

# Circulating Plasma DNA as marker of therapy response in ovarian cancer

A study of the OVCAD consortium

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# Circulating Cell-free DNA

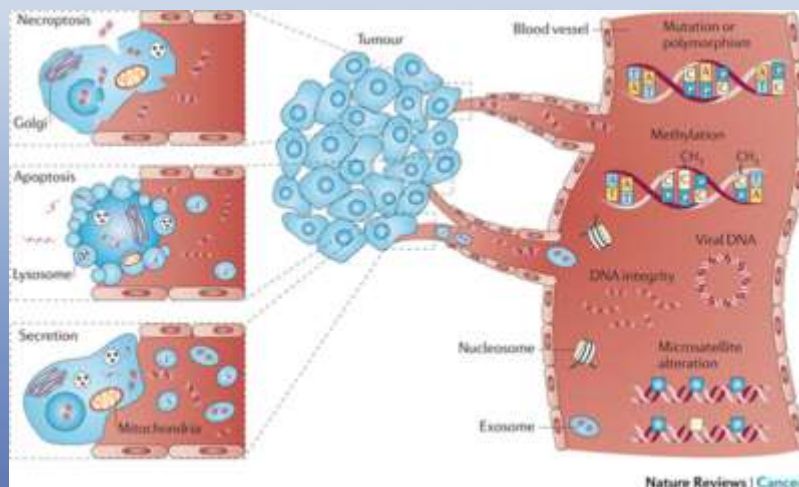
1948 Mandel et Métais

Apoptotic cell death

$10^{11}$ – $10^{12}$  cells per day (primarily haematogenous)

uniform DNA fragments (180-200bp)

100 ng/ml DNA in healthy blood plasma



Courtesy of Schwarzenbach et al.  
*Nature Reviews Cancer* 2011

# Elevated levels of cfDNA

## Cause

↑ cell death rates  
proliferation  $\neq$  cell death

## Conditions

### Physiological

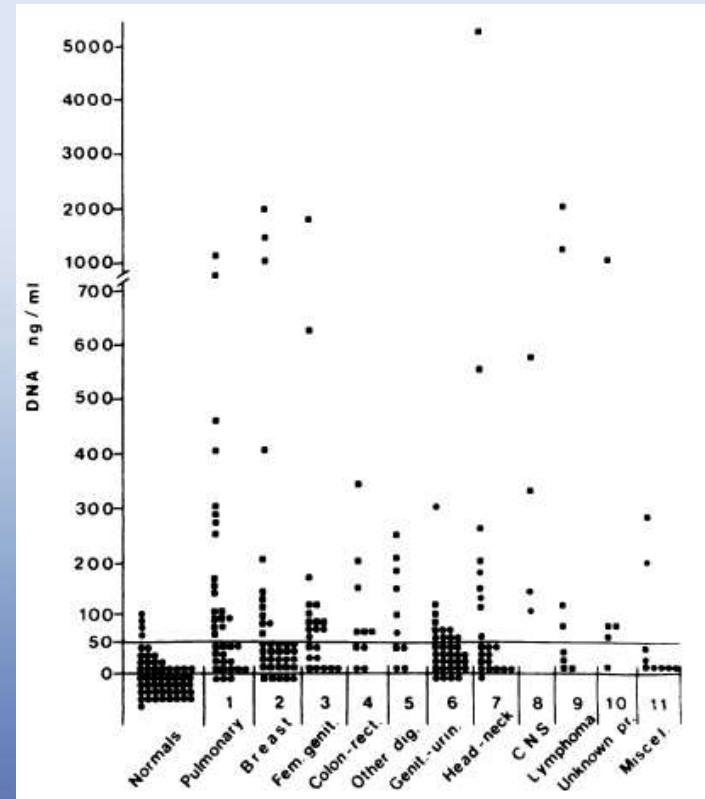
Old age, severe exercise,....

### benign diseases

SLE, trauma, burns, sepsis, diabetes,  
cerebral stroke, myocardial infarction,  
graft rejection,...

→ malignant diseases

Courtesy of Leon et al.  
*Cancer Res* 1977



# cfDNA as diagnostic tool

## Potential use

- diagnosis and staging
- monitoring the efficacy of cancer therapy
- estimating prognosis

## Challenge

- proportion of tumor- and nontumor-derived cfDNA

  - LOH, mutations (*TP53*, *KRAS*, *BRAF*), methylations,...

