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BEST PRACTICE

CIRCULATING TUMOUR DNA IN CANCER

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OUTLINE

Introduction to liquid biopsy

Overview of clinical applications of circulating tumour DNA:

- Gastrointestinal tumours
- Lung cancer
- Breast cancer
- Prostate cancer

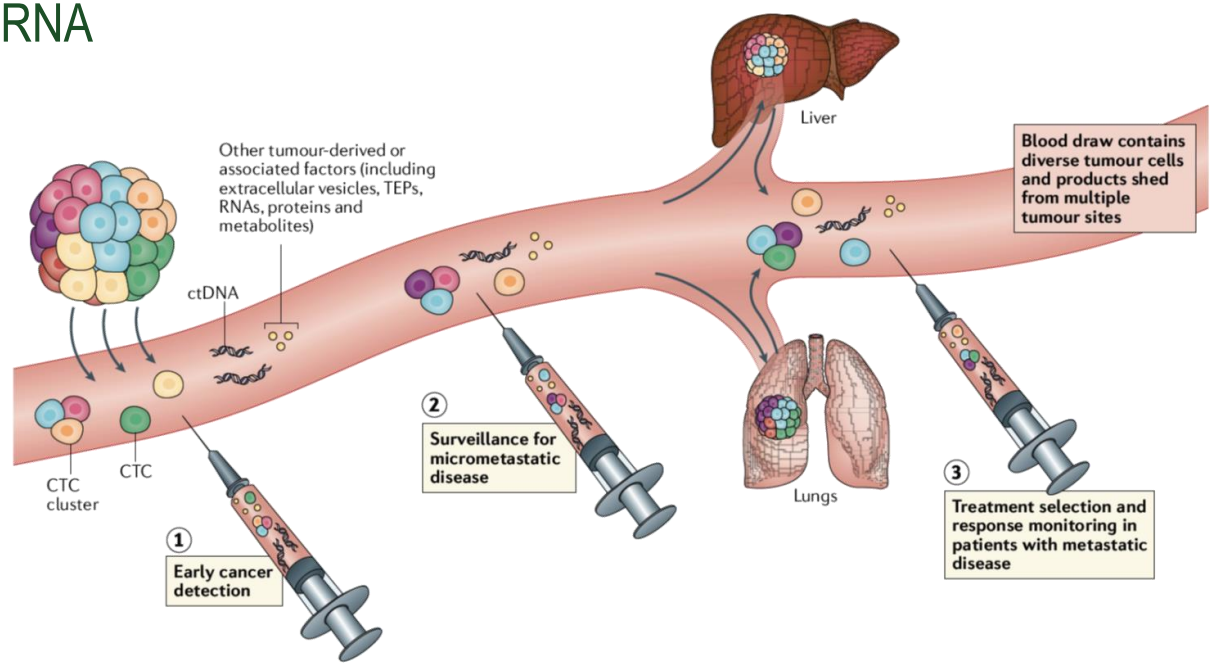
Take home messages

INTRODUCTION TO LIQUID BIOPSY

Tumour cell components that can be detected on the blood of cancer patients:

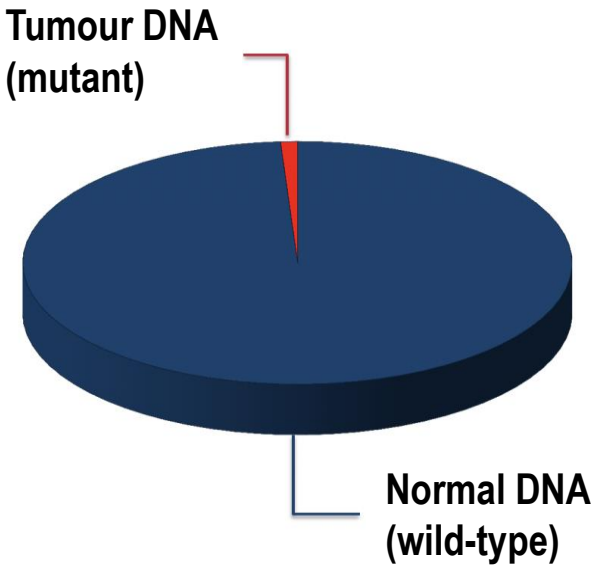
- Circulating tumour DNA (ctDNA)
- Circulating tumour cells (CTCs)
- Circulating tumour cells vesicles (exosomes)
- Circulating tumour RNA

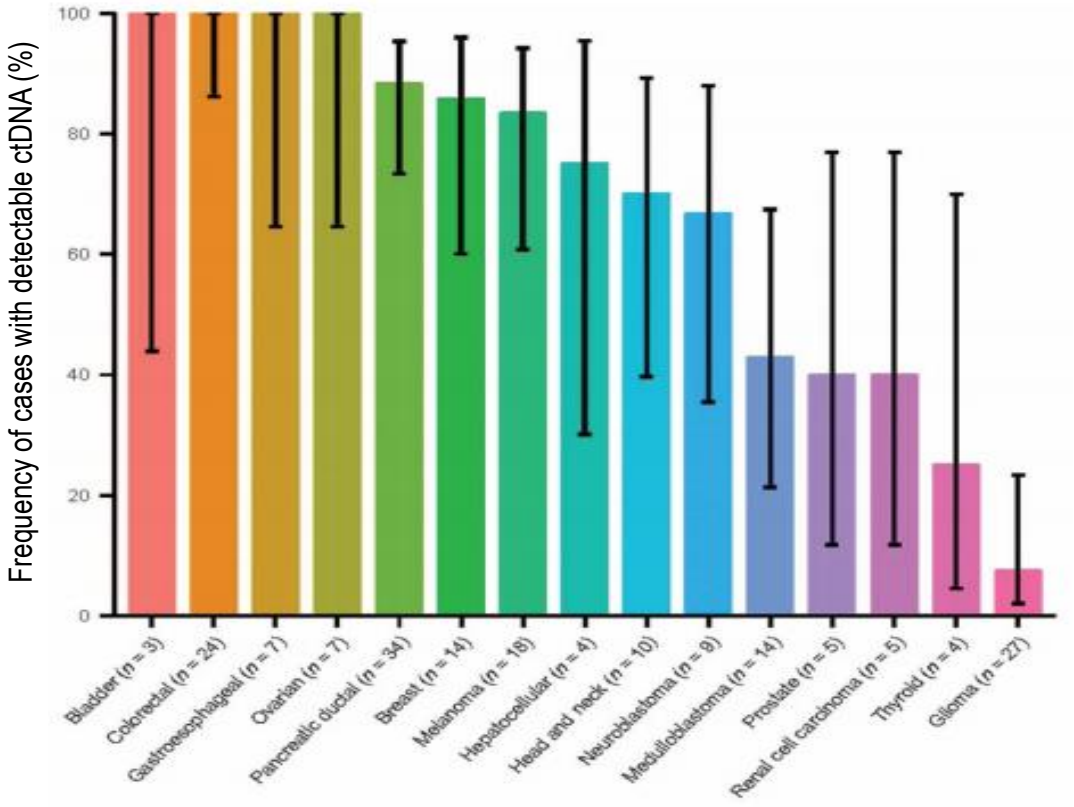
- **Liquid biopsies permit to have access to tumour genotype and phenotype with low harm**
- **The most useful source comes from ctDNA**



Ignatiadis M, *et al.* Nat Rev Clin Oncol 2021 Jan 20. doi: 10.1038/s41571-020-00457-x. Online ahead of print Published by Springer Nature. Reproduced with permission from Dr M Ignatiadis.

