

Application of oncoMonitor liquid biopsy assay for monitoring of therapy and minimal residual disease in various solid cancers

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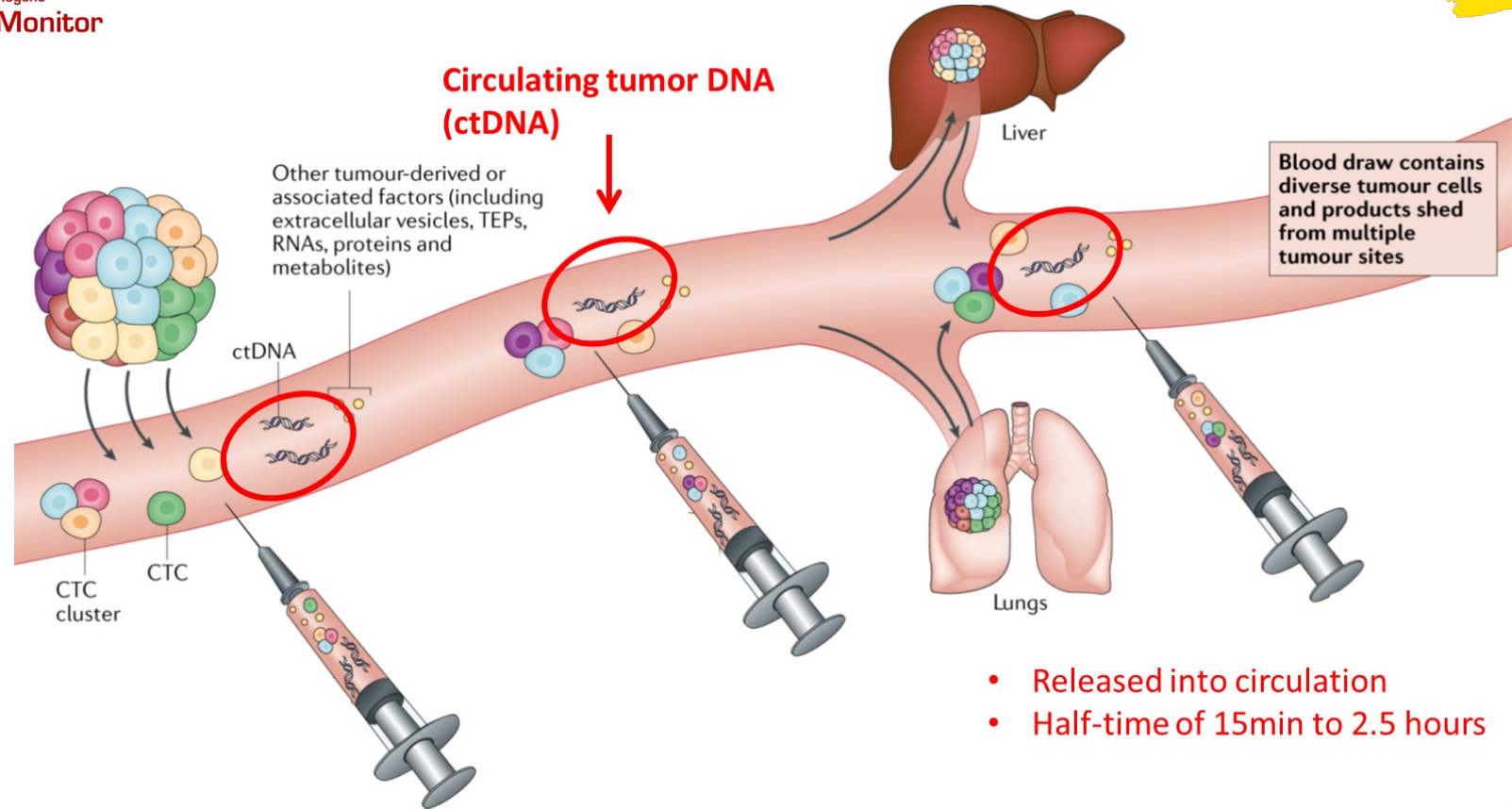
Disclosure



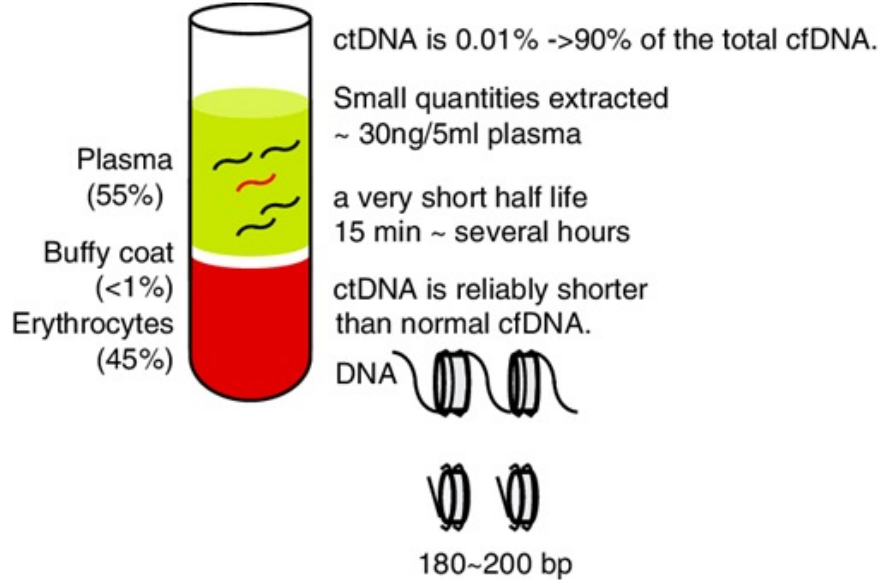
Ownership in Elphogene, s.r.o. company

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Liquid biopsy - evaluation of ctDNA



Liquid biopsy - evaluation of ctDNA



Key to success:

1. Efficient sampling and extraction
2. Detection sensitivity

	Mass of cfDNA in sample (ng)	
	10ng	1ng
Genome Equivalents	3,000	300
0.1% ctDNA	3 mutant copies	0.3 mutant copies

Liquid biopsy approaches



Tumor Uninformed Approach

No baseline tumor or cfDNA analysis

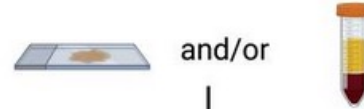


Same panel for all patients



Off the shelf assay
Shorter turnaround time
Can detect evolving clonal variants
Lower cost