  - copy number variation

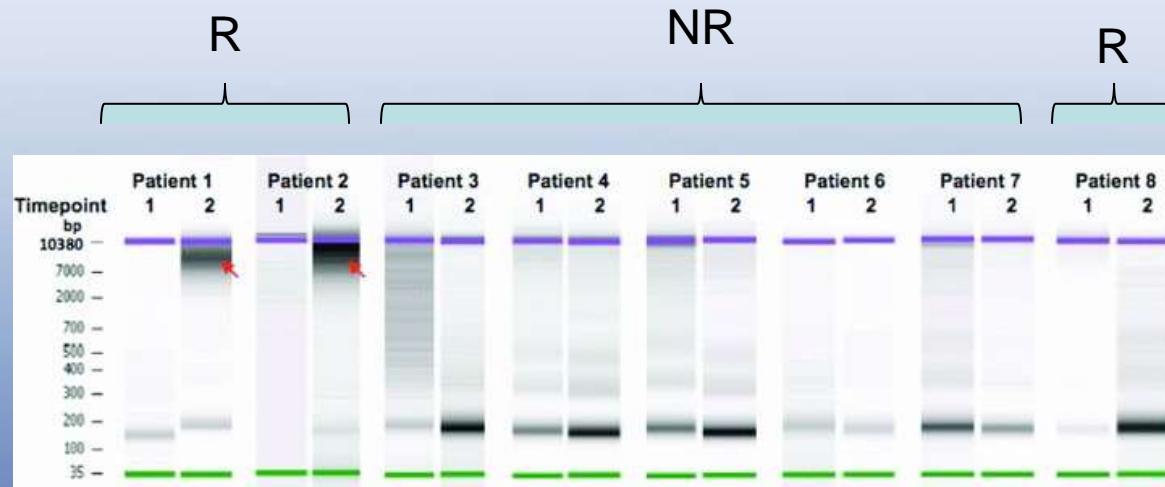
  - ⇒ **DNA integrity**

    - e.g. ALU and LINE1*

    - ratio of apoptotic (180 bp fragments) to necrotic cfDNA (long fragments)

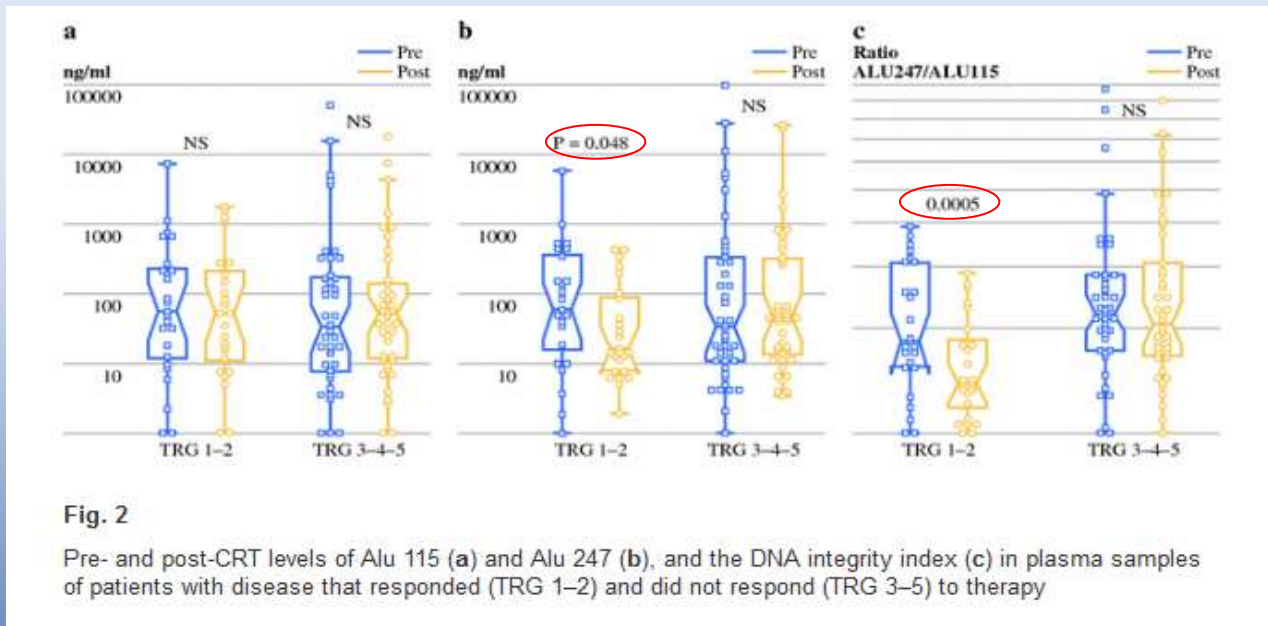
# DNA integrity reflects therapy response

