Memorial Sloan-Kettering Cancer Center

institutCurie
Together, let's beat cancer.

CTC in clinical studies:

Latest reports on GI cancers

François-Clément Bidard, MD PhD

GI cancers are characterized by

Multimodal treatment strategies

Treatments are adapted to tumor burden & prognosis

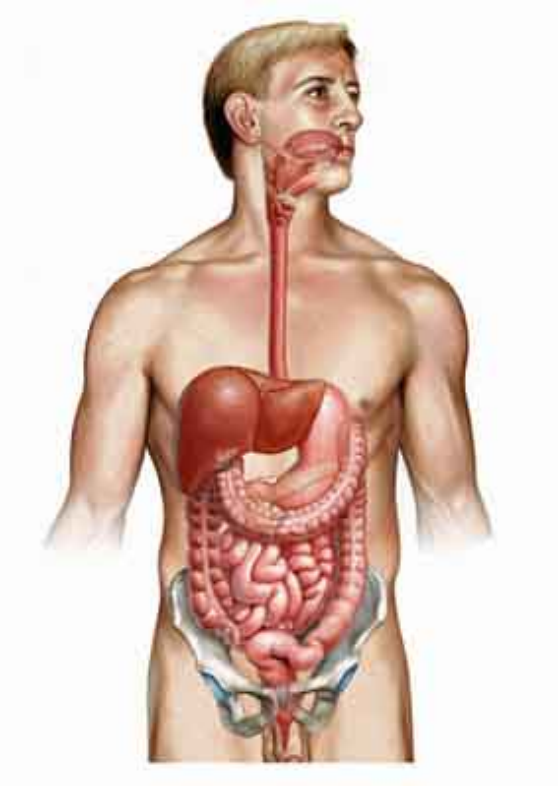
è a perfect setting to implement biomarkers to evaluate

Metastasis ability

Tumor burden / response to treatment

Strong clinical utility of somatic genomic markers

è KRAS/BRAF status in CRC



I. Colorectal ADK: metastatic setting

Colon cancer: metastatic setting – what do we know ?

Quantitative CTC count – clinical validity

CTC count before and during treatment is a prognostic marker

- Cohen *et al*, JCO 2008, N=430 patients, CellSearch, **LOE II**
- Tol *et al*, Ann Oncol 2010, N=467 patients, CellSearch, **LOE II**
- Sastre *et al*, Oncologist 2012, N=180 patients, CellSearch, **LOE III**

Recent subgroup analysis **Sastre *et al*, Clin Colorectal Cancer 2013**

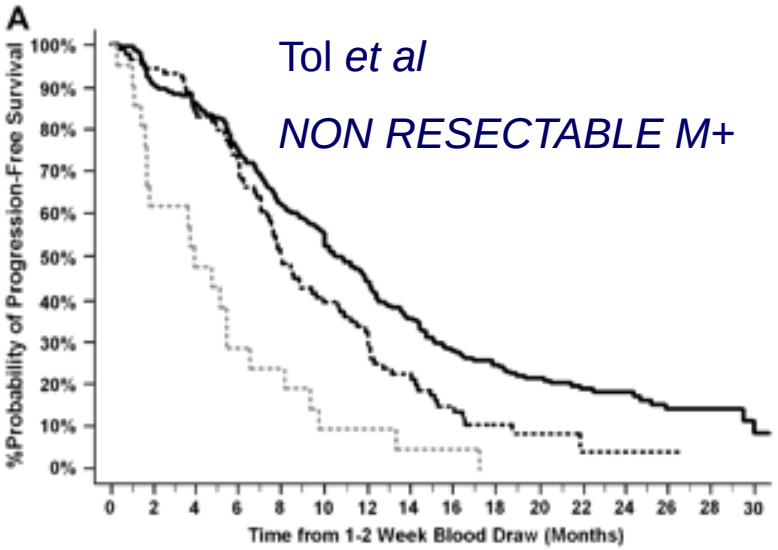
- **Gazzaniga *et al*, J Cancer Res Clin Oncol 2013, N=119 patients, CellSearch, LOE III**

Threshold >=3 vs >=1 ?

- Gazzaniga *et al*, J Cancer Res Clin Oncol 2013

Colon cancer: metastatic setting – what do we know ?

Quantitative CTC count changes – clinical utility ???



PFS

Tol *et al*

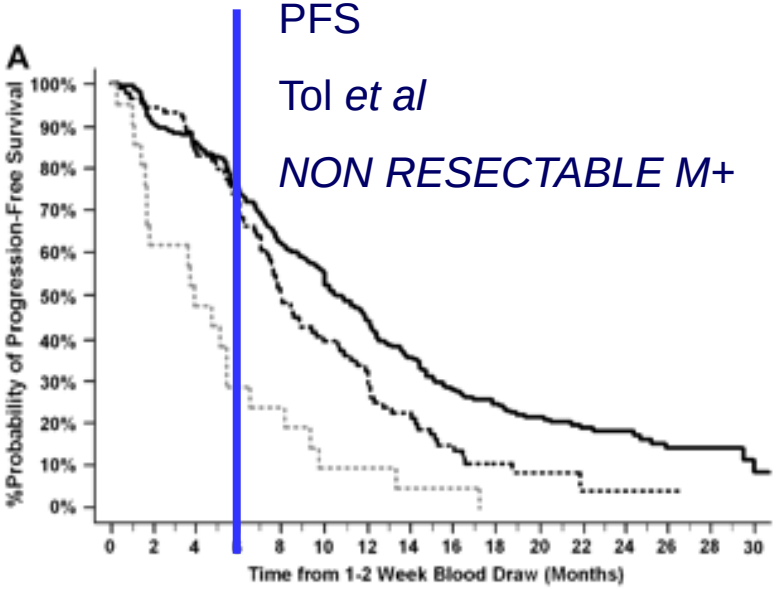
NON RESECTABLE M+

1 - Population size

-- / Pos: only 5% of patients !!!!

Colon cancer: metastatic setting – what do we know ?

Quantitative CTC count changes – clinical utility ???



PFS

Tol *et al*

NON RESECTABLE M+

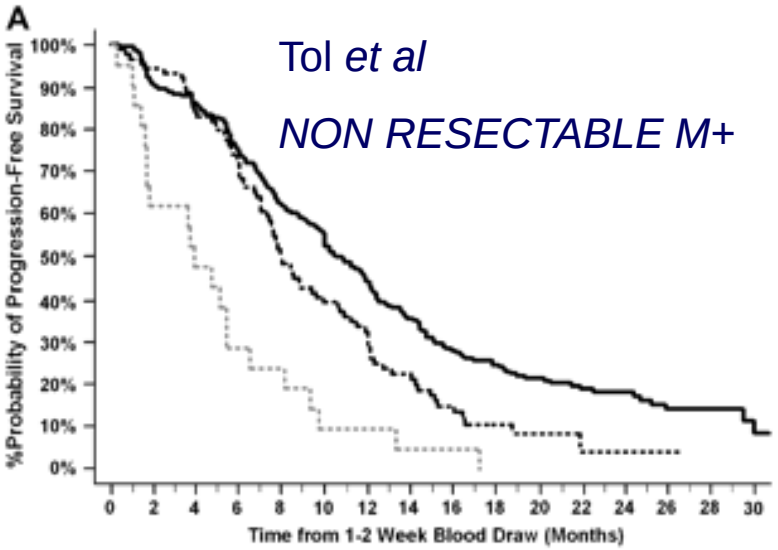
1 - Population size

2 – Detection of PFS < 6 months

Sp=80% & Se=14%

Colon cancer: metastatic setting – what do we know ?

Quantitative CTC count changes – clinical utility ???