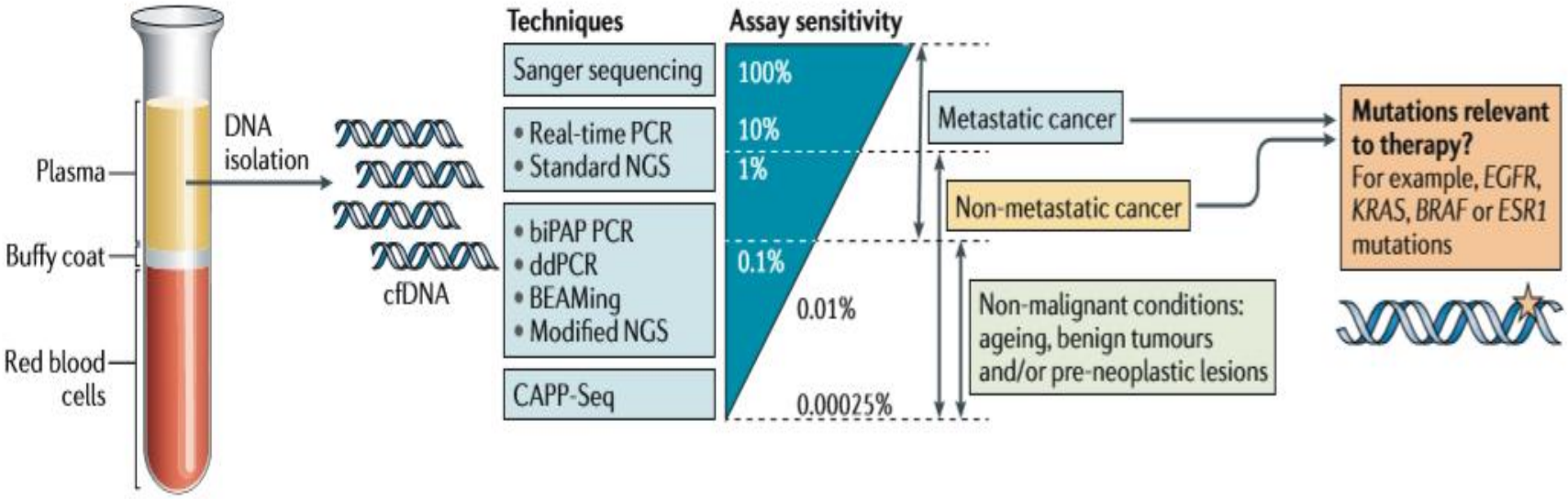
ctDNA ALLELE FRACTION VARIES WITH TUMOUR TYPE AND SETTING





From Bettegowda C, *et al.* Sci Transl Med 2014;6(224):224ra24. Reprinted with permission from AAAS.

PLATFORMS FOR ctDNA DETECTION

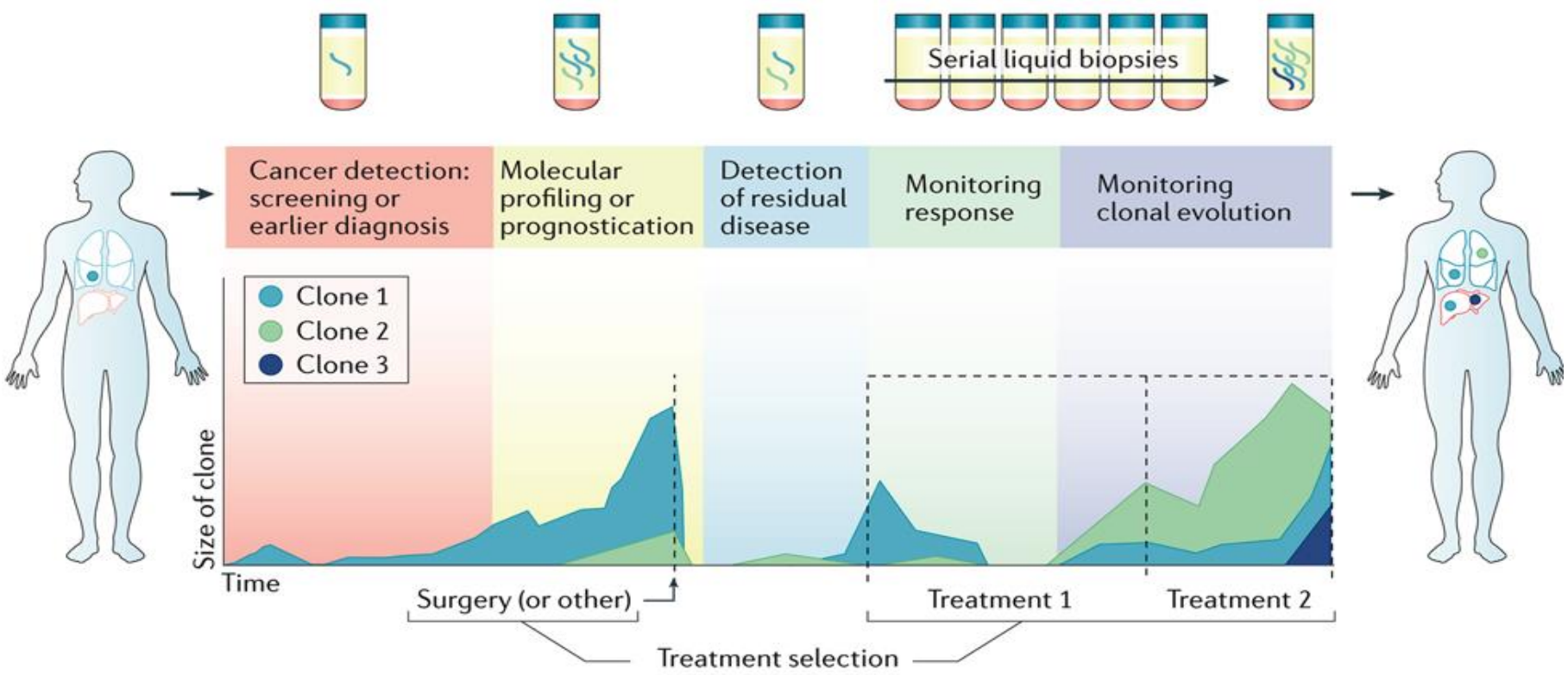


POTENTIAL APPLICATIONS OF LIQUID BIOPSY

Applications	Tumour biopsies	Liquid biopsies
Diagnosis of cancer	✓	✗
Monitoring residual disease	✗	✓
Assessing intratumour heterogeneity	✓	✓
Evaluation of early treatment responses	✗	✓
Identification of genetic determinants for targeted therapies	✓	✓
Tracking secondary (‘acquired’) resistance	✓	✓

Adapted from Diaz LA, Bardelli A. J Clin Oncol 2014;32(6):579–86.

USES OF ctDNA IN CANCER TREATMENT



METASTATIC SETTING

Concordance ctDNA-solid tissue:

PCR and digital PCR

Idylla QAS PCR: 88%–90%

BEAMING: 83%–99%

ddPCR 85%–95%

NGS

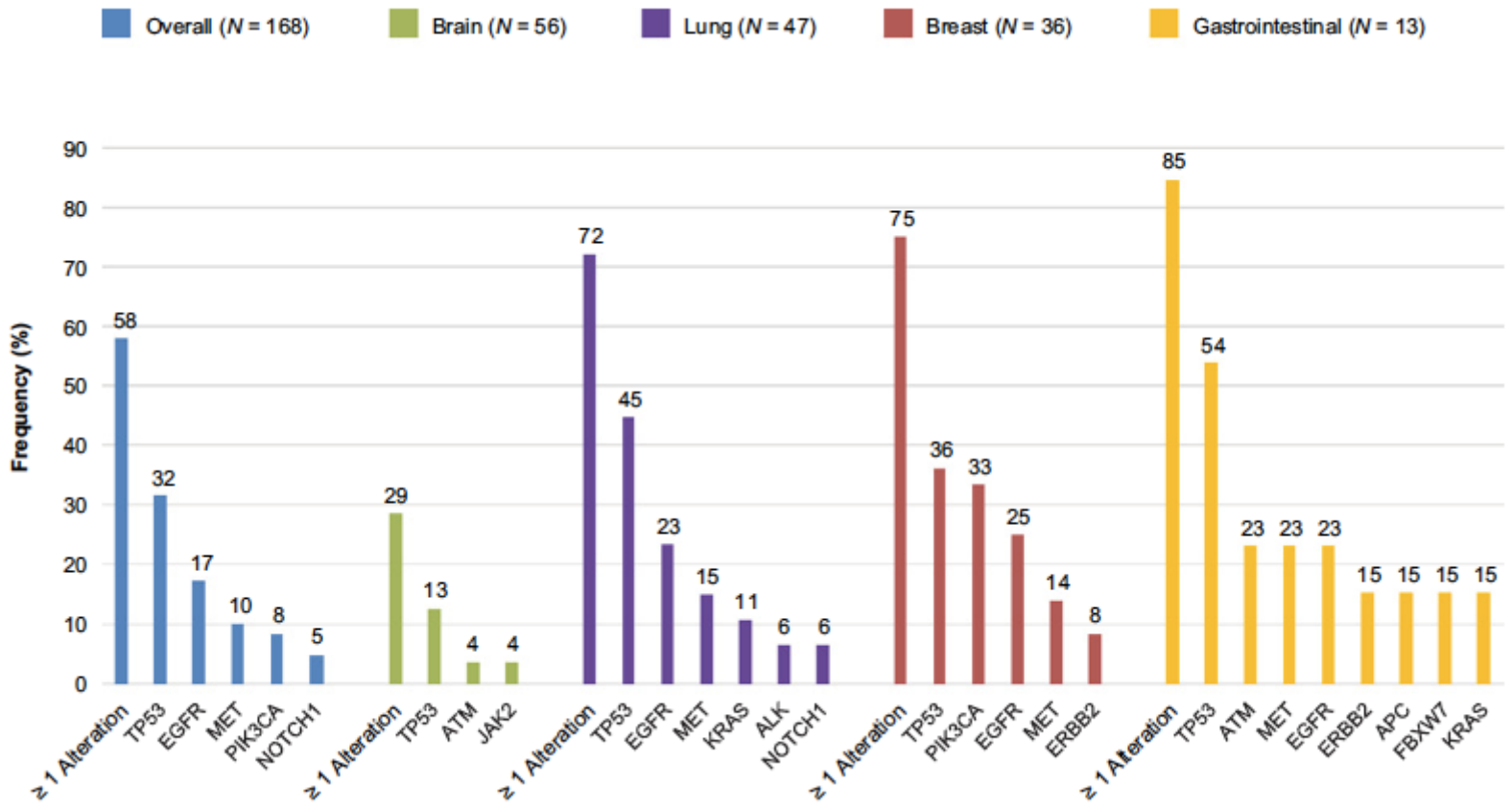
Concordance between ultra-deep NGS of plasma cfDNA and clinical molecular testing of archival tumour tissue for the 55 patients with advanced cancers

Type of agreement between plasma cfDNA and tumour tissue	No. of patients (%)
Complete detection	45 (82) ←
Partial detection	3 (5)
Aggregate complete and partial detection	48 (87) ←
Complete disagreement	7 (13)

Janku F, *et al.* Mol Cancer Ther 2016; Janku F, *et al.* Oncotarget 2015; Janku F, *et al.* Ann Oncol 2017; Janku F, *et al.* Clin Cancer Res 2017.

METASTATIC SETTING

Cross tumour type actionable mutations in plasma



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Reprinted from Clin Cancer Res, 2016, 22(22):5497–505, Schwaederle M, *et al.* Use of Liquid Biopsies in Clinical Oncology: Pilot Experience in 168 Patients, with permission from AACR.

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CLINICAL APPLICATIONS OF ctDNA IN GASTROINTESTINAL TUMOURS

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CLINICAL APPLICATIONS OF ctDNA IN COLON CANCER

Adjuvant setting:

- Detection of occult residual disease

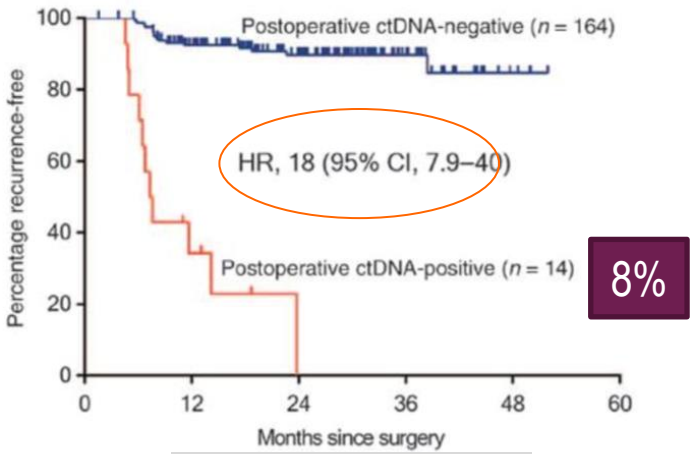
Metastatic setting:

- Detection of occult residual disease after metastatic resection
- Molecular characterisation of the tumour
- Monitoring of response and resistance

ADJUVANT SETTING

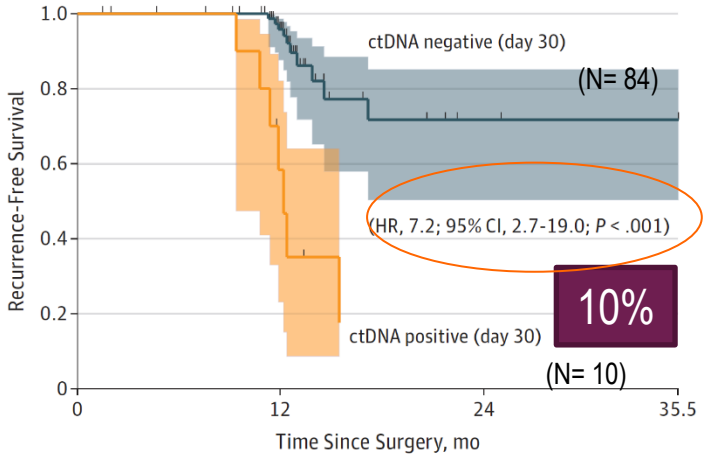
Colon cancer stage II-III patients not treated with adjuvant chemotherapy

Stage II post-surgery (4–10 weeks)[1]



78% of ctDNA+ relapse
10% of ctDNA- relapse

Stage I-III (mostly stage III) post-surgery (30 days)[2]

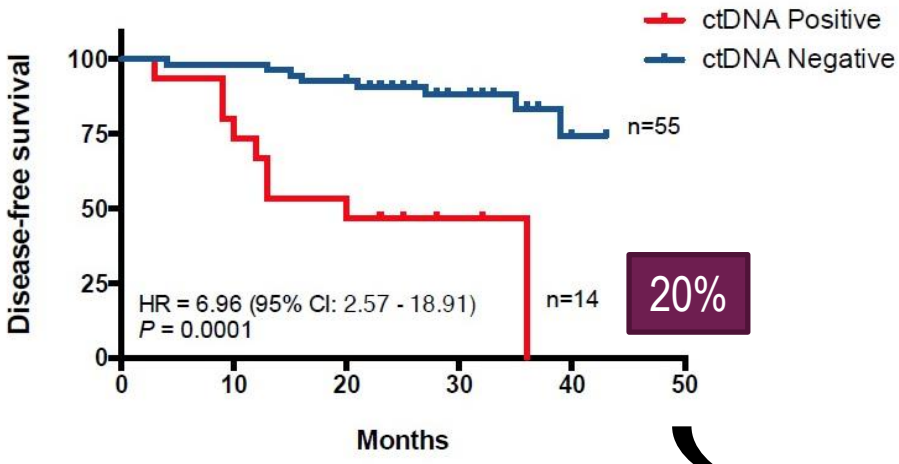


70% of ctDNA+ relapse
12% of ctDNA- relapse

1. From Tie J, *et al.* Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8(346):346ra92. Reprinted with permission from AAAS; 2. Reinert T, *et al.* JAMA Oncol 2019;5(8):1124–31. Reproduced under the terms of the CC-BY license (available at: https://creativecommons.org/licenses/by/2.0/; accessed Jan 2021);
Schøler ,*et al.* Clin Cancer Res 2017; Ng, *et al.* Sci Rep 2017; Wang *et al.* JAMA Oncol 2019.