Prostate cancer patients treated with docetaxel  
PET/CT to evaluate tumor response



Courtesy of Kwee et al.  
*Clin Transl Sci.* 2012

# Rectal cancer patients treated with chemoradiotherapy



Courtesy of Agostini et al.  
*Ann Surg Oncol.* 2011

# cfDNA in ovarian cancer ?



## OVARIAN CANCER – DIAGNOSIS OF A SILENT KILLER (OVCAD)

The 6th Framework Programme for Research, Technological Development and Demonstration

### Diagnosis

66.700 new diagnoses annually

80% advanced stage

5-year OS < 40%

### Treatment

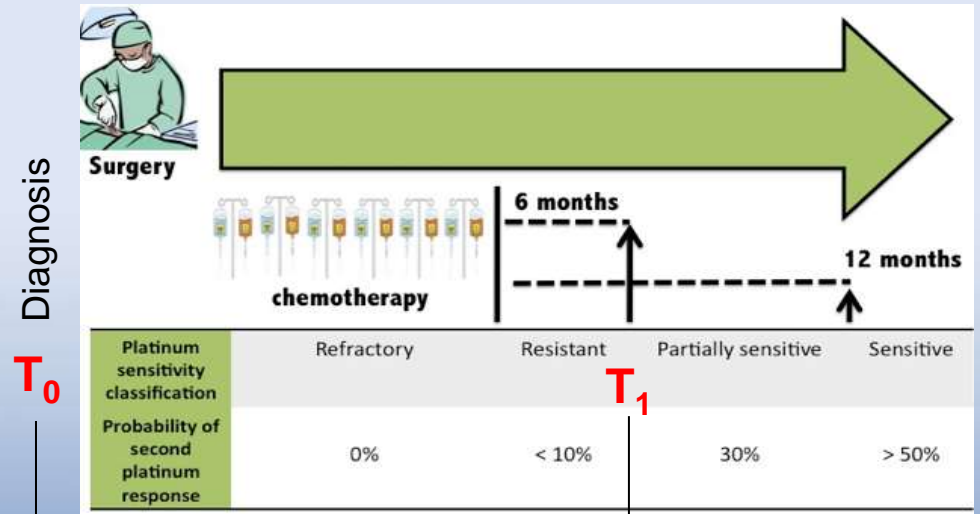
surgery & chemotherapy (Pt)

80% initial response rate

### Prognosis

70% relapse rate (DFS 18-24 months)

↓ chemoresponse



Gonzalo Tapia and Ivan Diaz-Padilla (2013). Molecular Mechanisms of Platinum Resistance in Ovarian Cancer, Ovarian Cancer - A Clinical and Translational Update

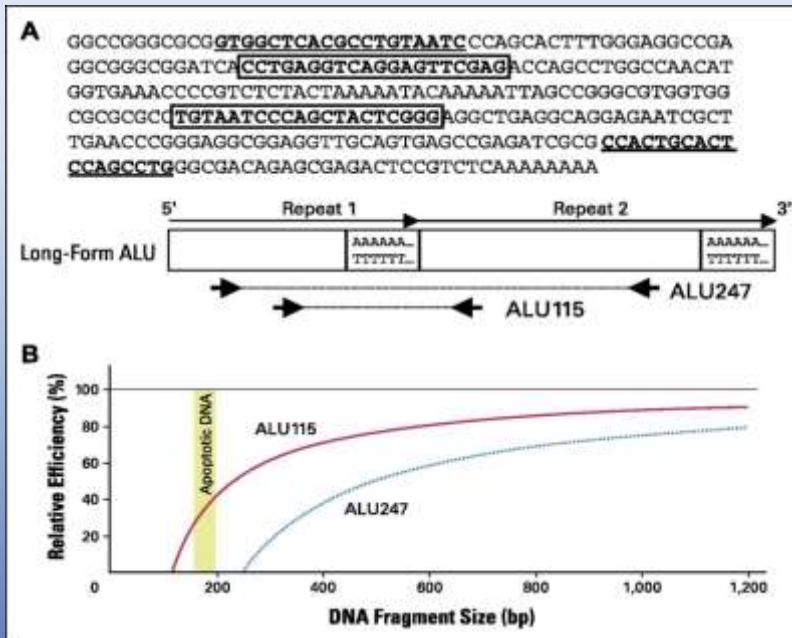
Prognostic value of cfDNA in ovarian cancer ?