PFS

Tol *et al*

NON RESECTABLE M+

1 - Population size

2 – Detection of PFS < 6 months

Sp=80% & Se=14%

3 – CEA changes are informative

Aggarwal *et al*, Ann Oncol 2013

Colon cancer: metastatic setting – what do we know ?

Quantitative CTC count changes – clinical utility ???

1 - Population size

2 – Detection of PFS < 6 months

Sp=80% & Se=14%

3 – CEA changes are informative

è **Probably marginal utility of CTC count changes in the general M+ population**

è **Trials similar to SWOG 500 & CirCe 01 would require thousands of patients**

Colon cancer: metastatic setting – what do we know ?

How to overcome these issues ?

From the CTC side:

Investigating other detection techniques in patients

è Same CTC definition, but more sensitive (e.g. microfluidics)

è Other CTC definition

EPISPOT Deneve *et al*, Clin Chem 2013

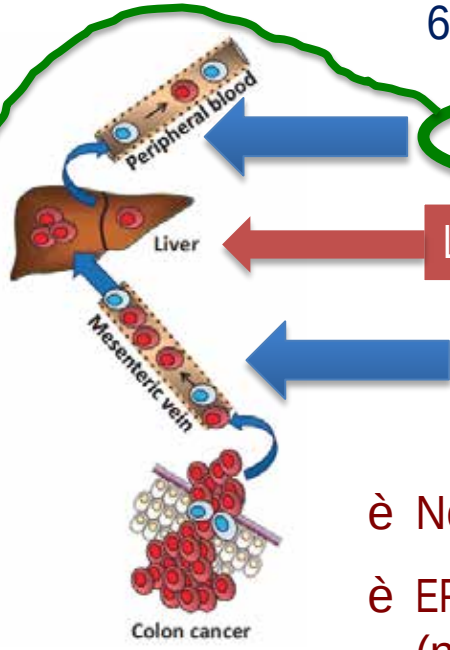
è More molecular characterization

Plastin 3, Yokobori *et al*, Cancer Res 2013

Capture of Viable Circulating Tumor Cells in the Liver of Colorectal Cancer Patients

Eric Denève,[1] Sabine Riethdorf,[2] Jeanne Ramos,[3] David Nocca,[1] Amandine Coffy,[4] Jean-Pierre Daurès,[4] Thierry Maudelonde,[5] Jean-Michel Fabre,[1] Klaus Pantel,[2] and Catherine Alix-Panabières[4,5,6*]

Clin Chem 2013



60 M0 and 15 M1 CRC before primary cancer surgery

EPISPOT detected more CTC than CellSearch

Liver as a filter for EpCam+ cells ?

EPISPOT & CellSearch detected same amount of CTC

è No concordance between the 2 techniques

è EPISPOT associated with poor differentiation & absence of emboli (not with M stage)

		EPISPOT	CellSearch
Metastatis		0.44	0.0181
M0	60	34/59 (57.6)	12/55 (21.8)
M+	15	7/15 (46.7)	8/14 (57.1)

Testing different thresholds, in M0 patients
EPISPOT showed a significant impact on cancer-specific survival (p=0.046)
CELLSEARCH didn't

Colon cancer: metastatic setting – what do we know ?

How to overcome these issues ?

From the clinical side:

Investigating clinically challenging subgroups

è **Resectable metastases**

Length & strengh of (neo)adjuvant treatment

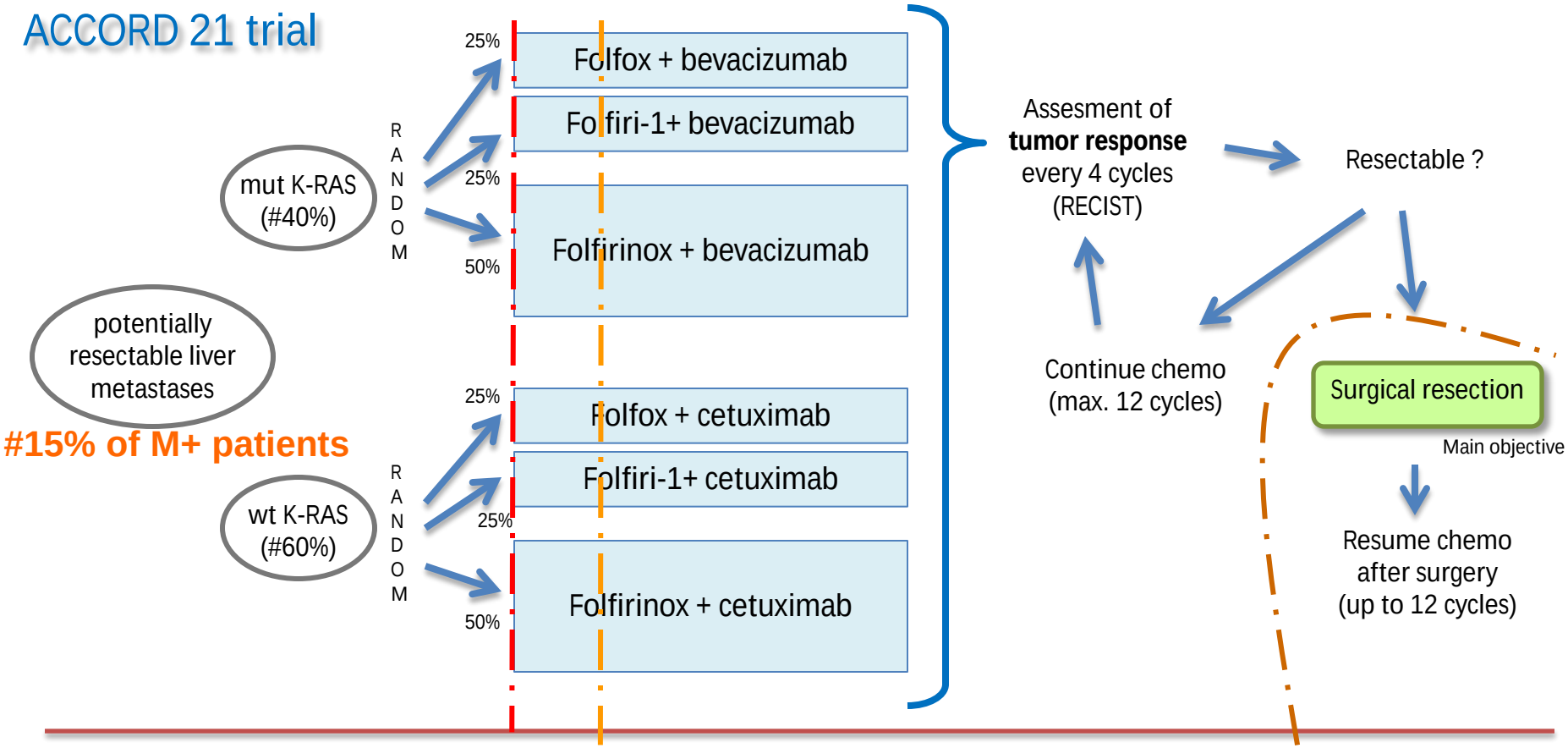
è **Potentially resectable metastases**

Length & strengh of first-line chemotherapy

Give an early indication about the future tumor response

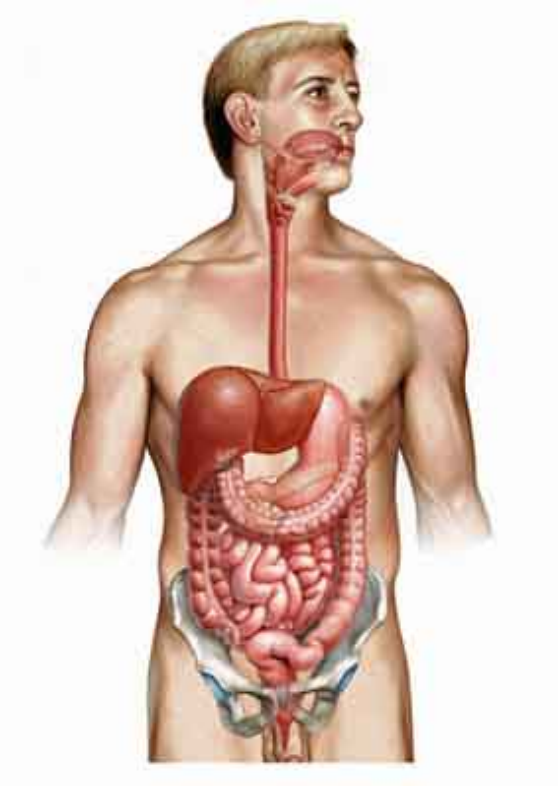
& whether surgical resection of metastases will become possible