Tumor Informed Approach



and/or

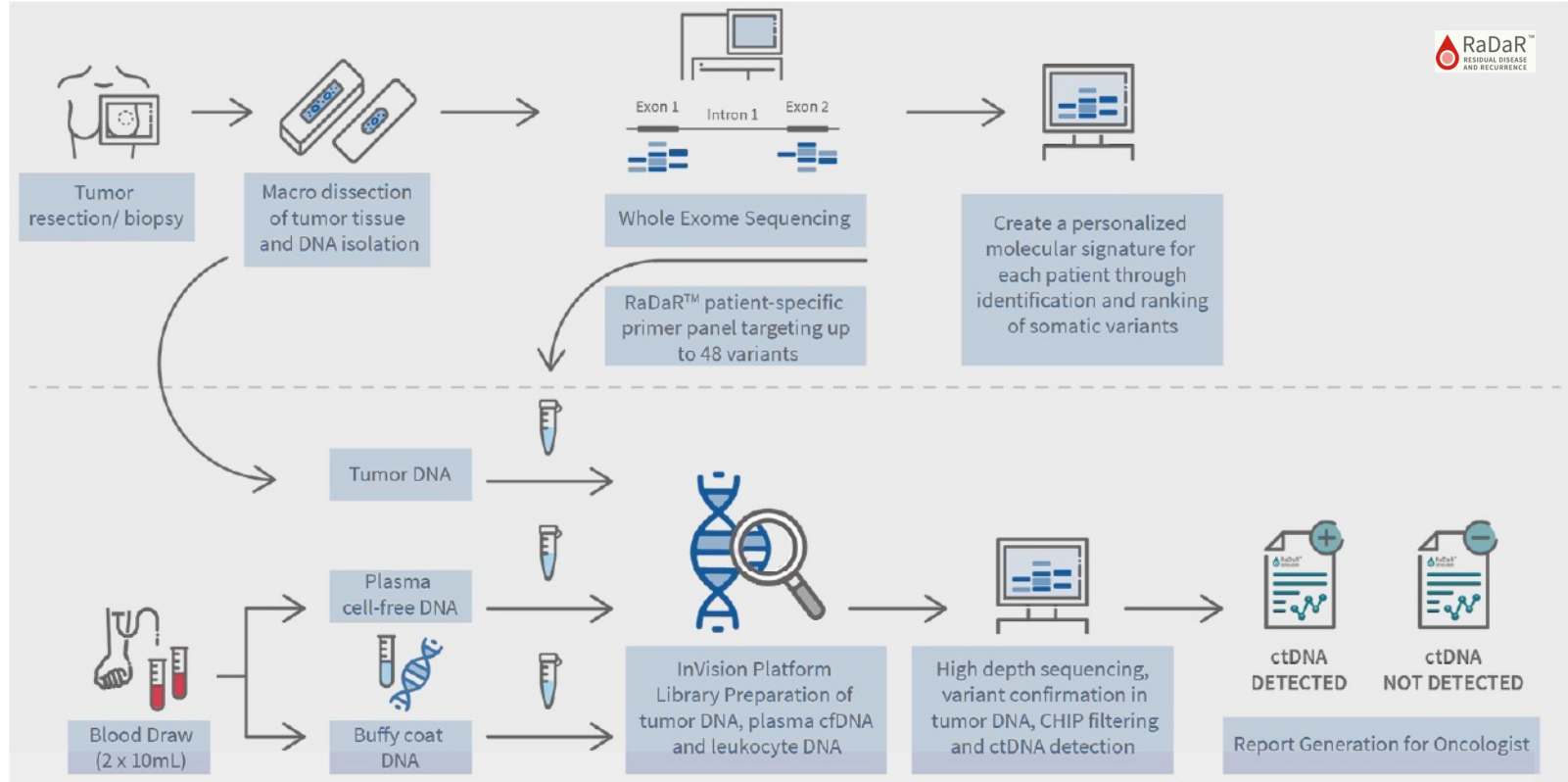


Personalised assay developed



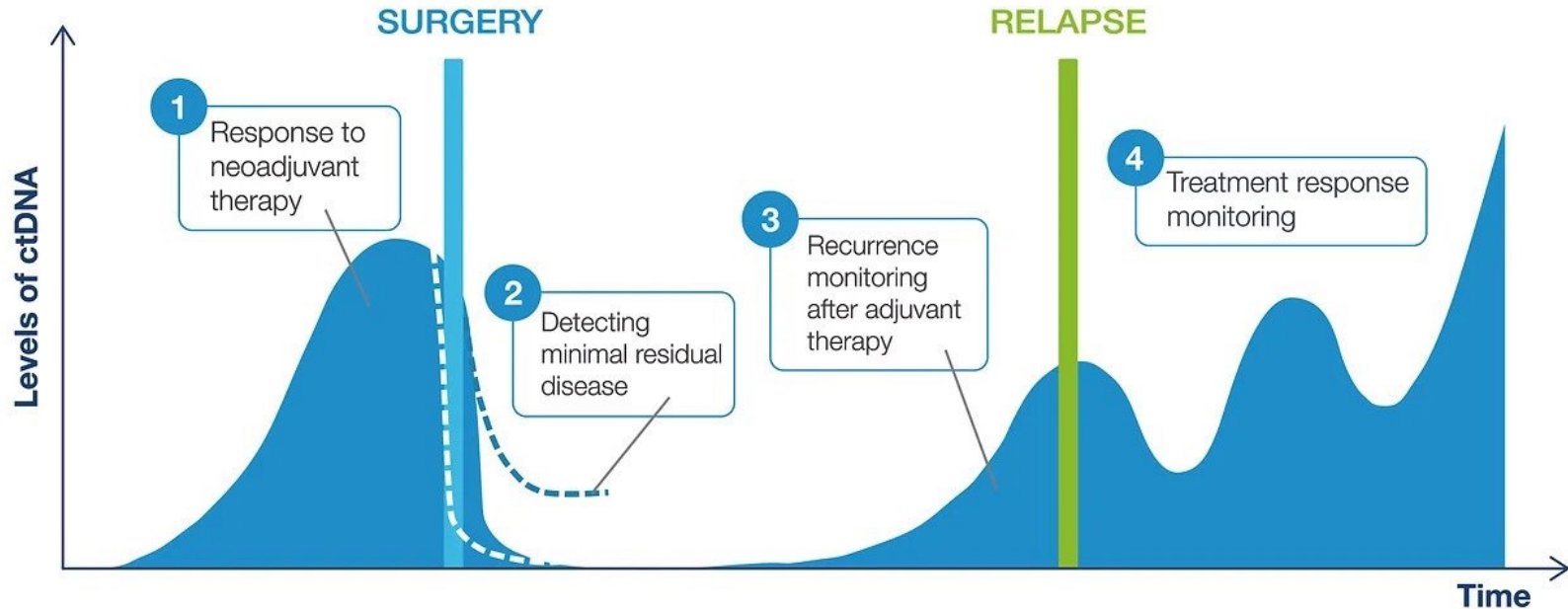
Requires prior knowledge of tumour genotype
Personalised assay
Longer turnaround time
Does not account for clonal evolution
More sensitive and specific