# Methodological approach

**ALU specific PCR**  
about 1.4 million copies

**DNA integrity index**  
Long/short fragments  
0 – 1



Courtesy of Umetani et al.  
JCO 2006

	ALU247	ALU115
specificity	tumoral	total
origin	necrotic	apoptotic
<b>DNA integrity index</b>	$\frac{ALU247}{ALU115}$	

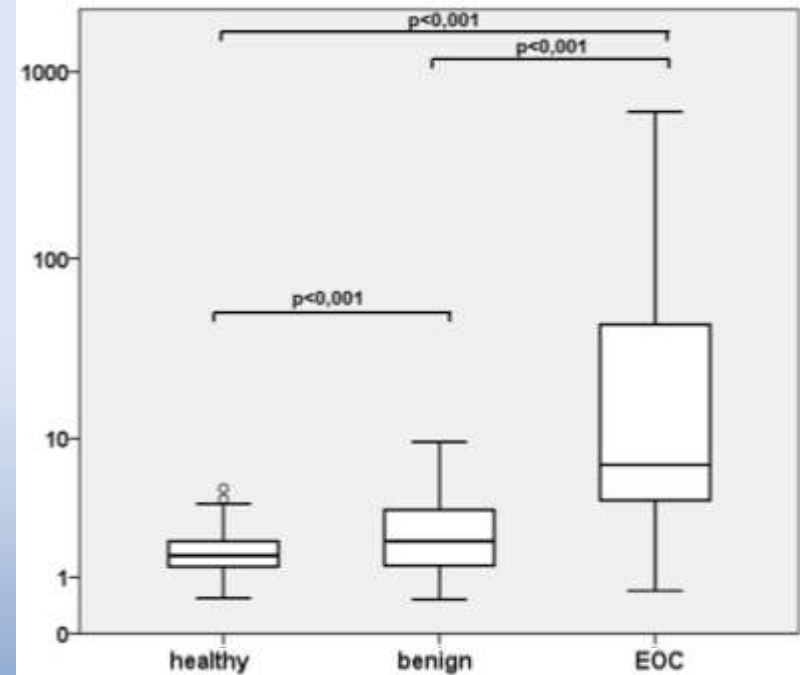
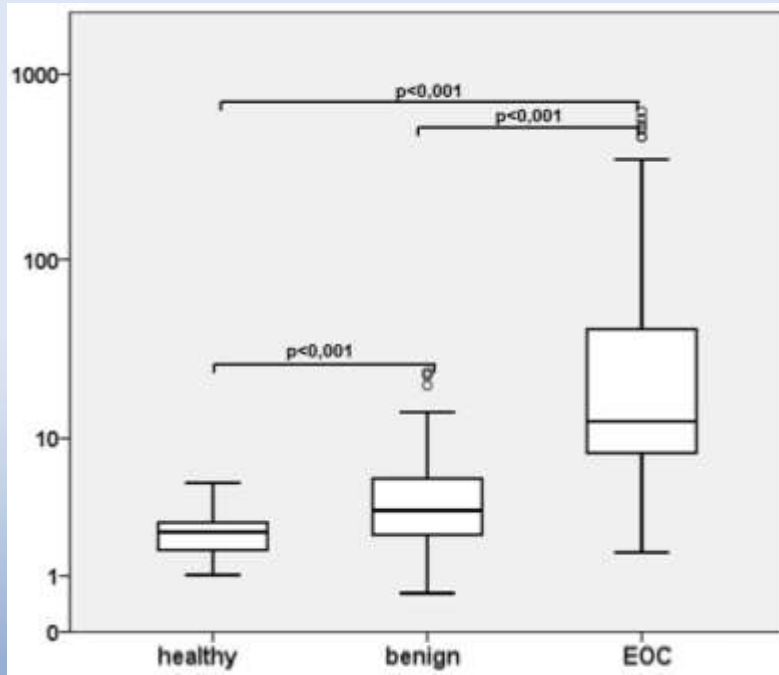
associated with