ACCORD 21 trial



CirCe 03 study

	Baseline	Before C2	Before surgery (if resectable after treatment)
	N=110 pts	N=86 pts	N=43 pts
CTC>0	50%	13%	13%
CTC>=3	31%	2%	2%
CTC>=10	10%	2%	2%



II. Colorectal ADK: adjuvant setting

Lu *et al*, Br J Cancer 2013

hTERT, CK19, CK20, CEA mRNA; 90 stage II-III pts

CTC after adjuvant FOLFOX is an independent prognostic marker (N=90 pts)

*So far, **no confirmation** of the previous report (Uen et al Ann Surg 2007)*

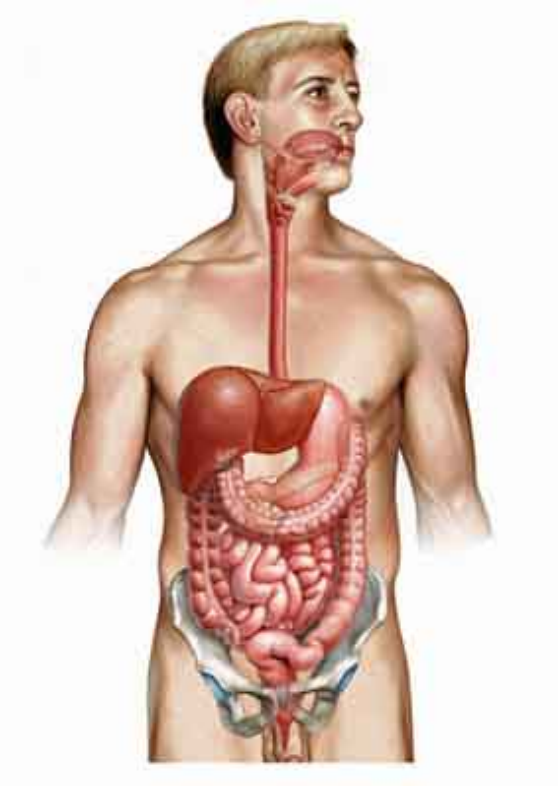
which strongly suggested that these mRNA can distinguish high risk stage II cancers

Gazzaniga *et al*, Tumor Biol 2013

CellSearch; 37 stage II-III pts

8 pts (22%) with ≥1CTC **after surgery**, before the start of adj chemotherapy

Correlated with N+ status



III. Colorectal ADK: molecular characterization

Determining KRAS – BRAF mutation status from CTC

Patient selection: everyday practice *vs* selected to have a "high" CTC count

CTC sorting	A few CTC	And
	single cell analysis	No leukocyte
	batch analysis	A few leukocytes
		A lot of leukocytes

Whole genome amplification : amplification consistent across the genome ?

Sequencing	Technique	Sensitivity	Min CTC/leuko ratio
	Standard PCR	5-10 %	10-20%
	- - -		
	Modified PCR (ASA, COLD…)	1 %	2 %
	Standard NGS	1 %	2 %
	- - -		
	Modified NGS (barcoding…)	<0.1 %	≈0.2 %
	BEAMing / PAP / ddPCR	<0.1 %	≈ 0.2 %

Clinical interpretation: differences in mutational status: artefact or reality ?

ctDNA è

Complex Tumor Genomes Inferred from Single Circulating Tumor Cells by Array-CGH and Next-Generation Sequencing

Ellen Heitzer[1], Martina Auer[1], Christin Gasch[6], Martin Pichler[3], Peter Ulz[1], Eva Maria Hoffmann[1], Sigurd Lax[5], Julie Waldispuehl-Geigl[1], Oliver Mauermann[6], Carolin Lackner[2], Gerald Höfler[2], Florian Eisner[3], Heinz Sill[4], Hellmut Samonigg[3], Klaus Pantel[6], Sabine Riethdorf[6], Thomas Bauernhofer[3], Jochen B. Geigl[1], and Michael R. Speicher[1]

Cancer Res 2013

6 metastatic CRC patients:

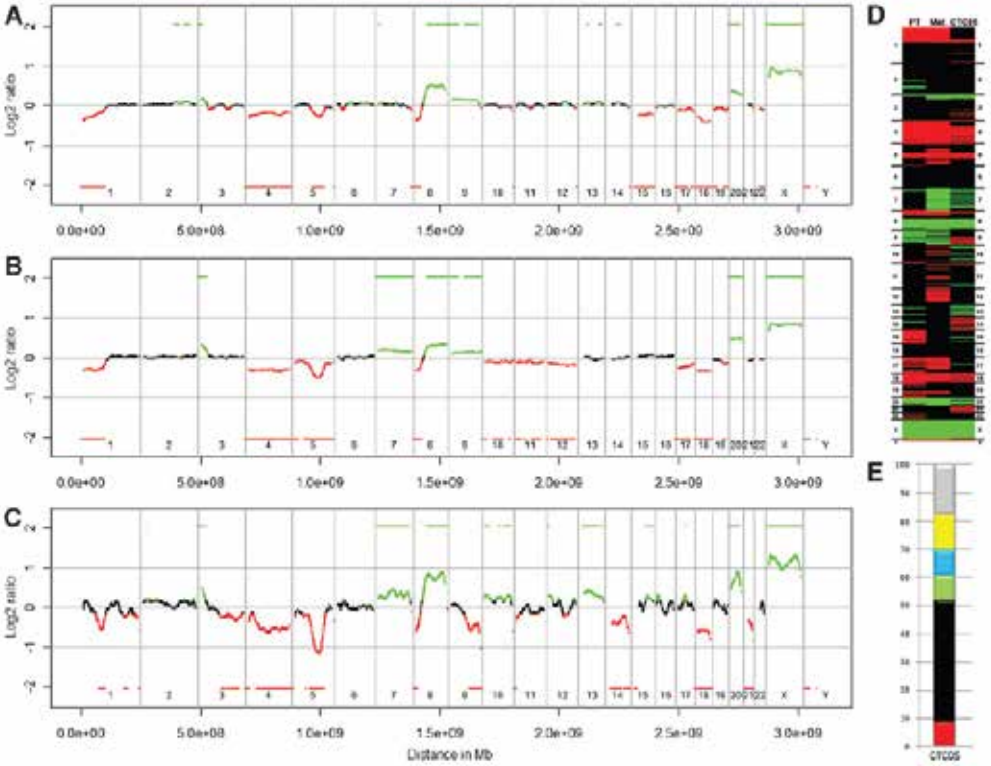
37 CTC isolated individually (post CellSearch sorting) è WGA è CGH & Sequencing

