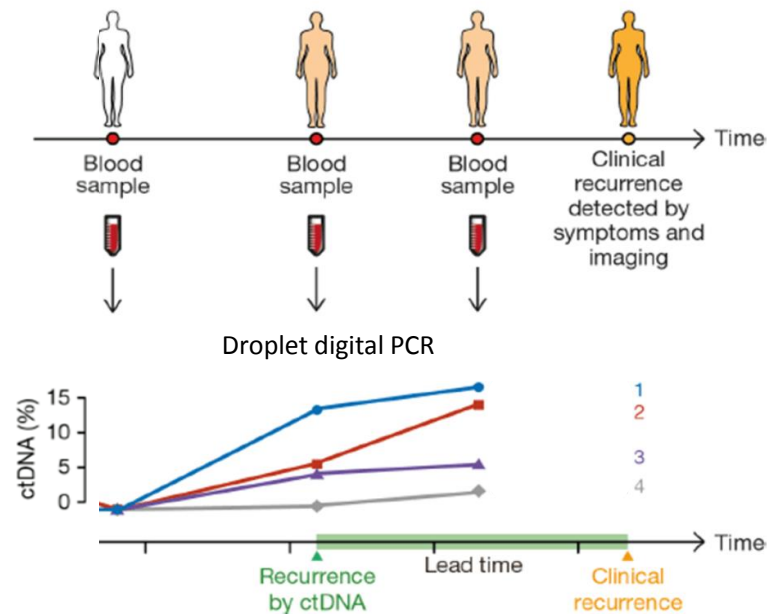
# Clinical validity demonstrated in SCCA

- **HPV ctDNA detection with a high sensitivity**
- **Localized and metastatic stage**
- **Strong prognostic of the residual detection after (radio)-chemotherapy**
- **No validated prognostic marker in metastatic anal cancer**
- **Useful for monitoring the efficacy of therapy.**



# Perspectives

- Other tumour localizations
- Clinical interest:
  - CirCA-HPV : follow-up of pelvic tumors after RCC
  - Prospective cohortes (anus, cervix, vulva)



# Perspectives

- **Other tumour localizations**
- **Clinical interest: follow-up**
- **Detection of other genotypes**
  - Detection for HPV45, 31, 33, 35, 52, 58



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