- Malignancy
- chemoresponse



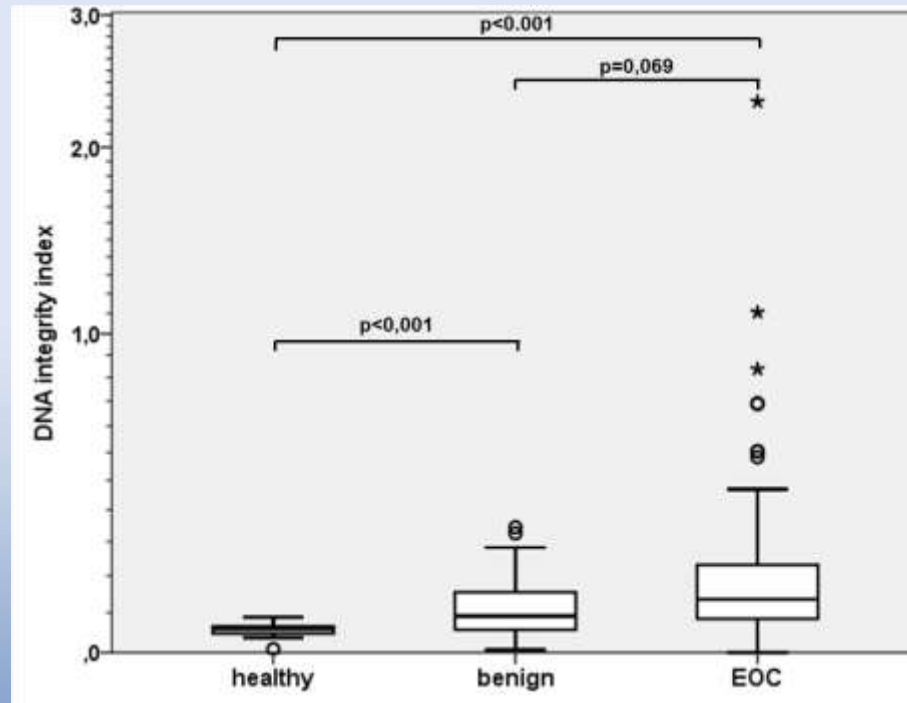
# total cfDNA

# tumoral cfDNA



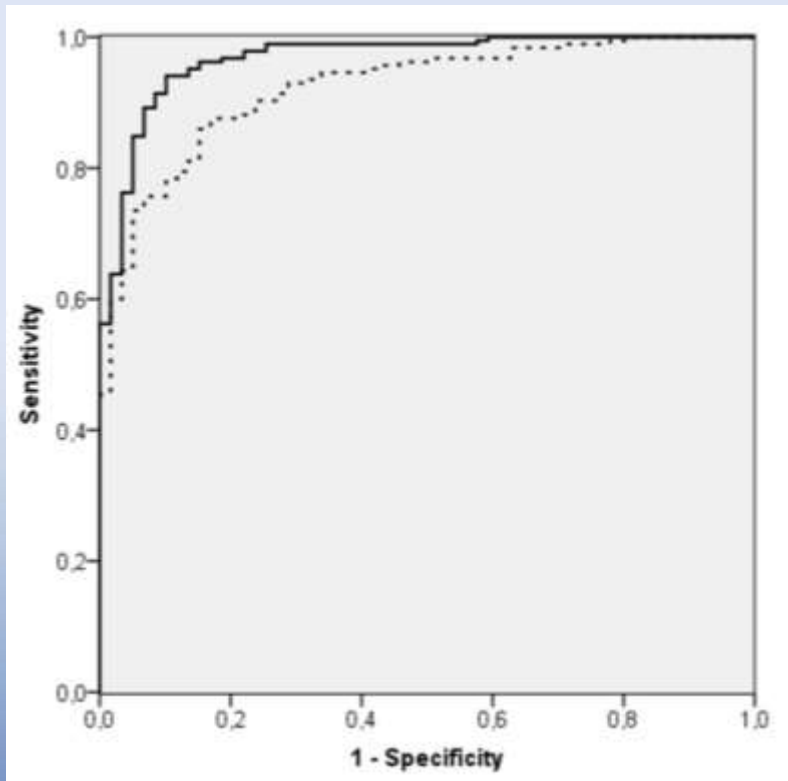
	Healthy (N=32)	Benign (N=82)	EOC (N=188)
<b>Total cfDNA (ALU 115)</b>	2.5 ng/ml (1.0 – 5.4)	3.5 ng/ml (0.6 – 23.9)	12.7 ng/ml (1.7 – 630.0)
<b>Tumoral cfDNA (ALU247)</b>	1.6 ng/ml (0.6 – 5.0)	2.1 ng/ml (0.5 – 9.6)	7.0 ng/ml (0.7 – 609.1)