Primary tumor

Metastasis

DNA copy number profiles

Globally similar



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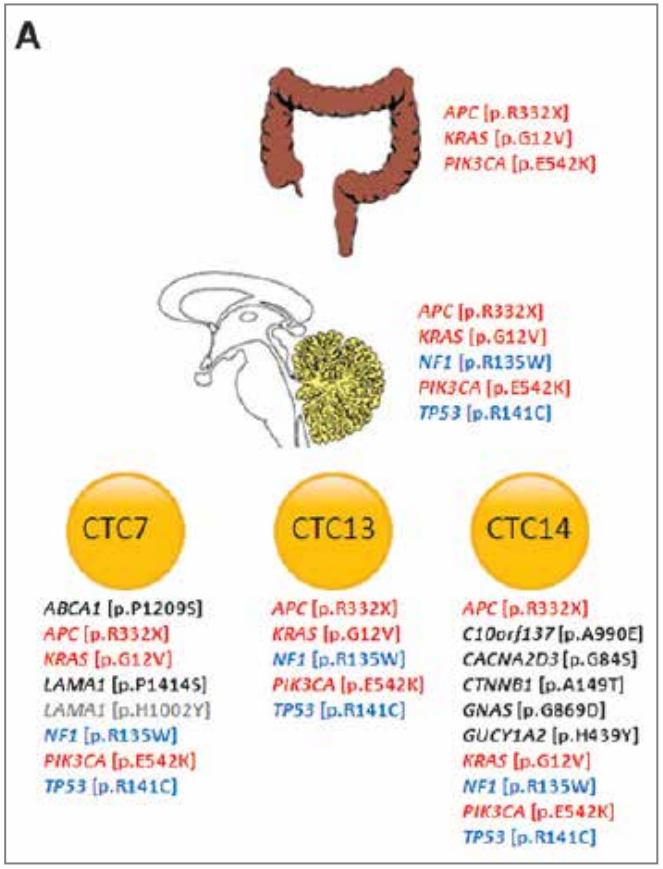
Cancer Res 2013

6 metastatic CRC patients:

37 CTC isolated individually (post CellSearch sorting) è WGA è CGH & Sequencing

Targeted sequencing 68 CRC-associated genes

- CTCs harbored some level of genetic heterogeneity
- Mutations harbored by CTC were present either at clonal or subclonal level in primary tumor & matched metastasis



è Use of CTC as a molecular surrogate (liquid biopsy)

Heterogeneity of Epidermal Growth Factor Receptor Status and Mutations of *KRAS/PIK3CA* in Circulating Tumor Cells of Patients with Colorectal Cancer

Christin Gasch,[1] Thomas Bauernhofer,[2] Martin Pichler,[3] Sabine Langer-Freitag,[4] Matthias Reeh,[5] Adrian M. Seifert,[5] Oliver Mauermann,[1] Jakob R. Izbicki,[5] Klaus Pantel,[1] and Sabine Riethdorf[1*]

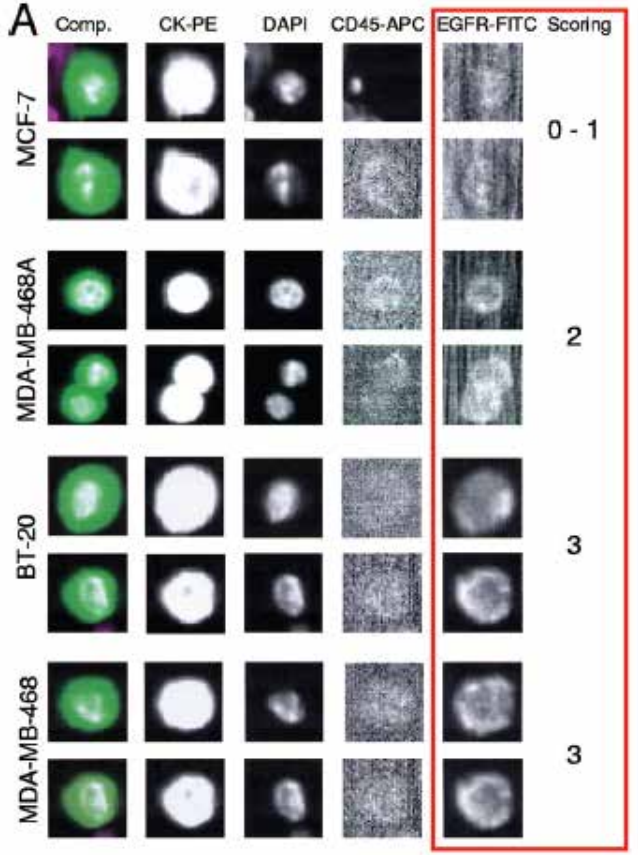
Clin Chem 2013

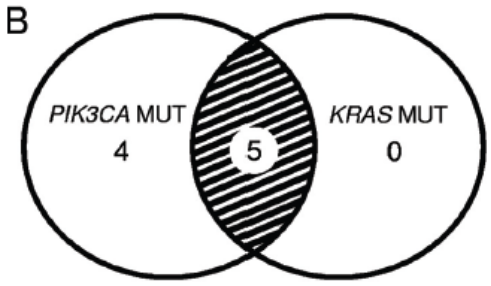
EGFR expression & amplification in cell lines & CRC samples

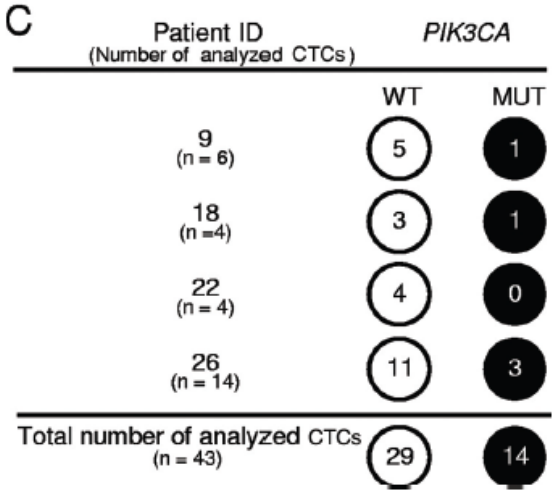
5 metastatic CRC patients:

CTC isolated individually (post CellSearch sorting)
è WGA è Sequencing (KRAS/BRAF/PIK3CA)

è Intrapatient genetic heterogeneity between CTCs







Detection and recovery of circulating colon cancer cells using a dielectrophoresis-based device: *KRAS* mutation status in pure CTCs

Francesco Fabbri[a,*], Silvia Carloni[a], Wainer Zoli[a], Paola Ulivi[a], Giulia Gallerani[b], Pietro Fici[c], Elisa Chiadini[a], Alessandro Passardi[d], Giovanni L. Frassineti[d], Angela Ragazzini[e], Dino Amadori[d]

[a] Biosciences Laboratory, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy
[b] Department of Morphology and Experimental Medicine, University of Ferrara, Ferrara, Italy
[c] Department of Internal Medicine, Aging and Renal Disease, University of Bologna, Section of Nephrology, Dialysis and Transplantation, Bologna, Italy
[d] Department of Medical Oncology, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy
[e] Unit of Biostatistics and Clinical Trials, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy

Cancer Lett 2013

DEParray in 40 metastatic CRC patients

15 to 20ml of blood è Oncoquick density gradient è panCK cocktail/Hoechst/CD45

è DEParray è aliquots of 1 then 5-10 epithelial CTCs

è WGA è KRAS exon 2 standard sequencing

- **Slightly higher detection rate**
(theoretical comparison % CellSearch)

- **Single CTC sequencing led to inconsistent results**
  - Þ ***batch analysis !!***
  - Þ ***Exclusion of pts with 1-2 CTC detected***

- **Poor accuracy for KRAS status determination in patients**
(cell lines were OK)

KRAS status in primary tissue and CTCs.