Tumor - informed workflow












Clinical applications of ctDNA testing for MRD assessment

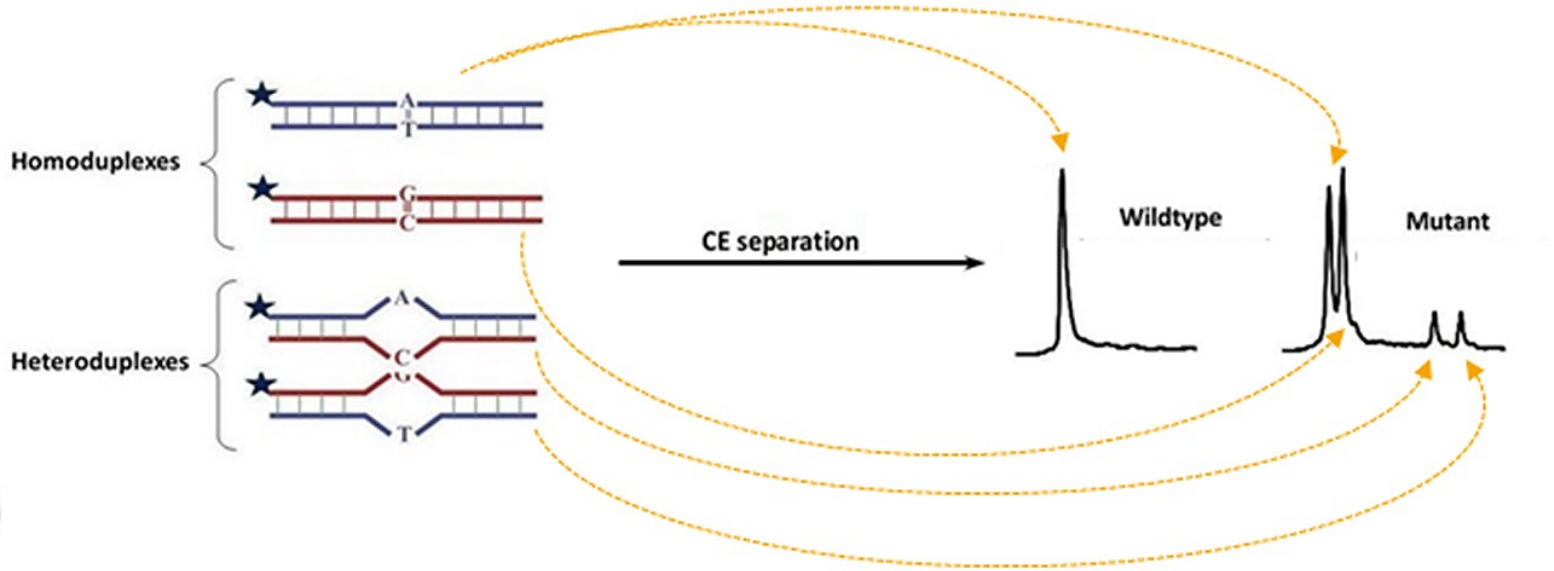


Current portfolio of MRD assays



Assay	Company	Method	Sensitivity (MAF)
Signatera	 natera®	WES, Multiplex PCR	0,01 %
RaDaR	 NEO GENOMICS	WES, Amplicon NGS	0,001 %
CAPP-Seq	 EXACT SCIENCES	Hybrid capture NGS	0,003 %
AVENIO	 Roche	Hybrid capture NGS	0,1 %
Reveal	 GUARDANT™	Hybrid capture + CpG	0,01 %
MRDetect	 C2i Genomics	WGS + „AI“	0,001 %
oncoMonitor	 Elphogene	Hybrid capture NGS + DCE	0,1 %

Denaturing Capillary Electrophoresis (DCE)



Sanger DNA sequencer



Elphogene
oncoMonitor

oncoMonitor assay - a two-tier method

frontiers
in Oncology

ORIGINAL RESEARCH
published: 24 July 2020
doi: 10.3389/fonc.2020.01028

Monitoring of Early Changes of Circulating Tumor DNA in the Plasma of Rectal Cancer Patients Receiving Neoadjuvant Concomitant Chemoradiotherapy: Evaluation for Prognosis and Prediction of Therapeutic Response

Filip Pazdrek¹, Marek Minarik^{1,2*}, Lucie Benesova¹, Tereza Halkova², Barbora Belsanova², Milan Macek¹, Lubomir Stepanek¹ and Jiri Hoch¹

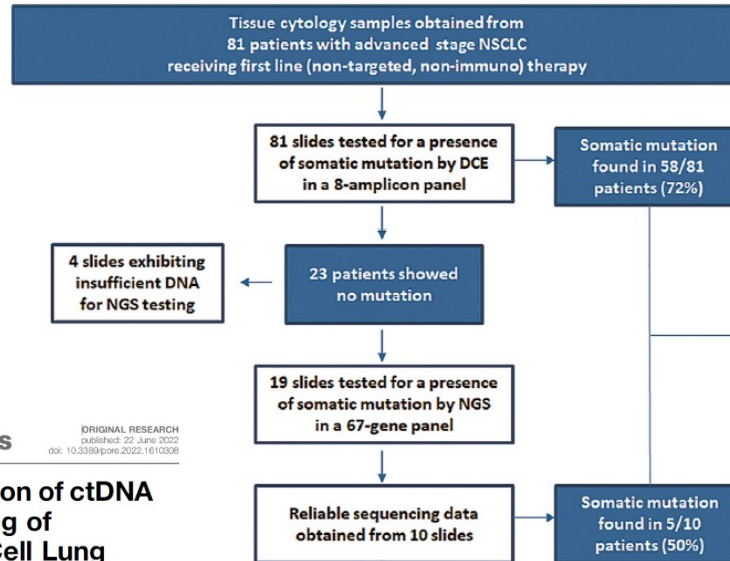
Pathology &
Oncology Research

frontiers

ORIGINAL RESEARCH
published: 22 June 2022
doi: 10.3389/fonc.2022.1010098

Detection and Quantification of ctDNA for Longitudinal Monitoring of Treatment in Non-Small Cell Lung Cancer Patients Using a Universal Mutant Detection Assay by Denaturing Capillary Electrophoresis