ADJUVANT SETTING

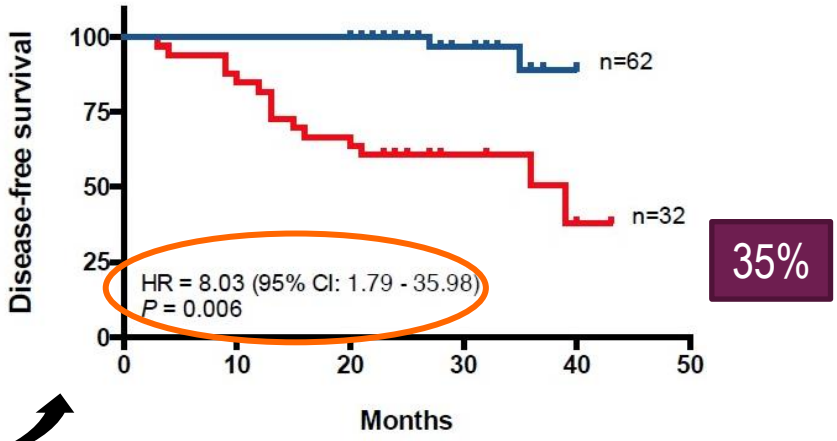
Post-operative (Week 6–8, N=69)



20%

57% of ctDNA+ relapse
10% of ctDNA- relapse

Mutation tracking (serial plasma samples; N=94)



35%

87% of ctDNA+ relapse
5% of ctDNA- relapse

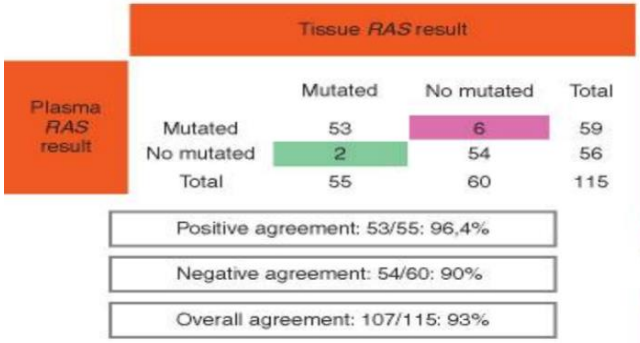
Increase predictive accuracy
(same cohort of patients?)

Tarazona N, *et al.* ESMO Annual Meeting 2019; abstract 2488. Reproduced with permission from Dr N Tarazona.

METASTATIC SETTING

Correlation tissue-ctDNA is high but still discordances may happen:

Citation	Cancer	Marker	Stage	Patient Number	Tissue Analysis	Plasma Analysis	Concordance
F. Jones *et al.*, Sysmex Inostics, Poster No. 2012 ECC 2015	mCRC	Extended RAS	IV	76	SOC RAS Panel	BEAMing	93.4%
Schmiegel *et al.* Poster No. 402 ECC 2015	CRC	Extended RAS	IV	50	SOC RAS Panel	BEAMing	92.6%
Diehl *et al.* Nature Medicine 2008	CRC	KRAS	IV	10	Sanger Sequencing	BEAMing	100%



RAS PLASMA MUT/ TISSUE WT

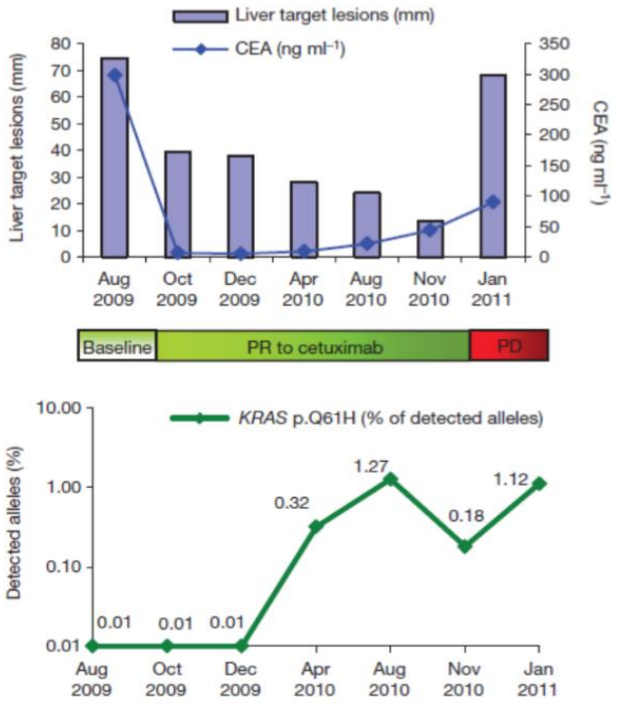
Pt n.	Codon	MAF	Site tumor biopsy	Primary tumor respected	Site of metastasis	Days between tissue – plasma collection	Systemic treatment before ctDNA	Treatment	Best response
1	KRAS 13	0,2458%	primary	yes	liver, lung	71	Yes	FOLFOX Panitumumab	PD
2	KRAS 12	0,128%	primary	yes	liver	138	No	FOLFOX Cetuximab	PR
3	KRAS 61	31,73%	primary	yes	liver, lung	122	No	XELOX	PD
4	KRAS 12	0,896%	primary	yes	liver	60	No	FOLFOX Cetuximab	PR
5	KRAS 61	0,316%	primary	no	liver	32	No	FOLFOX Cetuximab	PR
6	KRAS 13	0,05%	primary	no	liver, lung	21	No	FOLFOXIRI Bevacizumab	PR

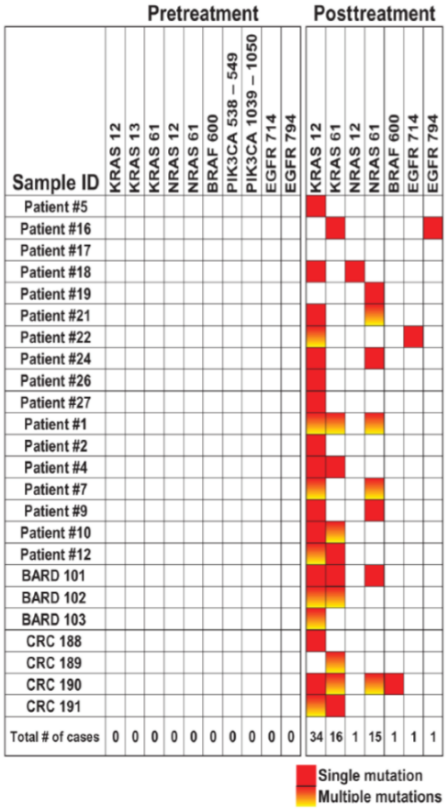
RAS PLASMA WT/ TISSUE MUT

Pt n.	Codon	MAF	Site tumor biopsy	Primary tumor resected	Site of metastasis	Days between tissue – plasma collection	Systemic treatment before ctDNA	Treatment	Best response
7	KRAS 12		primary	no	Peritoneum, lung	1195	No	no	
8	KRAS 12		primary	yes	Peritoneum	39	No	FOLFOX Bevacizumab	PR

METASTATIC SETTING

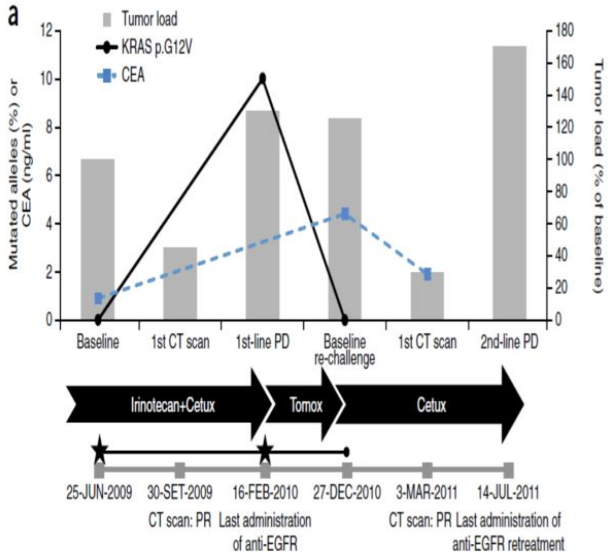
Monitor resistance to anti-EGFR mAbs:





Liquid biopsy for longitudinal monitoring of RAS mutations in blood of patients

Rechallenge with cetuximab



1. Reprinted by permission from Springer Nature: Nature, Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer, Misale S, *et al.* Copyright 2012; 2. rom Bettegowda C, *et al.* Sci Transl Med 2014;6(224):224ra24. Reprinted with permission from AAAS; 3. Reprinted by permission from Springer Nature, Nat Med, Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients, Siravegna G, et al. Copyright 2015; Díaz LA, *et al.* Nature 2012.

ctDNA IN PANCREATIC CANCER

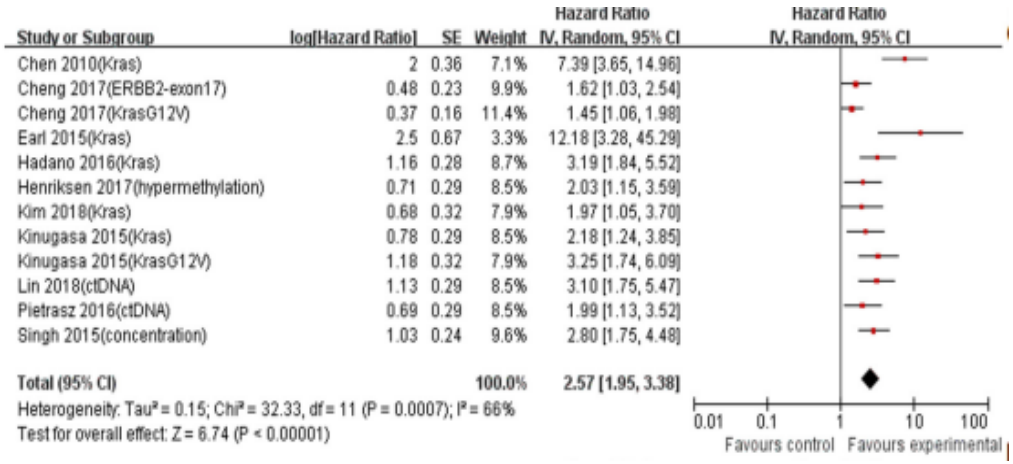
Insufficient sensitivity for screening and diagnostic use

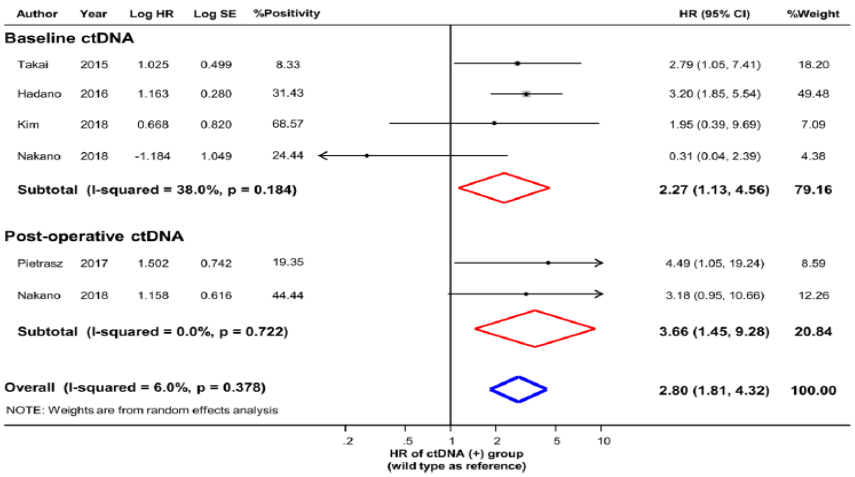
Tumour burden in metastatic setting

- ctDNA: negative prognostic factor for OS HR = 2.57 [1.95, 3.38]; n=1243 patients)

Detection of minimal residual disease after resection

- ctDNA at baseline or postoperatively: prognostic in resectable PC (HR=2.80 [1.81, 4.32])





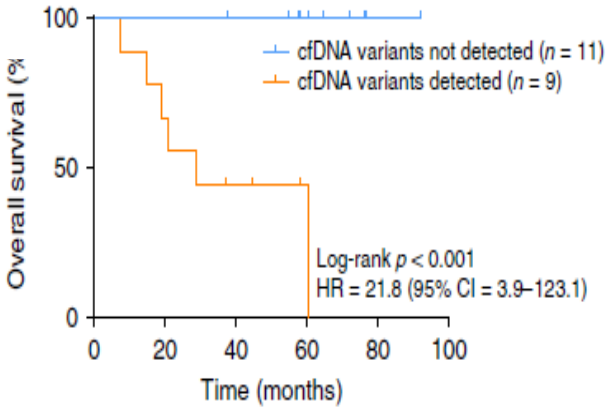
1. Reprinted from Gene, 679: Chen L, *et al.* Prognostic value of circulating cell-free DNA in patients with pancreatic cancer: A systemic review and meta-analysis, 328–3, Copyright 2018, with permission from Elsevier; 2. Lee J-S, *et al.* Sci Rep 2019;9(1):16971. Reproduced under the terms of the Creative Commons CC BY 4.0 license (available at: https://creativecommons.org/licenses/by/4.0/; accessed Jan 2021).

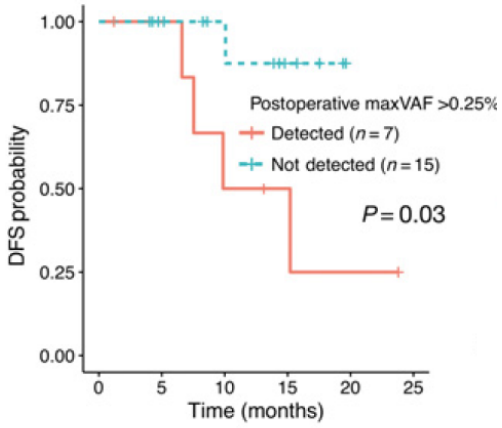
ctDNA IN GASTRO-OESOPHAGEAL CANCER

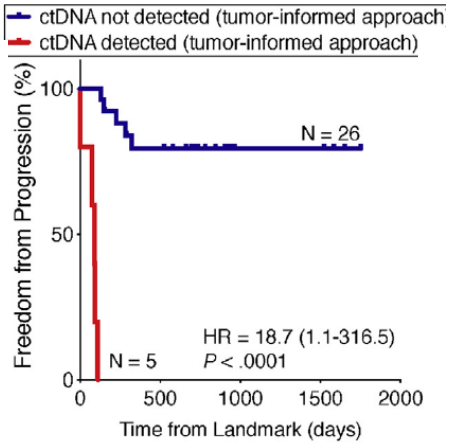
Limited data on the role of ctDNA in diagnosis, monitoring and prognosis in GE cancer

Detection of Minimal residual disease after curative Rx

Small studies (n=20-31). Post-therapeutic ctDNA associated with reduced OS and PFS







CLINICAL APPLICATIONS OF ctDNA IN LUNG CANCER

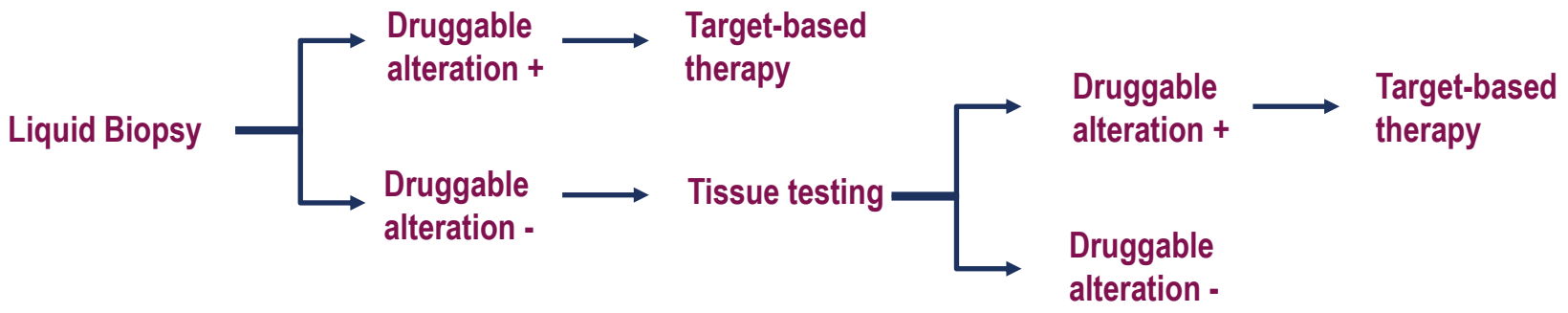
LIQUID AND TISSUE BIOPSY IN LUNG CANCER

EGFR T790M example

Best percentage change in target lesion diameter for evaluable patients (n=231)