Patient no.	*KRAS* status	
	Primary	CTC
32	**G12D**	**G13D; G12D; G12C**
40	**G12D**	**G12D**
2	**G13D**	WT
5	**G13D**	WT
7	**G13D**	WT
25	**G12C**	WT
29	**G12A**	WT
31	**G12D**	WT
33	**G12V**	WT
28	**G12V**	ne
14	**G12D**	ne
38	**G12D**	ne
11	WT	WT
22	WT	WT
24	WT	WT
30	WT	WT
36	WT	WT
37	WT	WT
39	WT	**G12D**
16	WT	ne
26	WT	ne

ne, not evaluable.

KRAS and *BRAF* mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue

Bianca Mostert[1], Yuqiu Jiang[2], Anieta M. Sieuwerts[3], Haiying Wang[2], Joan Bolt-de Vries[3], Katharina Biermann[4], Jaco Kraan[1], Zarina Lalmahomed[5], Anne van Galen[3], Vanja de Weerd[3], Petra van der Spoel[1], Raquel Ramírez-Moreno[6], Cornelis Verhoef[5], Jan N.M. IJzermans[5], Yixin Wang[2], Jan-Willem Gratama[1], John A. Foekens[3], Stefan Sleijfer[1] and John W.M. Martens[3]

Int J Cancer 2013

43 metastatic CRC patients with primary tumor available, at time of liver met resection

30ml of blood è Ficoll è CellProfile (EpCam+) i.e. *few CTC within 1,000s leukocytes*

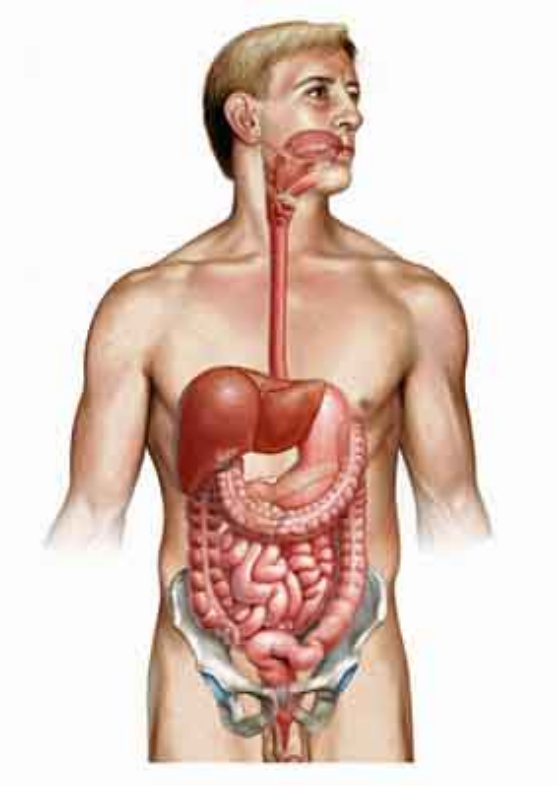
Comparison of 3 techniques for rare mutant allele detection (KRAS & BRAF):

COLD PCR + ASA PCR = ASB PCR

- **Best concordance obtained by ASB PCR** (sensitivity up to 0.2% in *in vitro* assays)

- **In the 20 pts with concordant status between primary & met**
  è ***in 13 patients wt, 12 were wt according to CTC analysis***
  è ***in 6 patients mut, 1 was mut according to CTC analysis (low CTC number in <u>that</u> setting)***

- **In the 11 pts *(>30% !)* with discordant status between primary & met**
  è ***CTC-based mutation status was wt in 7 pts (reality or poor sensitivity ?)***

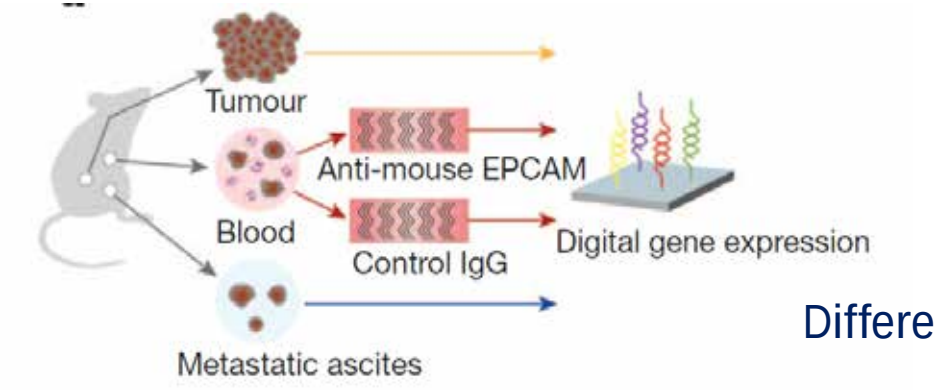


V. Pancreatic ADK

RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis

Min Yu[1,2*], David T. Ting[1*], Shannon L. Stott[1], Ben S. Wittner[1], Fatih Ozsolak[3], Suchismita Paul[1], Jordan C. Ciciliano[1], Malgorzata E. Smas[1], Daniel Winokur[1], Anna J. Gilman[1], Matthew J. Ulman[1], Kristina Xega[1], Gianmarco Contino[1], Brinda Alagesan[1], Brian W. Brannigan[1], Patrice M. Milos[3], David P. Ryan[1], Lecia V. Sequist[1], Nabeel Bardeesy[1], Sridhar Ramaswamy[1], Mehmet Toner[1], Shyamala Maheswaran[1] & Daniel A. Haber[1,2]

Nature 2012



EpCAM+ CK+ CTC Chip

↓

RNA Seq

↓

Differential analysis *vs* cells trapped with control IgG

↓

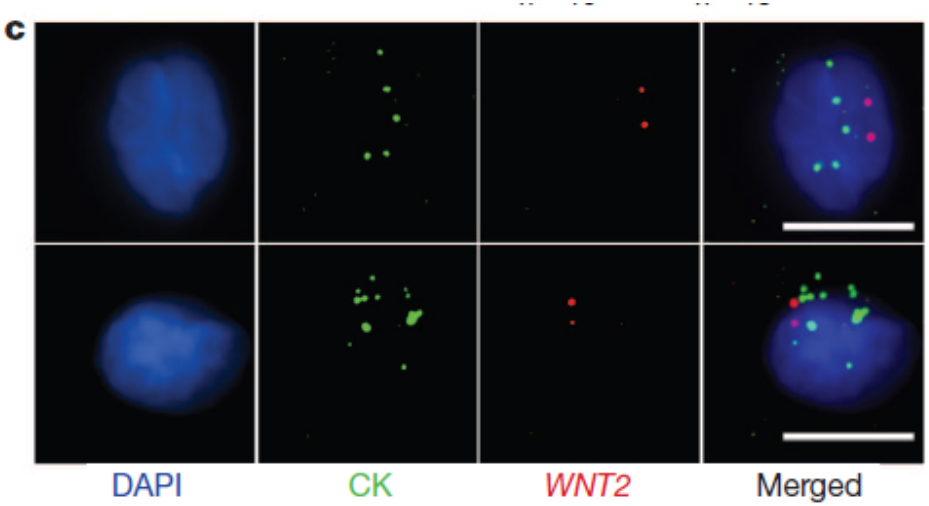
Wnt2

↙

Functional validation of **Wnt2 role in metastasis**

↓

Wnt2 expression (RNA-ISH) in CTC from patients



Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial

F. C. Bidard[1,2*], F. Huguet[3], C. Louvet[4], L. Mineur[5], O. Bouché[6], B. Chibaudel[7], P. Artru[8], F. Desseigne[9], J. B. Bachet[10], C. Mathiot[11], J. Y. Pierga[1,2] & P. Hammel[12]

Ann Oncol 2013

Locally advanced M0 pancreatic cancer patients (non surgically resectable)

? Focus on the visible tumor mass (i.e. with radiation therapy)

Focus on the invisible systemic disease (i.e. with full dose systemic therapy)

Knowing that metastases are detected in most patients few months after initiation of therapy