Lucie Benesova¹, Renata Ptackova¹, Tereza Halkova¹, Anastasiya Semyakina¹, Martin Svaton², Ondrej Fiata^{3,4}, Milos Pesek² and Marek Minarik^{4,5*}



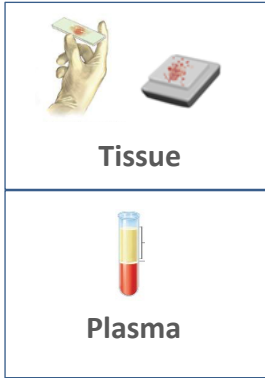
small DCE panel
(10 – 20 hotspots)

large NGS panel
(128 genes)

AmoyDx

A tumor-informed liquid biopsy

(Capillary Electrophoresis heteroduplex assay)



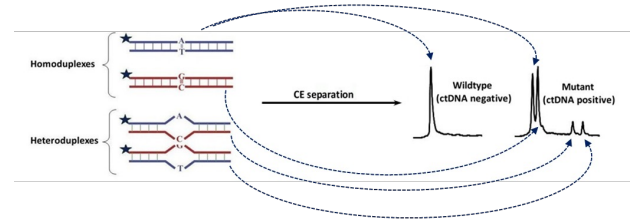
AmyDx ddCap[®] Comprehensive NGS panel Tissue or plasma (128 genes)

Detection of SNVs, Indels, Fusions, CNVs (130 genes)																	Detection of Polymorphisms (53 genes)																																																																																																													
AKT1	AKT2	AKT3	ALK	APC	AR	ARAF	ARID1A	ATM	ATR	AURKA	ARCB1	MTOR	BAP1	BCL2L1	BRCA1	BRCA2	CCND1	CENPE	CD274	CDK12	CDK4	CDK6	Cdher34	SEMA3C	CDKN2A	CDKN2B	CREBBP	CTNNA1	CTNNA1	DDR2	EGFR	EIF1AX	EPAS1	EPCAM	ERBB2	ERBB3	CD4	SLC28A3	ERBB4	ESR1	ETSE1	PANCA	FBXW7	FGF19	FGF3	FGFR1	FGFR2	FGFR3	FGFR4	CHP13A1	SOX2	FLCN	FLT3	GNAS	HIF1A	KRAS	DHPL	DHPL	GDF19	JAK1	JAK2	JAK3	CYP26B1	TSP1	KDM5C	KDR	KIT	KRAS	MMP9K1	MMP9K1	MET	MLH1	MRE11	MSH2	MSH4	DPYD	UGT1A1	MTOR	MYC	NF1	NF2	NOTCH1	NRAS	NRG1	NTXK1	NTXK2	NTXK3	PALB2	BINC2H1	UMPS	PIK3A	PDCD1	PDGFRA	PDR	PKNOX1	PKNOX1	PIK3R1	PIK3R1	PIK3R2	PIK3R3	PIK3R4	PTEN	RAF1	RASAI	RASAI	RBBP1	RET	RICTOR	RIT1	RPL1	RPL1	SPR1	GSTP1	XRCC1	SMAD4	SMAD4	SMO	STK11	TEK1	TOP2A	TP53	TRCC1	TRCC2	TSHR	VHL	MTORR1

SNV, Indel • SNV, Indel, CNV • SNV, Indel, Fusion • SNV, Indel, Fusion, CNV • Fusion •

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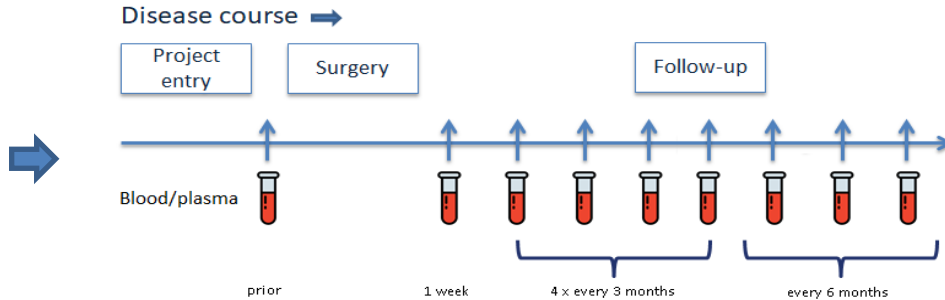
oncoMonitor (denaturing CE)



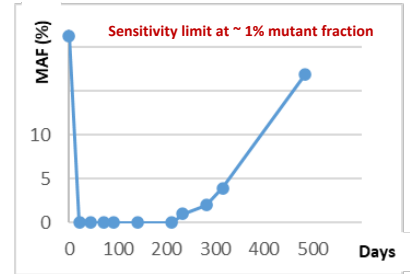
1. Tissue/plasma mutation profiling by NGS
2. Simple PCR design on any mutants
3. ctDNA detection by partially-denaturing CE



Cancer patient



Low-cost minimal residual disease monitoring by analysis of specific mutation(s)

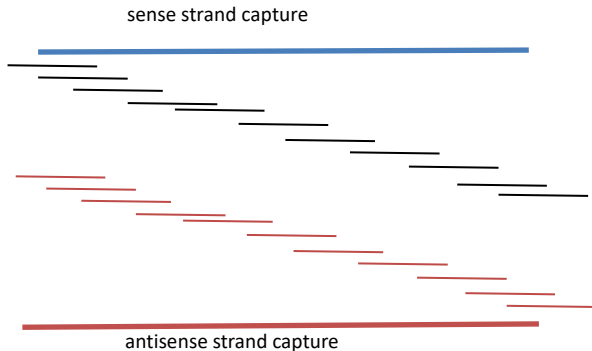


Early detection of recurrence



ddCap[®] Comprehensive Panel

Dual: probes targeting double strands



Total turnaround time

- 5h extraction and quality check
- 24 - 48h NGS library prep
- 24h Illumina sequencing (NextSeq/NovaSeq)
- 2 - 3h Data processing and analysis