	ORR (95% CI)	p-value
Tumor T790M+	62% (54, 70)	<0.001
Tumor T790M–	26% (15, 39)	
Plasma T790M+	63% (55, 70)	0.011
Plasma T790M–	46% (36, 56)	

POSSIBLE ALOGRITHM IN NSCLC



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Oxnard J, *et al.* J Clin Oncol 2016;34(28):3375–82.

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LIMITATIONS OF ctDNA IN LUNG CANCER

ctDNA can detect well

- single nucleotide variants: e.g., mutations in *EGFR, KRAS, BRAF*

ctDNA has less utility for detection of

- copy number variations: *MET* amplification
  - problem: there is no good mechanism to relate increase in copy numbers in ctDNA to increase in copy numbers in tumour tissue
- rearrangements: *ALK* fusions, *ROS* fusions, *RET* fusions
  - problem: fusions have different partners and breakpoints, which are difficult to detect with DNA based technologies

If liquid biopsy is negative for targetable genomic aberrations, it might be reasonable to obtain tissue testing if feasible

CLINICAL APPLICATIONS OF ctDNA IN BREAST CANCER

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ctDNA STUDIES IN METASTATIC BREAST CANCER (MBC)

Numerous small studies performed, main findings suggest that:

High ctDNA molecule numbers (expressed as variant allele frequency or mutant copies/mL) at baseline associated with poor outcome and probably reflects tumour load/tumour aggressiveness

Decrease in ctDNA molecule numbers during treatment is associated with a better outcome compared with patients with an increase in ctDNA molecule numbers during treatment

A high number of different mutated variants is associated with poor outcome and probably reflects disease heterogeneity

The presence/emergence of mutated variants of genes associated with resistance (i.e., ESR1 variants in MBC patients treated with aromatase inhibitors) precedes radiological/clinical progression

Based on these studies:

High need for consensus on standard procedures on how to determine ctDNA and express ctDNA molecule numbers (variant allele frequency or mutant copies/mL)

Kruger DT, *et al.* Mol Oncol. 2020; Turner NC, *et al.* Clin Cancer Res 2020; Clatot F, *et al.* Breast Cancer Res. 2020; Beije N, *et al.* Mol Oncol 2018.

1ST ctDNA FDA APPROVED TEST IN MBC

The Therascreen® PIK3CA RGQ PCR Kit to identify patients who can be eligible for treatment with alpelisib and fulvestrant

Detects 11 PIK3CA mutations in tumour tissue or plasma

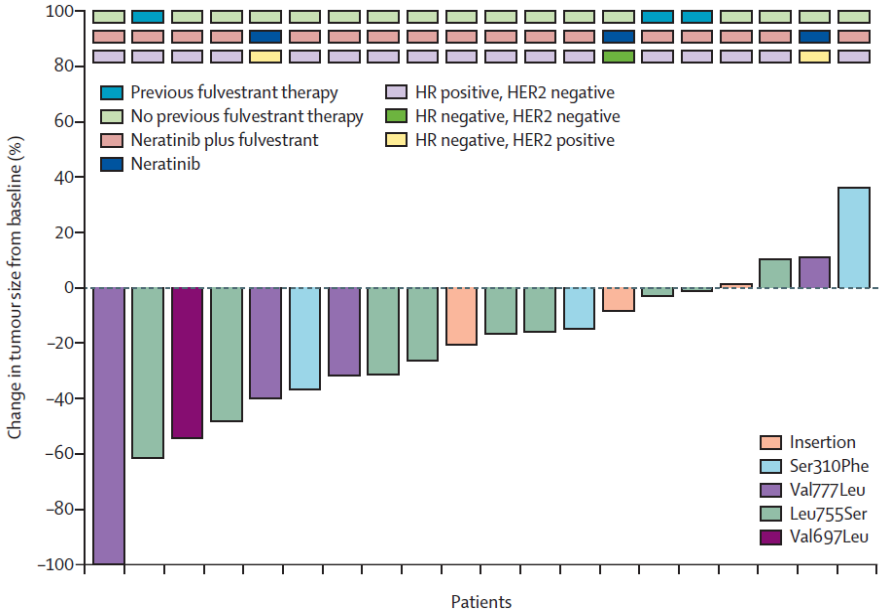
If not detected in plasma, then PIK3CA status must be determined in tumour tissue

Alpelisib–fulvestrant improves PFS in PIK3CA-mutated, HR-positive, HER2-negative MBC patients who previously received endocrine therapy

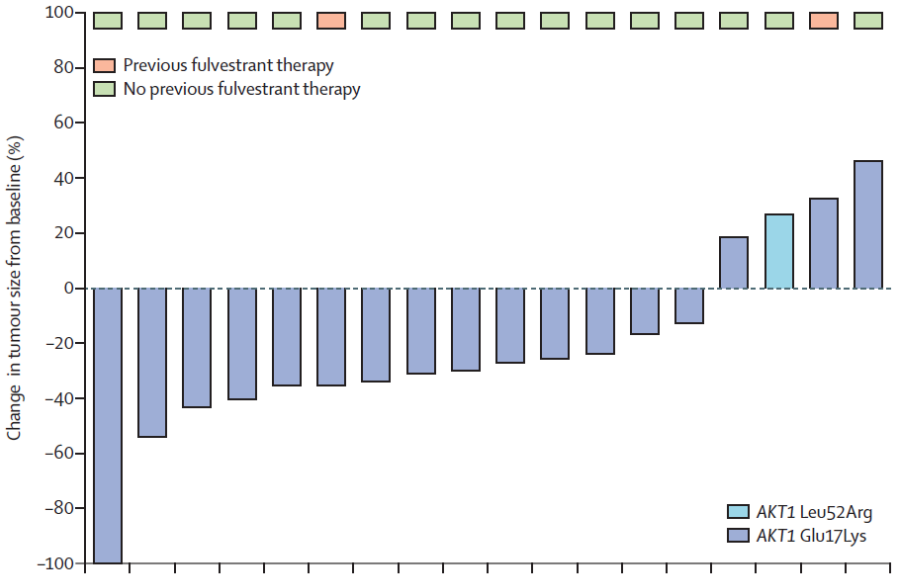
André F, *et al*. NEJM 2019.