è **CTC as a guiding tool ???** ***(something that ctDNA probably can't do)***

N=79 patients, 2 sample time (baseline & 2 months) CellSearch system

- **Global CTC detection rate of 9% (≥1 CTC) – all CTCs were EGFR+**

Baseline: **4 pts / 75** (5%)
After 2 months of gemcitabine +/- erlotinib: **5 pts / 56** (11%) with ≥1 CTC
Range of CTCs : **1-2 CTC in 8 pts, 15 CTCs in 1 pt**

- **All detected CTCs were EGFR+** (CellSearch immunostaining)

Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial

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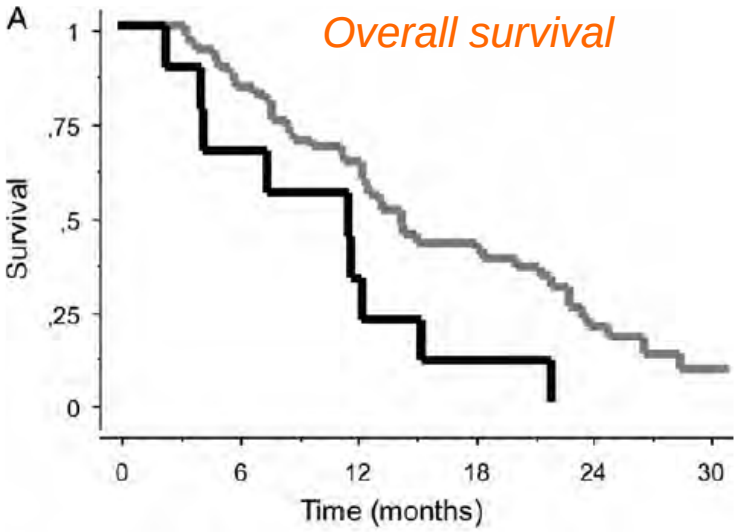
Ann Oncol 2013

- **CTC detection correlated with poor differentiation**

And not with any other patient characteristic

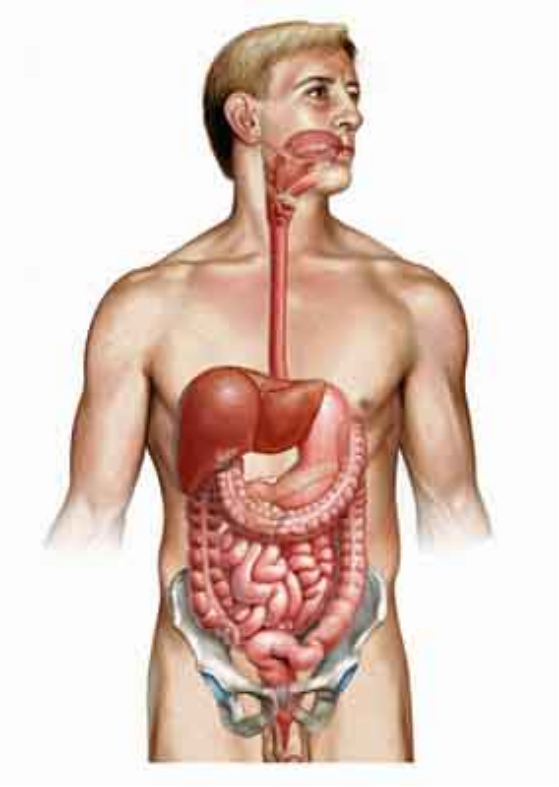
homogeneous population "fit" for the trial (good general condition & liver function)

- **Univariate analysis**



- **Multivariate analysis**

	Relative risk (95%CI)	*P* value
Model with CTCs at baseline ($N = 75$ patients)		
≥1 CTC/7.5 ml at baseline ($n = 4$ patients at risk)	3.1 (1.0–8.8)	**0.04**
Hb < 110 g/l ($n = 12$ patients at risk)	3.2 (1.4–7.4)	**0.008**
ALP > ULNV ($n = 30$ patients at risk)	1.7 (0.9–2.9)	0.07
Model with CTCs at 2 months ($N = 56$ patients)		
≥1 CTCs/7.5 ml at 2 months ($n = 5$ patients at risk)	2.2 (0.8–6.0)	0.11
Hb < 110 g/l ($n = 7$ patients at risk)	3.2 (1.1–8.7)	**0.02**
ALP > ULNV ($n = 21$ patients at risk)	1.6 (0.8–3.1)	0.20
Model with CTC at baseline and/or 2 months ($N = 79$ patients)		
≥1 CTC/7.5 ml ($n = 9$ patients at risk)	2.5 (1.2–5.4)	**0.01**
Hb < 110 g/l ($n = 13$ patients at risk	3.4 (1.4–7.9)	**0.005**
ALP > ULNV ($n = 32$ patients at risk)	1.6 (0.9–2.9)	0.07



VI. Neuroendocrine tumors

Circulating Tumor Cells As Prognostic Markers in Neuroendocrine Tumors

Mohid S. Khan, Amy Kirkwood, Theodora Tsigani, Jorge Garcia-Hernandez, John A. Hartley, Martyn E. Caplin, and Tim Meyer

J Clin Oncol 2013

CellSearch before a new line of therapy

N=176 pts with mesurable NET (101 midgut & 47 pancreatic)

Table 2. Baseline CTC Counts

Characteristic	Patients With CTCs (%) ≥ 1	≥ 2	≥ 5	≥ 10	≥ 50	Range of CTC Counts
Primary site						
All NETs (n = 175)	49	42	30	22	9	0-3,731
Midgut (n = 101)	51	47	32	24	6	0-294
Pancreatic (n = 42)	36	24	19	17	12	0-430
Bronchial (n = 17)	41	29	24	18	12	0-452
Unknown (n = 12)	92	92	67	33	17	0-3,731
Tumor burden, %						
≤ 25	33	24	17	10	2	
> 25	64	59	42	34	14	
P	< .001	< .001	< .001	< .001	.005	
Tumor grade						
1	40	31	24	22	4	
2	54	49	27	13	6	
3	66	59	55	45	28	
P	.036	.014	.006	.003	< .001	
ECOG PS						
0	49	43	30	22	7	
> 1	50	38	38	38	1	
P	1.0	1.0	.70	.379	.519	
CgA, pmol/L						
≤ 120	29	23	19	16	5	
> 120	64	57	39	27	11	
P	< .001	< .001	.005	.1	.275	

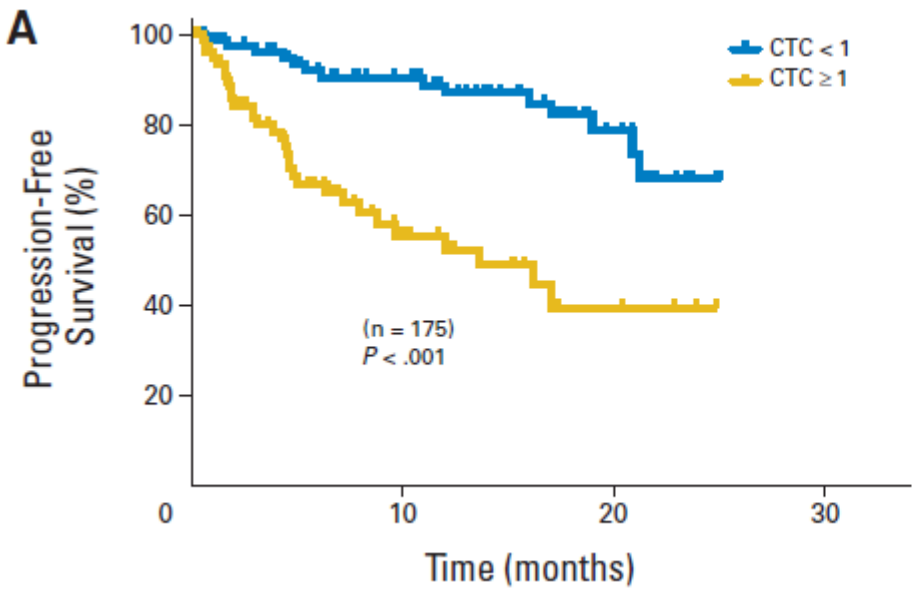
Circulating Tumor Cells As Prognostic Markers in Neuroendocrine Tumors