# DNA integrity at baseline



	Healthy (N=32)	Benign (N=82)	EOC (N=188)
DNA integrity ALU247/ALU115	0.05 (0.01 - 0.08)	0.08 (0.01 - 0.31)	0.12 (0.0 - 2.31)

# Diagnostic power



**Area Under the Curve**

Test Result Variable(s)	Area	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Q115_Quant_VD	,890	,024	,000	,843	,937
Q247_Quant_VD	,857	,026	,000	,806	,909
DNAint_Q	,639	,040	,001	,561	,717
CA125	,922	,018	,000	,887	,957
Predicted probability	,967	,012	,000	,944	,990

a. Under the nonparametric assumption  
b. Null hypothesis: true area = 0.5

# Prognostic value of cfDNA (T<sub>0</sub>)

## clinical/histopathological parameters

	n	Total cfDNA median (Q1; Q3)	p	Tumoral cfDNA median (Q1; Q3)	p	DNA integrity median (Q1; Q3)	p
<b>Distant metastasis</b>			0,018		0,002		0,067
M0	92	11,7 (7,2; 18,7)		6,0 (4,1; 9,7)		0,12 (0,10; 0,21)	
M1	38	15,6 (9,8; 102,5)		12,7 (4,9; 91,1)		0,11 (0,07; 0,16)	
<b>Peritoneal carcinomatosis</b>			0,124		0,015		0,095
no	60	12,3 (7,1; 20,5)		5,9 (3,8; 11,4)		0,14 (0,09; 0,25)	
yes	120	12,8 (8,8; 54,2)		8,4 (4,4; 82,5)		0,11 (0,08; 0,18)	

Age, FIGO, grade, residual tumor mass after debulking, ascites  
CTCs (Obermayr et al., *Gyn Oncol.* 2013)