PLASMA MATCH TRIAL

Targeting rare HER2 mutations in ctDNA with neratinib



Targeting rare AKT12 mutations in ctDNA with capivasertib + fulvestrant



EXAMPLES OF ONGOING STUDIES

To demonstrate clinical utility of ctDNA in MBC

PADA-1 (NCT03079011): Phase 3 study; ER+/HER2– MBC; treatment with CDK4/6inh + AI; patients with rising ESR1 and without tumour progression randomised between continuation of CDK4/6inh + AI *vs,* switch to CDK4/6 + fulvestrant; endpoint PFS

INTERACT study (NCT04256941): randomised Phase 2; ER+ MBC; treatment with CDK4/6inh + AI for at least 12 months. Presence of ESR1 variants. Randomisation CDK4/6 inh + AI *vs.* CDK4/6 inh + fulvestrant; endpoint PFS

ctDNA STUDIES IN EARLY BREAST CANCER

Study	Disease subtype	Setting of ctDNA monitoring	ctDNA technology used	Patients (N)	Patients with evaluable ctDNA results (n)	Median lead time from ctDNA relapse to clinical relapse (months)	DFS/RF[a]	pCR[b]
Olsson, *et al.* (2015)	All	Adjuvant	ddPCR	20	20	11	Yes	NA
Riva, *et al.* (2017)	TNBC	Neoadjuvant	ddPCR	46	38	NR	Yes	No
Chen, *et al.* (2017)	TNBC	Adjuvant	134-gene NGS panel	38	33	<8	Yes	NA
Garcia-Murillas, *et al.* (2015 and 2019)	All	Neoadjuvant and/or adjuvant	ddPCR	225	144	10.7	Yes	NR
Rothé, *et al.* (2019)	HER2$^+$	Neoadjuvant	ddPCR	119	69	NR	No	Yes
Coombes, *et al.* (2019)	All	Adjuvant	Signatera assay	50	49	8.9	Yes	NA
McDonald, *et al.* (2019)	All	Neoadjuvant/ adjuvant	TARDIS	33	33	NR	NA	Yes
Zhang, *et al.* (2019)	All	Adjuvant	68-gene NGS panel 136-gene NGS panel	102	102	NR	NR	NA
Radovich, *et al* (2020)	TNBC	Post-neoadjuvant	FoundationACT or FoundationOne Liquid CDx	196	142	22.8	YES	NA

ctDNA, circulating tumour DNA; ddPCR, droplet digital polymerase chain reaction; DFS/RFS, disease-free survival or relapse-free survival; NA, not applicable; NGS, next-generation sequencing; NR, not reported; pCR, pathological complete response; TNBC, triple-negative breast cancer.

[a]Association between ctDNA detection during follow-up surveillance after neoadjuvant and/or adjuvant chemotherapy and surgery and unfavourable DFS/RFS.

[b]Association between ctDNA detection before and during the administration of neoadjuvant chemotherapy and a lower pCR rate.

Ignatiadis M, *et al*. Nat Rev Clin Oncol 2021 Jan 20. doi: 10.1038/s41571-020-00457-x. Online ahead of print.

ctDNA RELAPSE

A new space for drug development in breast cancer?

Setting	ADJUVANT	NEO-ADJUVANT	POST-NEO-ADJUVANT	ctDNA RELAPSE	METASTATIC
Curability	Curable	Curable	Curable	Unknown	Uncurable
Target	Treatment-naive MRD	Primary tumour and treatment-naive MRD	Treatment-resistant MRD	Treatment-resistant MRD	Metastases
Monitoring treatment efficacy	No	pCR	No	ctDNA clearance?	RECIST
Trial endpoints	iDFS, OS	EFS, OS	iDFS, OS	iDFS, OS	PFS, OS

EFS, event- free survival; iDFS, invasive disease-free survival; MRD, minimal residual disease.

Ignatiadis M, *et al*. Nat Rev Clin Oncol 2021 Jan 20. doi: 10.1038/s41571-020-00457-x. Online ahead of print.

CLINICAL APPLICATIONS OF ctDNA/CTCs IN PROSTATE CANCER

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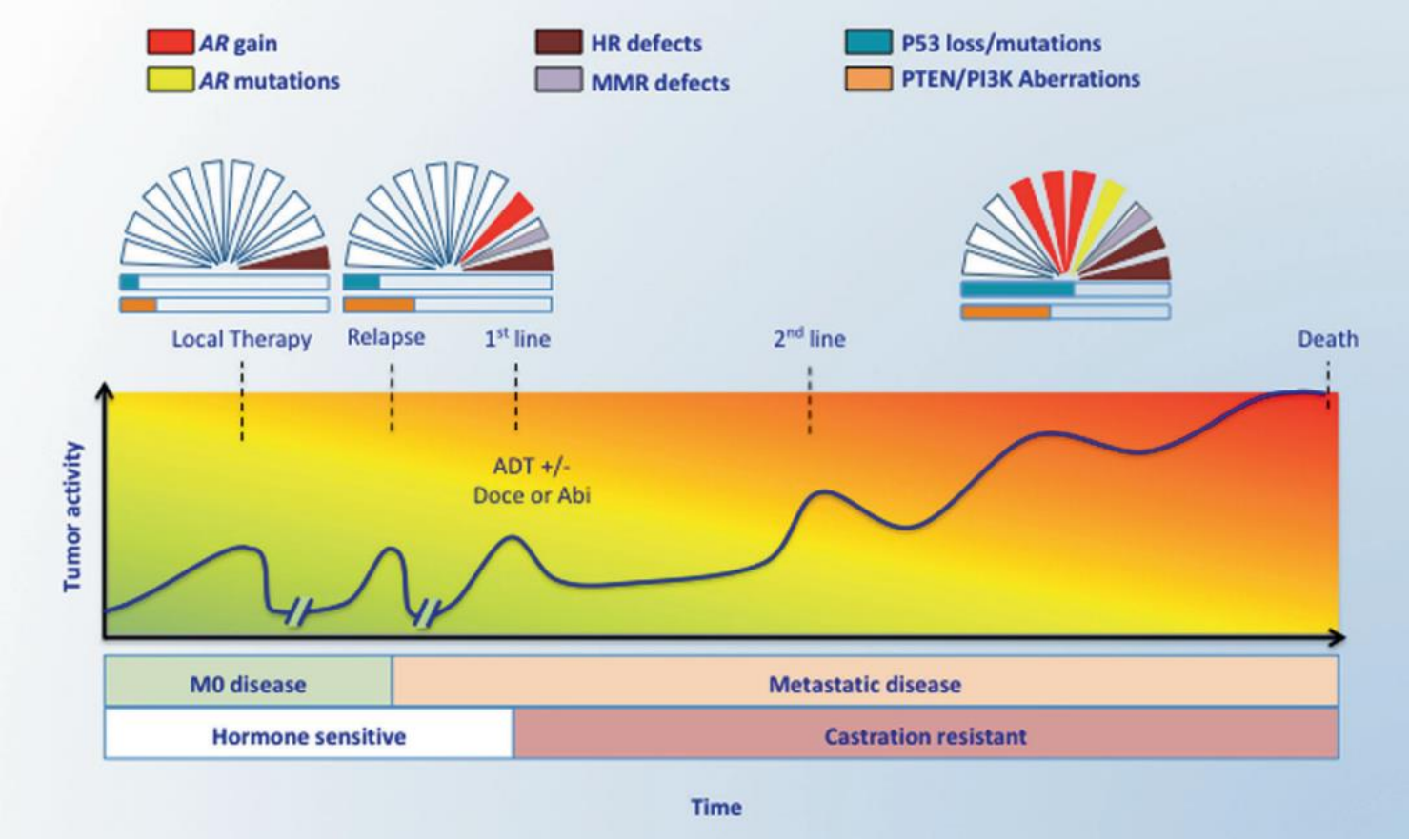
LIQUID BIOPSY