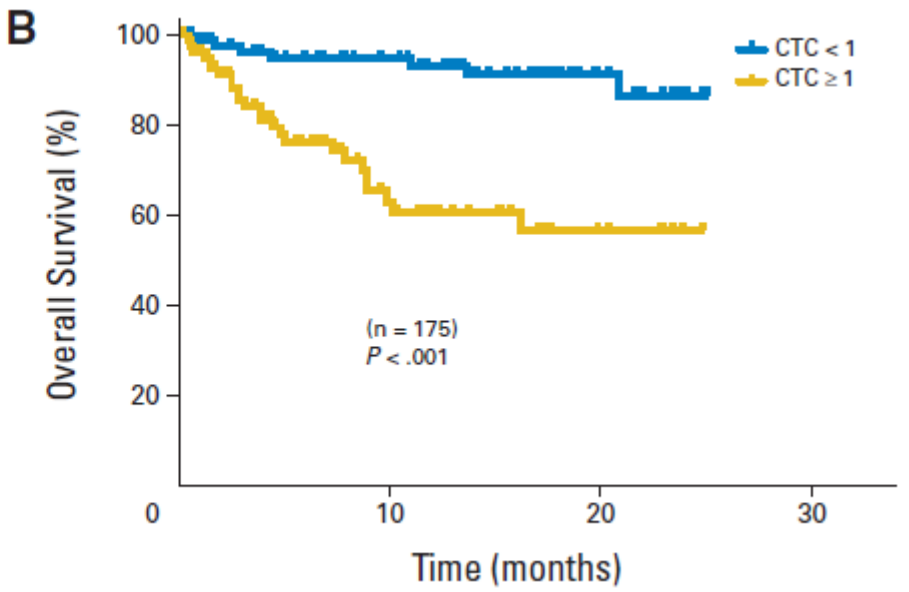
Mohid S. Khan, Amy Kirkwood, Theodora Tsigani, Jorge Garcia-Hernandez, John A. Hartley, Martyn E. Caplin, and Tim Meyer

J Clin Oncol 2013

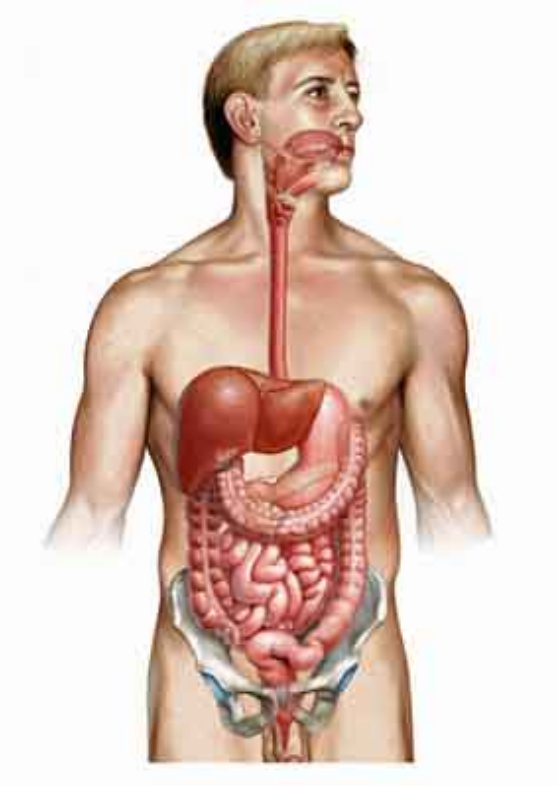
CellSearch before a new line of therapy

Prognostic value in univariate & multivariate analyses





Significant in the whole population, but also in grade I and grade II NET



VII. Esophageal & gastric cancers

Clinical Significance of Circulating Tumor Cells in Peripheral Blood From Patients With Gastric Cancer

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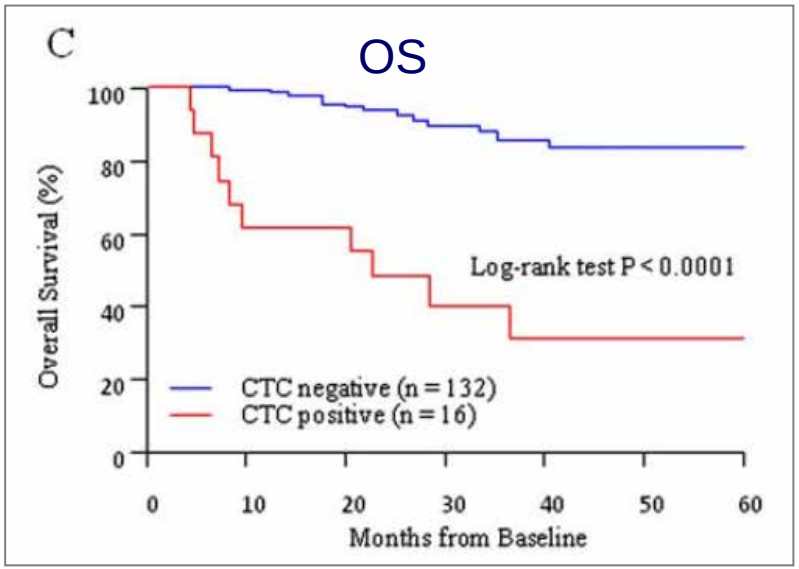
Cancer 2013