} Not significantly associated

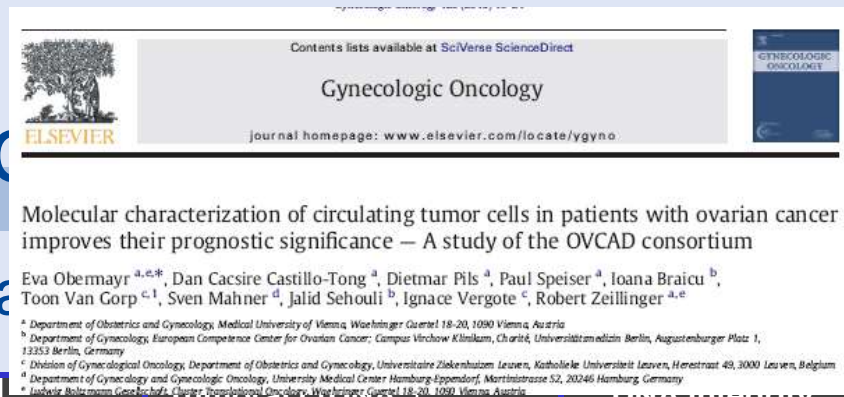
response to treatment

Survival

disease free survival  
overall survival

} Not significantly associated

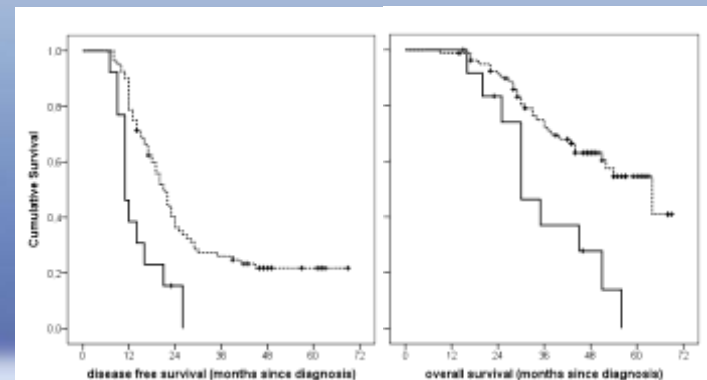
# Prognostic value of clinical/histopathological parameters



	n	Total cfDNA median (Q1; Q3)	p	Tumoral cfDNA median (Q1; Q3)	p	DNA integrity median (Q1; Q3)	p
<b>Distant metastasis</b>			<b>0.025</b>		0.055		0.831
M0	70	7,1 (4,1; 12,2)		4,5 (2,5; 8,5)		0,17 (0,12; 0,23)	
M1	17	10,4 (5,4; 26,0)		6,3 (4,5; 15,0)		0,20 (0,12; 0,23)	
<b>Chemoresponse</b>			0,521		0,227		<b>0,005</b>
yes	90	7,3 (4,2; 18,2)		4,8 (2,6; 16,8)		0,15 (0,11; 0,21)	
no	19	8,9 (5,1; 14,3)		5,1 (3,3; 7,8)		0,21 (0,15; 0,26)	
<b>CTCs</b>			0,695		0,834		<b>0,018</b>
positive	17	9,8 (6,0; 17,4)		5,5 (3,5; 10,0)		0,22 (0,17; 0,28)	
negative	84	8,2 (4,3; 18,2)		5,0 (2,8; 17,8)		0,15 (0,11; 0,20)	

Age, FIGO, grade, residual tumor mass after debulking, ascites, peritoneal carcinomatosis .....n.s.

	DFS	OS
<b>PPIC+</b>	11 months	30 months
<b>PPIC-</b>	21 months	64 months
<i>p</i> (log-rank)	0.001	0.001



# cfDNA at T<sub>0</sub>/T<sub>1</sub> and tumor response

Response to therapy	parameter	Baseline (Q1;Q3) (ng/ml)	Follow-up (Q1;Q3) (ng/ml)	p
Yes (N=62)	Total cfDNA	11.3 (6.8; 22.8)	5.8 (3.7; 12.6)	0.009
	Tumoral cfDNA	5.2 (3.8; 9.1)	4.1 (2.4; 9.9)	0.272
	DNA integrity	0,15 (0,10; 0,24)	0,16 (0,11; 0,21)	0.956
No (N=13)	Total cfDNA	10.4 (8.1; 12.0)	8.9 (6.0; 14.9)	0.924
	Tumoral cfDNA	5.0 (4.2; 6.4)	5.1 (4.2; 7.9)	0.527
	DNA integrity	0.13 (0.09; 0.16)	0.20 (0.14; 0.24)	0.073

# Prognostic value of cfDNA (T<sub>1</sub>)

Predictor	Progression free survival				Overall survival			
	HR	95% CI		p	HR	95% CI		p
Age		n.s.			1.044	1.016	1.073	0.002
FIGO stage	2,377	1,045	5,408	0,039	2,401	1,210	4,763	0,012
Residual disease		n.s.				n.s.		
Peritoneal carcinomatosis	4,610	2,519	8,437	<0,001	3,275	1,440	7,447	0.005
DNA integrity	1,284	1,016	1,623	0,037	1,415	1,068	1,843	0,010

# Summary and conclusions

- cfDNA levels are elevated in EOC
- Additive value to CA-125 alone to identify malignant tumor masses
- Increased levels correlate with
  - T0: disease stage (FIGO, peritoneal carcinomatosis)
  - T1: chemoresponse, residual disease (CTC)
- Therapy monitoring
- Prognostic value



# Thank you!



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