To capture the genomic landscape of prostate cancer

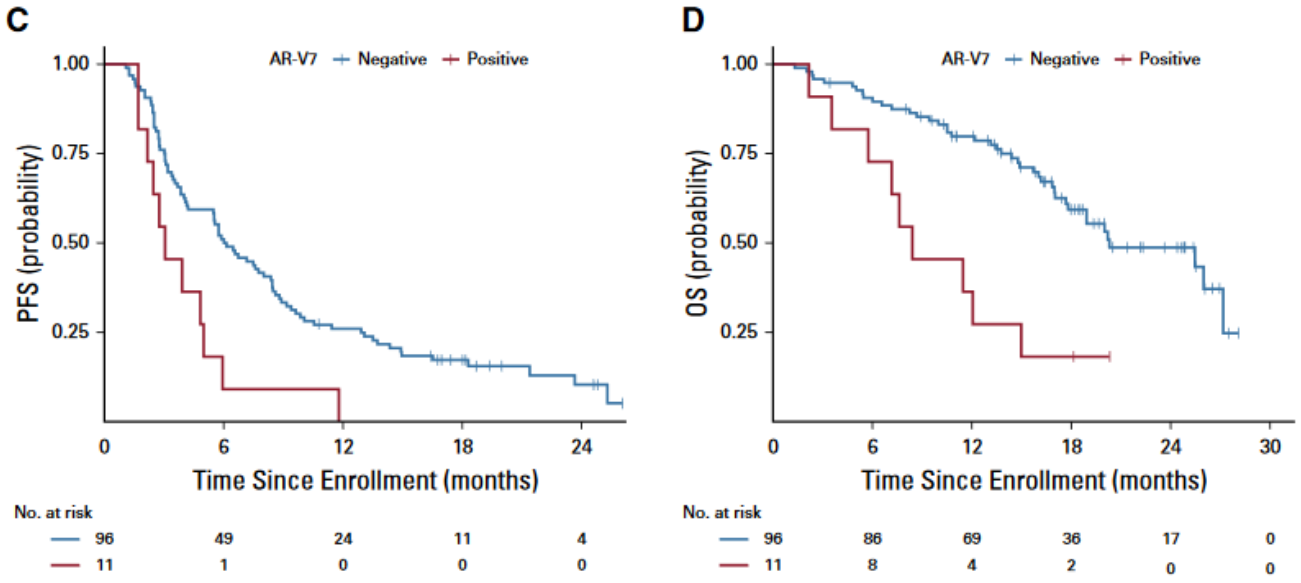


Reprinted by permission from Springer Nature, Prostate Cancer Prostatic Dis, Circulating tumor DNA in advanced prostate cancer: transitioning from discovery to a clinically implemented test, González-Billalabeitia E, *et al.* Copyright 2019.

PROSPECTIVE VALIDATION OF ANDROGEN RECEPTOR SPLICE VARIANT 7 (AR-V7)

The Prophecy Study

Detection of AR-V7 in Circulating Tumour Cells in men with metastatic castration resistant prostate cancer is associated with shorter PFS and OS with abiraterone or enzalutamide; these patients remain sensitive to docetaxel



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TAKE HOME MESSAGES

Advances in circulating tumour (ctDNA) have led to the introduction of liquid biopsy in the clinic

Single and multi-gene ctDNA assays are now reimbursed for treatment selection for several indications in metastatic solid tumours

Assays for the detection of ctDNA relapse (detection of rising ctDNA without imaging detected relapse) are currently being evaluated for their clinical utility

DISCLOSURES

- **Michail Ignatiadis** has reported honoraria for Advisory Boards: Novartis, Seattle Genetics. Research funding as Trial Chair from Pfizer, Roche (Institutional financial interests), from Natera Inc., as Coordinating PI (Institutional no financial interest).
- **Dirk Arnold** has reported honoraria for Advisory Boards: Bayer Healthcare, Amgen, Merck Sharp & Dhome, Merck Serono, Eli Lilly, Bristol Myers Squibb, Servier, Roche, Terumo, Sirtex, Boston Scientific. Honoraria for presentations: Bayer Healthcare, Amgen, Servier, Roche, Terumo, Astellas, Biocompatibles, Sirtex, ArtTempi Media, Prime Oncology, TRM Oncology. Support for congress travel: Bristol Myers Squibb, Roche, Sanofi. Consulting board role IQVIA (paid to his Institution). Research Funding: Documentation fees with clinical trials, paid to his Institution by Sanofi, AstraZeneca, Incyte, Merck Sharp & Dohme. Non-financial interests: Flatiron. Principal Investigator of phase III trial with MOLOGEN. Planned as principal investigator with SFJ, Pleasanton (acting as trial sponsor for Merck Serono). Planned as principal investigator with Oncolytics. Scientific Advisory Board for Oncolytics, Biotech, SFJ, Munich Biotech. Leadership roles/Membership: ECCO Member of the Executive Board 2015-2017 (on behalf of ESMO), membership of the Finance Committee. AIO in DKG: Member since 2003, Chairperson of Colorectal Cancer Working Group 2010-2018, membership in other steering committees. EORTC: Member of GI Cancer Group, Steering Committee Member since 2008; Task Force lead for Rectal Cancer and Anal Group since 2016.
- **Ahmad Awada** has reported Advisory role, research grants to his institute. Speaker for Roche, Lilly, Amgen, EISAI, BMS, Pfizer, Novartis, MSD, Genomic Health, Ipsen, AstraZeneca, Bayer, Leo Pharma.
- **Paul Morten Mau-Sørensen** has reported Advisory Boards for Roche and Genmab. He has received research grant from Karyopharm. He has conduced sponsored trials with AstraZeneca, Bioclin, BMS, Cantargia, Genmab, Incyte, Loxo, Merck, Novartis, Pfizer, Puma biotechnology,Roche, Symphogen, Alligator Bioscience, Karyopharm, MSD, AbbVie, Sanofi-Aventis, Orion, Eli Lilly, (financial support paid directly to his institution).

DISCLOSURES

- **Emiliano Calvo** has reported Honoraria or consultation fees from: Astellas, Novartis, Nanobiotix, Pfizer, Janssen-Cilag, GLG, PsiOxusTherapeutics, Merck, Medscape, BMS, Gilead, Seattle Genetics, Pierre Fabre, Boehringer Ingelheim, Cerulean Pharma, EUSA, GehrmannConsulting, AstraZeneca, Roche Guidepoint, Servier, Celgene, Abbvie, Amcure, OncoDNA, Alkermes. Leadership role: Director, Clinical Research, START Madrid, Director, Clinical Research, HM Hospitals Group, Madrid. Stocks or ownership: START, OncoartAssociated, International Cancer Consultants. Direct research funding as project lead: Novartis, AstraZeneca, Beigene. Institutional financial support from clinical trials: Abbvie, ACEO, Amcure, AMGEN, AstraZeneca, BMS, Cytomx, GSK, Genentech/Roche, H3, Incyte, Janssen, Kura, Lilly, Loxo, Nektar, Macrogenics, Menarini, Merck, Merus, Nanobiotix, Novartis, Pfizer, PharmaMar, Principia, PUMA, Sanofi, Taiho, Tesaro, BeiGene, Transgene, Takeda, Incyte, Innovio, MSD, PsiOxus, Seattle Genetics, Mersana, GSK, Daiichi, Nektar, Astellas, ORCA, Boston Therapeutics, Dynavax, DebioPharm, BoehringenIngelheim, Regeneron, Millenium, Synthon, Spectrum, Rigontec. Non-financial interests: Scientific board at PsiOxus. Leadership in medical society: Founder and president, non-for-profit Foundation INTHEOS (Investigational Therapeutics in Oncological Sciences). Memberships: SEOM, EORTC, ESMO, ASCO10. Other relationships: HM Hospitals Group and START, Program of Early Phase Clinical Drug Development in Oncology, Employee: Medical Oncologist, Director, Clinical Research. Methods in Clinical Cancer Research (MCCR) Workshop, Zeist, Netherlands (Joint ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer, Research), Co-director.
- **Stefan Sleijfer** has reported travel reimbursement from Astra Zeneca to speak at post-ASCO event, Supervisory board (fee for institute) from Skyline Dx, financial support as PI from Ab Science, Servier, Philips, Sanofi and Blue Medicine.
- **Alain Hendlisz** has reported no conflicts of interest

DISCLOSURES

- **Felip Janku** has reported to have a research support from Novartis, Deciphera, Symphogen, Piqur, Roche, BioMedValley Discoveries and Upsher-Smith Laboratories; he is on the Scientific Advisory Boards of Deciphera, Illumina and Guardant Health, he provides paid consulting for Immunoment, IFM Therapeutics and Trovagene and has ownership interest in Trovagene.
- **Guillem Argiles** has reported no conflicts of interest
- **Patrick Pawels** has reported honoraria for public speaking, advisory role from BMS, Pfizer, Roche, MSD, Takeda, Boehringer, Novartis, AstraZeneca, Diocartes, Amgen (advisory role, expert testimony). Research funding to his institution from AstraZeneca, Roche, Biocartes.