CellSearch before any treatment

N=148 pts with resected tumor followed by adjuvant S1

è 11% with ≥1CTC

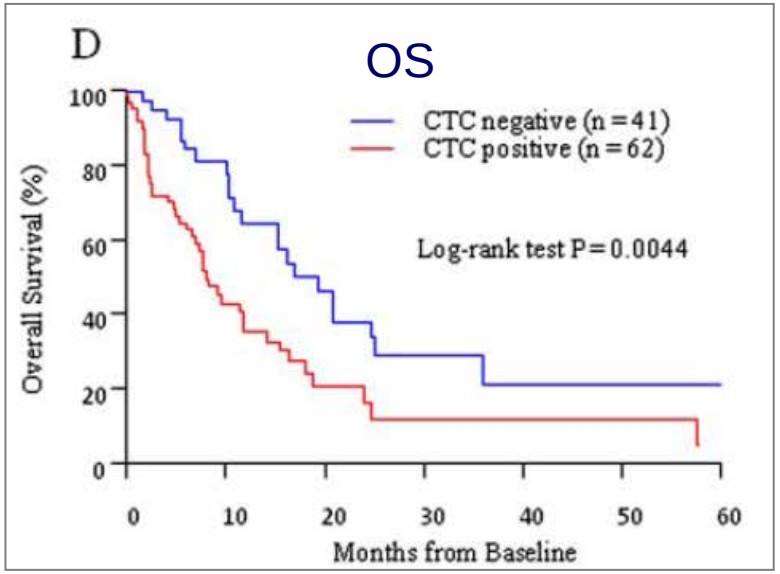
≥1CTC correlated with T, N & M status

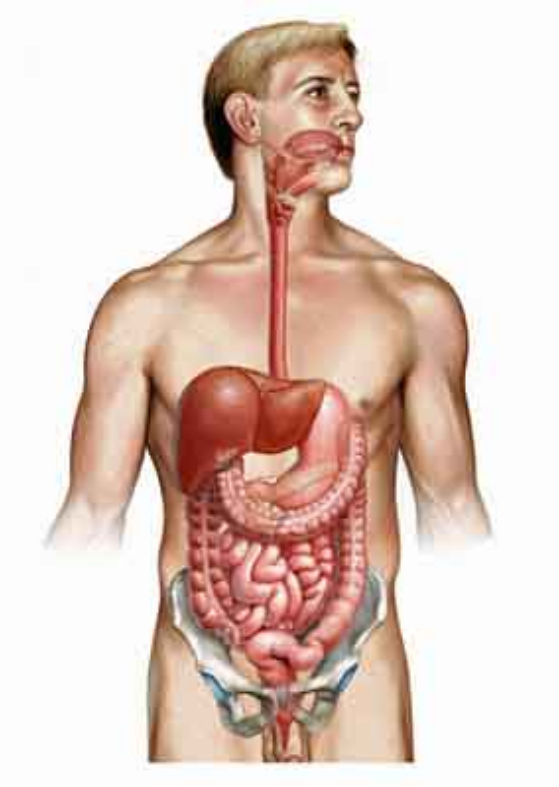


HR = 1.73 [1.08-2.77] in multiv. analysis

N=103 pts with non-resectable tumor (M+)

è 60% with ≥1CTC





VIII. Liver cancer

Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma

Kornelius Schulze[1], Christin Gasch[2], Katharina Staufer[3], Björn Nashan[4], Ansgar W. Lohse[1], Klaus Pantel[2], Sabine Riethdorf[2]* and Henning Wege[1]*

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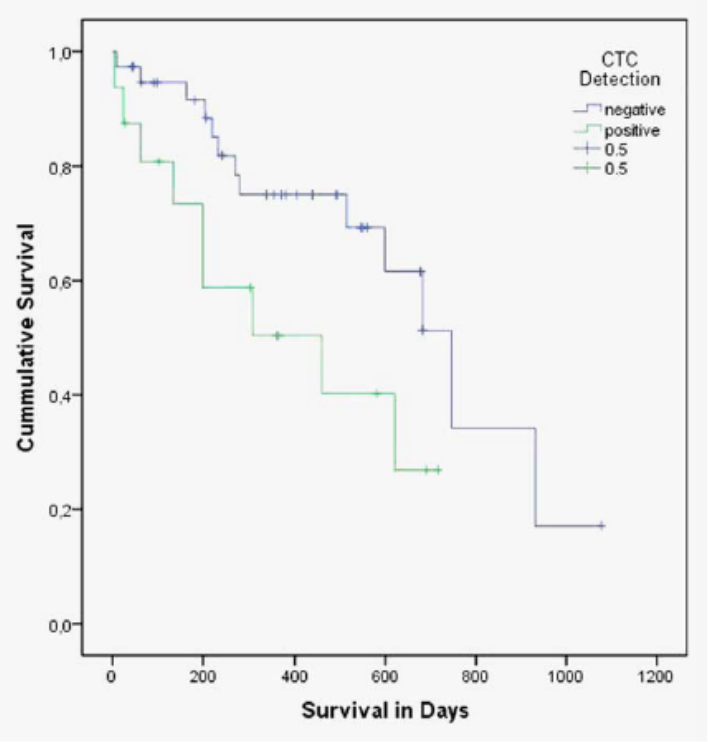
CTC detection in 59 HCC patients (stage I to IVB), with CellSearch + control pts

18 HCC pts (30%) had 1-5 CTC; 1 non-HCC pt (5%) had 1 CTC

Overall Survival, p=0.02

Correlated with

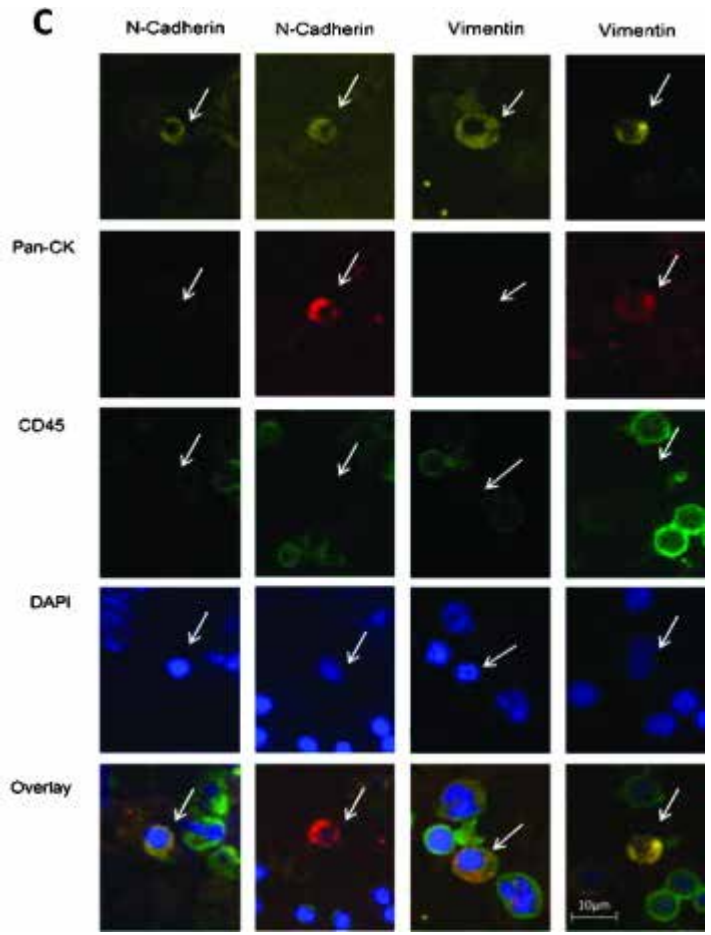
- **Tumor stage**
- **AFP**
- **Tumor vascular invasion**



Individual Profiling of Circulating Tumor Cell Composition and Therapeutic Outcome in Patients with Hepatocellular Carcinoma[1]

Ivonne Nel*, Hideo A. Baba†, Judith Ertle‡, Frank Weber§, Barbara Sitek¶, Martin Eisenacher¶, Helmut E. Meyer¶, Joerg F. Schlaak‡ and Andreas-Claudius Hoffmann*

Transl Oncol 2013



N=11 HCC patients

CD45 immunodepletion

followed by immunocytofluorescence

è Phenotypic heterogeneity

è Trend toward shorter TTP

for CTC with EMT phenotype

From a clinical perspective…
… where are we going with CTC in GI cancer ?

Prognostic value in M1 CRC (non resectable patients)

Clinical validity : LOE II with CellSearch

No ongoing trial to demonstrate clinical utility

ctDNA prognostic value under assessment

è dead end (?)

KRAS/BRAF status assessment in M1 CRC

Struggling with low CTC/leukocyte ratio & rare mutant detection

Efficient techniques are time consuming

è ctDNA will become the gold standard in clinics within a few years

From a clinical perspective…
… where are we going with CTC in GI cancer ?

CTC to measure intratumor genetic/phenotypic heterogeneity

Numerous evidences from M1 CRC patients

ctDNA (single mutation detection) does not quantify intratumor heterogeneity, but can detect mutant (resistant) subclones

è No clear clinical use so far

CTC to influence multimodal strategies in M1 tumors

M1 GI NET (LOE III)

M1 resectable / potentially resectable mCRC

CTC to guide the treatment decision ?

è To be followed

From a clinical perspective…
… where are we going with CTC in GI cancer ?

Metastatic potential in M0 cancers

M0 gastric cancers (LOE III)

M0 LA pancreatic cancers (LOE III)

M0 Hepatocellular carcinomas (LOE IV)

EMT: not mandatory for prognosis assessment ?

ctDNA may be of lower interest than CTC in M0 tumors

è To be followed