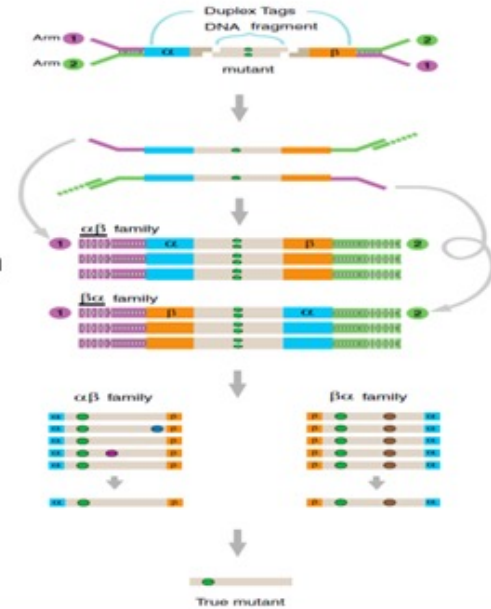
Digital: Unique molecular identifiers

1. Adapter Ligation

2. PCR Amplification
Error Rate 10^{-3}

3. Single UMI
Error Rate 10^{-5}

4. Double UMI
Error rate 10^{-6}





ddCap® Comprehensive Panel

Detection of SNVs, InDels, Fusions, CNVs (110 genes)												Detection of Polymorphisms (19 genes)	
AKT1	AKT2	AKT3	ALK	APC	AR	ARAF	ARID1A	ATM	ATR	AURKA	ABCB1	MTRR	
BAP1	BCL2L1	BRAF	BRCA1	BRCA2	CCND1	CCNE1	CD274	CDK12	CDK4	CDK6	C8orf34	SEMA3C	
CDKN2A	CDKN2B	CREBBP	CTNNB1	DDR2	EGFR	EIF1AX	EPAS1	EPCAM	ERBB2	ERBB3	CDA	SLC28A3	
ERBB4	ESR1	ETS2	FANCA	FBXW7	FGF19	FGF3	FGFR1	FGFR2	FGFR3	FGFR4	CYP19A1	SOD2	
FLCN	FLT3	GNAS	HIF1A	HRAS	IDH1	IDH2	IGF1R	JAK1	JAK2	JAK3	CYP2D6	TP53 ¹	
KDM5C	KDR	KIT	KRAS	MAP2K1	MAPK1	MET	MLH1	MRE11	MSH2	MSH6			
MTOR	MYC	NF1	NF2	NOTCH1	NRAS	NRG1	NTRK1	NTRK2	NTRK3	PALB2			
PAX8	PDCD1	PDGFRA	PGR	PIK3CA	PIK3R1	PMS2	POLD1	POLE	PSMD4	PTCH1			
PTEN	RAF1	RASA1	RASAL1	RB1	RET	RICTOR	RIT1	ROS1	RSF1	SF3B1			
SMAD4	SMARCA4	SMO	STK11	TERT	TOP2A	TP53 ¹	TSC1	TSC2	TSHR	VHL			

SNV, InDel ● SNV, InDels, CNV ● SNV, InDel, Fusion ● SNV, InDel, Fusion, CNV ● Fusion ●

1. TP53: SNVs, InDels and polymorphisms

Parameter	Specifications
Technology	ddCAP®
Target Regions	128 genes and MSI
Alterations Detected	SNV, Indel, Fusion, CNV, SNP, MSI *
Tumor Type	Cross-tumor
Sample Type	FFPE tumor tissue, liquid biopsy
DNA Input	FFPE DNA: optimal 100 ng (minimum 50 ng) Plasma cfDNA: optimal 30 ng (minimum 10 ng)
Limit of Detection (LoD)	FFPE DNA: 5% allele frequency; 20% tumor content Plasma cfDNA: 0.5% allele frequency
Data Output per Sample	FFPE DNA: 1.5 Gb/sample Plasma cfDNA: 8 Gb/sample
Sequencing Type	PE150
Sequencer	Illumina NextSeq 500, NovaSeq 6000
TAT for Library Preparation	2 d (hands-on time 4 h)
TAT from Sample to Report	5 days

€330,- /tissue
€490,- /plasma

Amoy NGS Data Analysis Server (ANDAS)



illumina® NovaSeq 6000

The next era in sequencing starts now

NextSeq™ 500





Colorectal cancer

Colorectal cancer (metastatic disease)



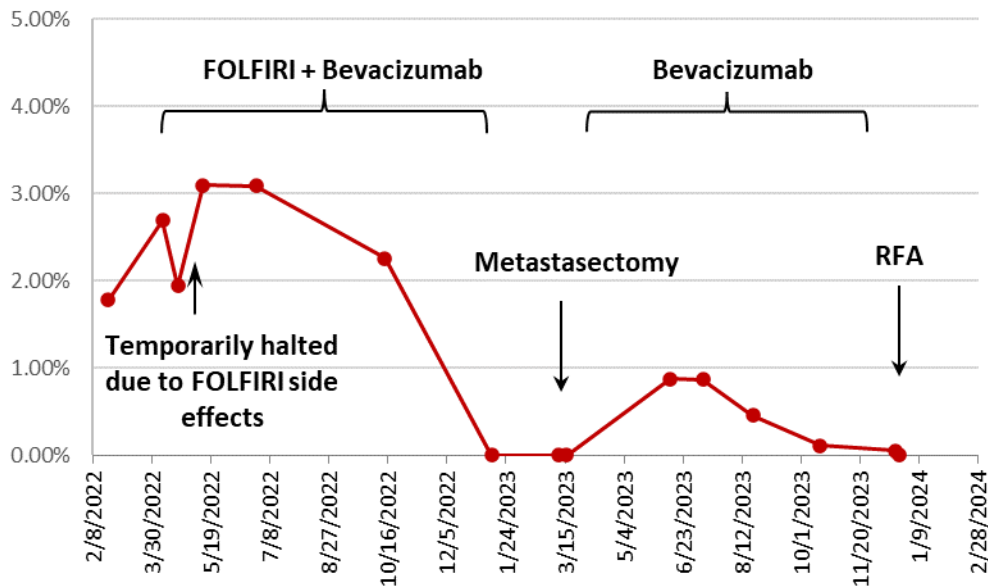
Female patient (*1967)

09/2020 Rectal cancer (*KRASmut*) with multiple liver meta
 11/2020 FOLFOX therapy
 02/2021 Bevacizumab
 08/2021 Complete remission

oncoMonitor ctDNA monitoring start

02/2022 New meta in liver (>8x)
 FOLFIRI+Bevacizumab therapy start
 03/2022 Therapy paused due to neutropenia
 04/2022 Bevacizumab therapy re-start
 08/2022 CT/NMR regression (only scars in meta sites)
 10/2022 CT/NMR stabilization (only scars in meta sites)
 01/2023 Continuing stabilization
 03/2023 **Surgery performed on liver with R1 resection (incomplete)**
 03/2023 Therapy stop
 06/2023 CT/NMR progression (residual lesions reported)
 06/2023 Bevacizumab therapy re-start
 08/2023 CT/NMR stabilization
 12/2023 Radiofrequency ablation performed in liver

Monitoring of ctDNA (*KRAS A146T*) during a multimodal therapy of mCRC



PLEASE COME SEE OUR POSTER



Tight monitoring of ctDNA in patient undergoing multimodal treatment of metastatic colorectal cancer

oncMonitor ctDNA monitoring start:

- 14.10.2023 - Rectal cancer (distant metastases) with multiple liver metastases
- 16.10.2023 - FOLFOX therapy
- 20.10.2023 - Treatment
- 28.10.2023 - Treatment

oncMonitor ctDNA monitoring start:

- 14.10.2023 - Treatment
- 16.10.2023 - Treatment
- 18.10.2023 - Treatment
- 20.10.2023 - Treatment
- 22.10.2023 - Treatment
- 24.10.2023 - Treatment
- 26.10.2023 - Treatment
- 28.10.2023 - Treatment
- 30.10.2023 - Treatment
- 31.10.2023 - Treatment
- 02.11.2023 - Treatment
- 04.11.2023 - Treatment
- 06.11.2023 - Treatment
- 08.11.2023 - Treatment
- 10.11.2023 - Treatment
- 12.11.2023 - Treatment
- 14.11.2023 - Treatment
- 16.11.2023 - Treatment
- 18.11.2023 - Treatment
- 20.11.2023 - Treatment
- 22.11.2023 - Treatment
- 24.11.2023 - Treatment
- 26.11.2023 - Treatment
- 28.11.2023 - Treatment
- 30.11.2023 - Treatment
- 02.12.2023 - Treatment
- 04.12.2023 - Treatment
- 06.12.2023 - Treatment
- 08.12.2023 - Treatment
- 10.12.2023 - Treatment
- 12.12.2023 - Treatment
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- 16.12.2023 - Treatment
- 18.12.2023 - Treatment
- 20.12.2023 - Treatment
- 22.12.2023 - Treatment
- 24.12.2023 - Treatment
- 26.12.2023 - Treatment
- 28.12.2023 - Treatment
- 30.12.2023 - Treatment

TIGHT MONITORING OF CTDNA IN PATIENT UNDERGOING MULTIMODAL TREATMENT OF METASTATIC COLORECTAL CANCER

Referencs R. J., Stopyeva P., Cheredasova A., Pudel I., Hoshidze L., Polyns R., Arkovik M. J.
©Pogreba, s.l.o., Popava
 Department of Surgery, 2nd Faculty of Medicine, Charles University and Military University Hospital, Prague
 Department of Clinical and Radiation Oncology, Hospital Havlíčkův Brod

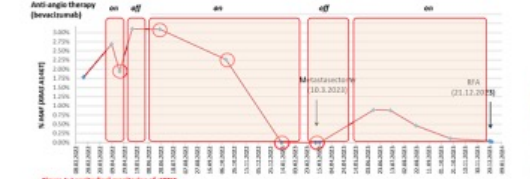


Background
 We here present a case study of a patient treated for advanced colorectal cancer who is undergoing a multimodal treatment of liver metastases. We have applied longitudinal liquid biopsy testing throughout the course of therapy by oncMonitor™ ctDNA assay for monitoring of the treatment outcome and early detection of a possible disease progression.

Patient
 A 64 year old female colorectal cancer patient has been monitored over the course of the past 24 months. The patient was diagnosed with multiple metastatic sites in the liver at the time of the craniotomy. During the previous therapy patient displayed positive response to the anti-angiogenic therapy by Avastin® (Bevacizumab) resulting in a temporary complete remission.

Methods
 The ctDNA is tracked by oncMonitor® ctDNA assay based on denaturing capillary electrophoresis directed at KRAS A147T mutation found in the primary tumor tissue as reported by the cooperating oncology department. In addition, during the monitoring a separate comprehensive profiling of the ctDNA mutation was performed by 128 gene plasma NGS panel (SeqSight).

Patient journey
 Initial cancer (distant metastases with multiple liver metastases)
 Bevacizumab + FOLFOX therapy
 Ongoing monitoring of ctDNA by oncMonitor
 Avastin® + FOLFOX therapy
 Surgery of liver metastases
 Ongoing monitoring of ctDNA by oncMonitor
 Bevacizumab + FOLFOX therapy
 Ongoing monitoring of ctDNA by oncMonitor
 Ongoing monitoring of ctDNA by oncMonitor
 Bevacizumab + FOLFOX therapy
 Ongoing monitoring of ctDNA by oncMonitor



Methods
 Following the initial 22-month period of gradual regression of the liver tumor mass on anti-angiogenic therapy by Bevacizumab (confirmed by imaging) the patient was elected for resection of remaining liver metastases based on the clearance of ctDNA from plasma. The initially complete eradication of ctDNA was followed by its re-emergence within 3 months after the surgery (see figure 1). This was in correlation with the EGFR resection radicality. Subsequently patient received adjuvant bevacizumab therapy after which the ctDNA has, again, been significantly reduced. Currently the patient is in stable disease with maximum in vivo activity of the remaining metastatic tissue. In order to exclude false result due to the tumor heterogeneity a separate effort, we have confirmed a mutation-stable profile by closely related dynamics of additional 5 somatic mutations found by NGS testing of ctDNA positive plasma samples: APC (c.9286G>T), MMR2 (c.206A>T) and SMAD2 (c.1777-T>G). Dynamics of all mutations was in concordance.

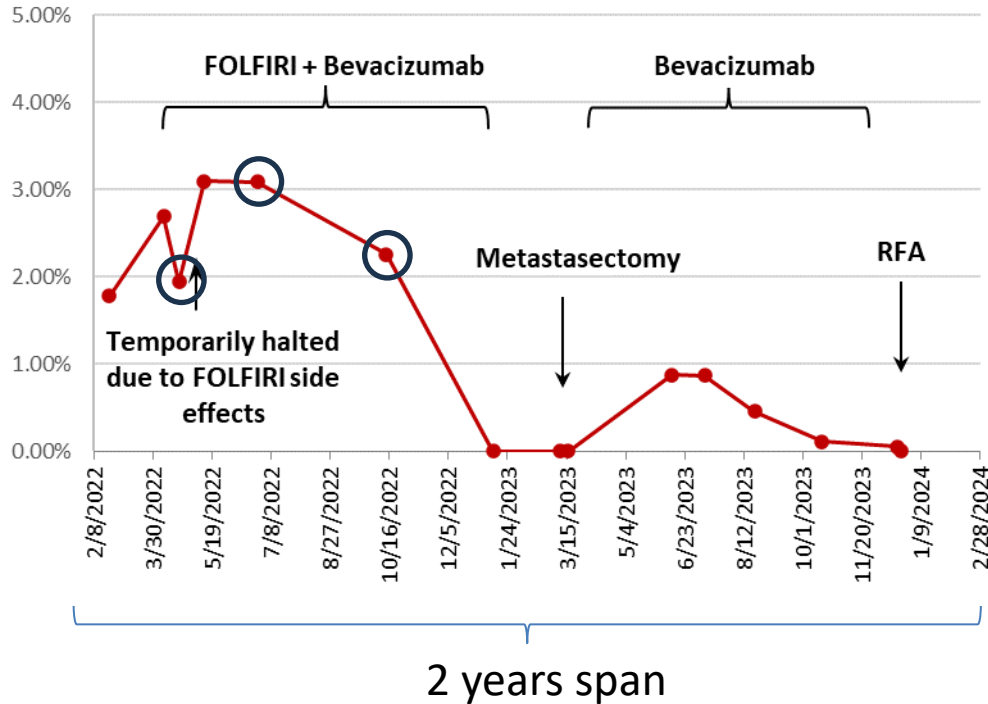
Conclusion
 The presented case demonstrates clinical utility of longitudinal liquid biopsy-ctDNA testing with frequent plasma sampling, which we refer to as "liquid monitoring". We see its main application for patients undergoing multimodal treatment where decisions can be made based on knowledge of therapy response and an exclusion of residual disease.

References
 [1] San-Gadea J et al. Monitoring and adapting cancer treatment using circulating tumor DNA kinetics. Current research, opportunities, and challenges. *Int J Mol Sci* 2023, 24, 6, doi:10.3390/ijms24063480.

Evolution of mutation profile over time



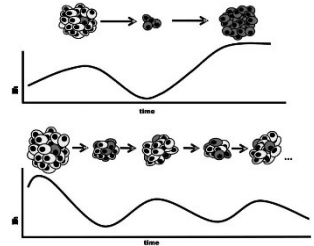
Monitoring of ctDNA (KRAS A146T) during a multimodal therapy of mCRC



Evolution (N Y). 2011 December ; 4(4): 624-634. doi:10.1007/s12052-011-0373-y.

How cancer shapes evolution, and how evolution shapes cancer

Matias Casás-Selves and James DeGregori

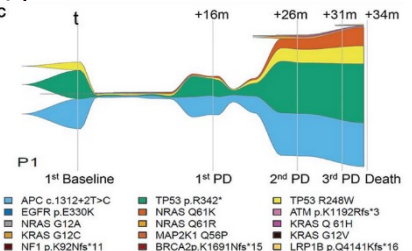


frontiers
in Oncology

Longitudinal Circulating Tumor DNA Profiling in Metastatic Colorectal Cancer During Anti-EGFR Therapy

Wentao Yang^{1,2}, Jianfeng Zou^{1,2}, Ye Li^{2,3}, Ruijiao Liu^{1,2}, Zhengping Yang¹, Shiqing Chen⁴, Xiaoying Zhao^{1,2}, Weijian Guo^{1,2}, Mingzhu Huang^{1,2}, Xi Xiaodong Zou^{1,2} and Zhijun Chen^{1,2*}

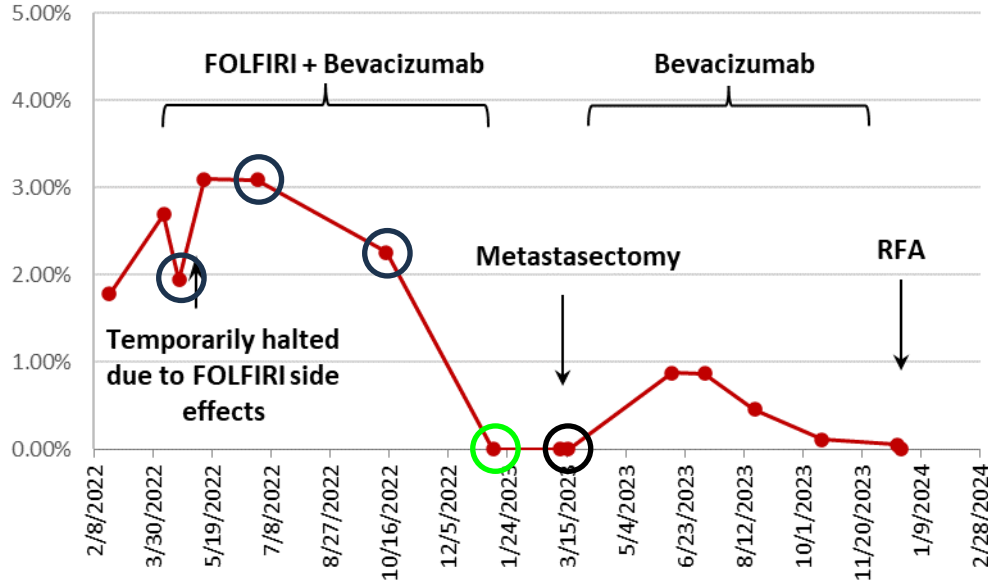
ORIGINAL RESEARCH
published: 24 February 2022
doi: 10.3389/fonc.2022.838816



Evolution of plasma mutation profile



Monitoring of ctDNA (KRAS A146T) during a multimodal therapy of mCRC



CP -1P	APC (4,84%), RASA1 (1,3%), JAK2 (6,14%)
CP- 2P	APC (0,86%), RASA1 (3,0%), JAK2 (6,24%)
CP- 3P	APC (0,96%), RASA1 (3,8%), JAK2 (5,64%)
CP- 4P	APC (0,39%), RASA1 (2,1%), JAK2 (0,00%)
CP-5P	APC (0,52%), RASA1 (1,8%), JAK2 (4,29%)

↑
A better early marker
of recurrence?



Lung cancer

Lung cancer (metastatic disease)



FIGURE 5 | DCE longitudinal MRD monitoring for advanced NSCLC patients undergoing chemotherapy (MAF— % of mutated minor allele fraction). The red arrows denote clinically confirmed disease progression.

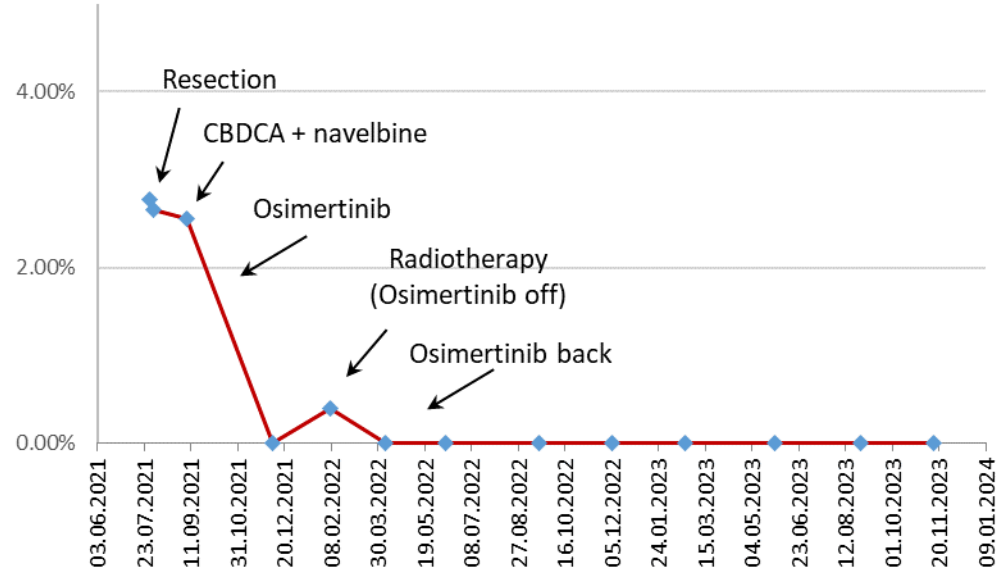
Benesova L., Ptackova R., Halkova T., Semyakina A., Svaton M., Fiala O., Pesek M., Minarik M., Detection and Quantification of ctDNA for Longitudinal Monitoring of Treatment in Non-Small Cell Lung Cancer Patients Using a Universal Mutant Detection Assay by Denaturing Capillary Electrophoresis. *Pathol Oncol Res.* 2022; 28: 1610308.

Pac. 51 years old, lung adenocarcinoma, right T2aN2MO IIB, pos lymph nodes in hill and mediastinum, *EGFR+*, *ALK+*

ctDNA (*EGFRdel19*, *TP53mut*)

1. Right lower lobectomy
2. Adjuvant therapy CHT CBDCA + navelbine
3. Osimertinib
4. Radiotherapy with temporal cessation of osimertinib
5. Osimertinib reinstated

Changes in ctDNA (*EGFRdel19*)



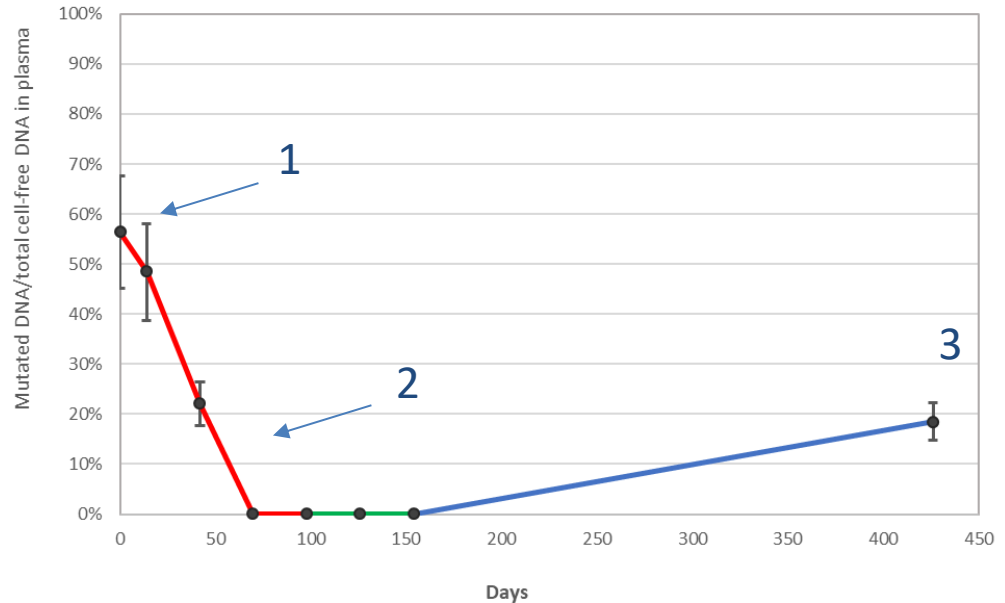


Breast cancer

Pac. 69 years old. NST triple negative breast cancer grade 3, BRCA 1 positive cT4, cN0, M2 (generalization to lungs, skeleton, liver, peritoneum), Ki-67: 20%.

1. Palliative chemotherapy started (Paclitaxel, Avastin)
2. PET CT - the finding significantly partially regresses both volumetrically and metabolically
3. PET CT - visible minimal volume progression of some lesions, metabolic progression of the liver deposit and, due to the new deposit, higher metabolic activity in the left acetabulum, overall this is a progression of findings

Patient (PC04) Minimal Residual Disease monitoring
ctDNA levels (*TP53* mut) in plasma



Summary



- Due to low levels of ctDNA liquid biopsy methods require high yield and sensitivity
- MRD by tumor-informed approach offers cost control with possibility of repeated sampling
- oncoMonitor is a tumor-informed assay based on NGS tissue mutation detection followed by targeted plasma mutation detection by Denaturing Capillary Electrophoresis
- oncoMonitor main use is for MRD in early detection of metastatic recurrence it can trace virtually any point mutation
- Concurrent monitoring of multiple mutations is beneficial for prevention of false negativity and early detection of recurrence
- Currently applied for colorectal, lung, pancreatic and breast cancer patient follow-up

Thank you



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MUDr. Alena Bulová



MUDr. Alice Tašková



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RNDr. Martina Putzová



RNDr. Lucie Benešová, Ph.D.
Mgr. Tereza Hálková



Mgr. František Snítily



2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA



UNIVERZITA KARLOVA
Přírodovědecká fakulta



Charles
University

T A
Č R

Technology
Agency
of the Czech Republic

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Thank you for your attention!