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

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6th Central - Eastern European congress on cell free DNA and medical practice
7. March 2024
Clarion congress center, Olomouc

Disclosure

Ownership in Elphogene, s.r.o. company

Owership in Carolina Biosystems, s.r.o. company

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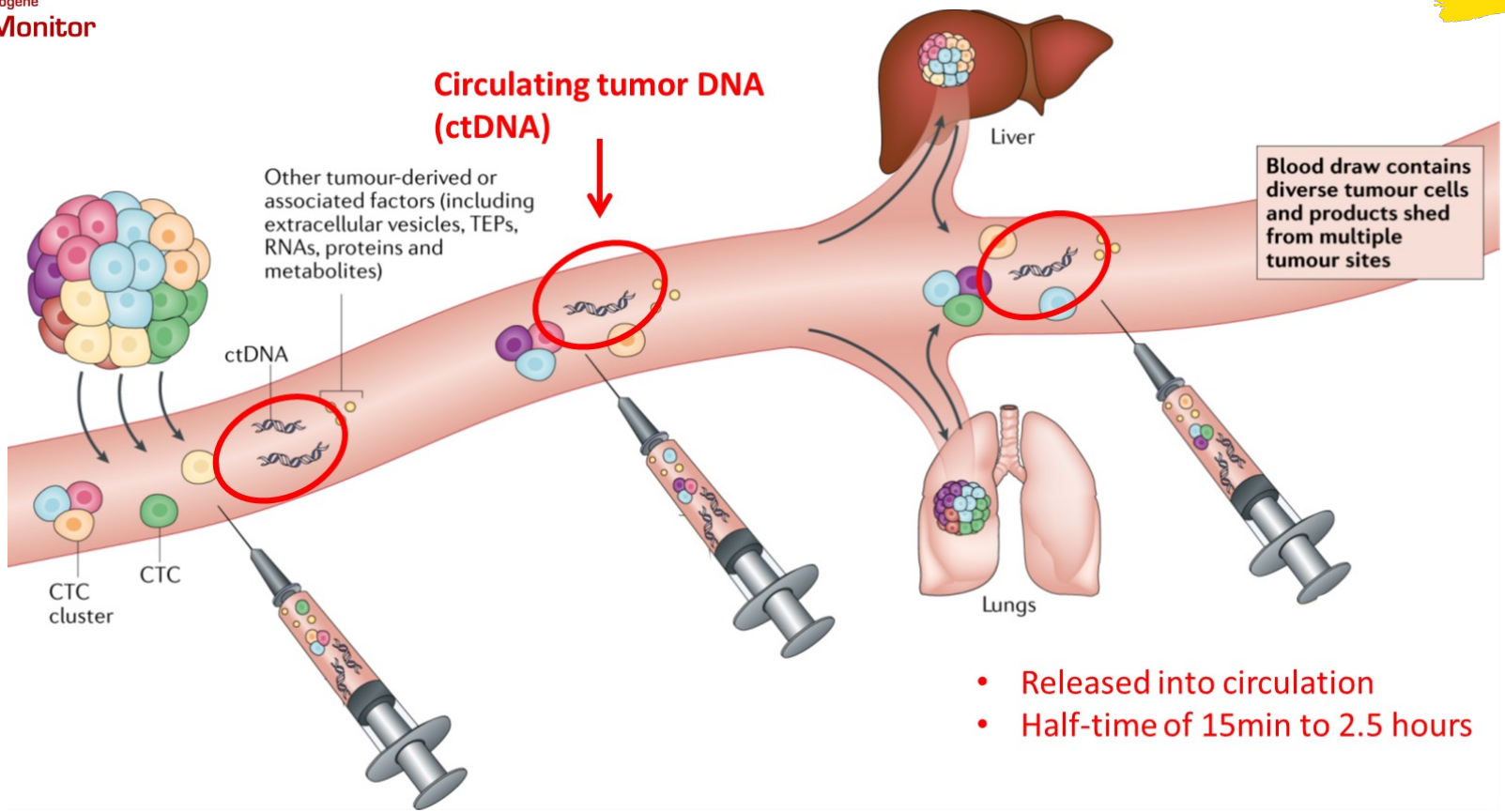
Liquid biopsy - evaluation of ctDNA



- Released into circulation
- Half-time of 15min to 2.5 hours

Adapted from: Ignatiadis, M., Sledge, G.W. & Jeffrey, S.S. Liquid biopsy enters the clinic — implementation issues and future challenges. Nat Rev Clin Oncol 18, 297–312 (2021).

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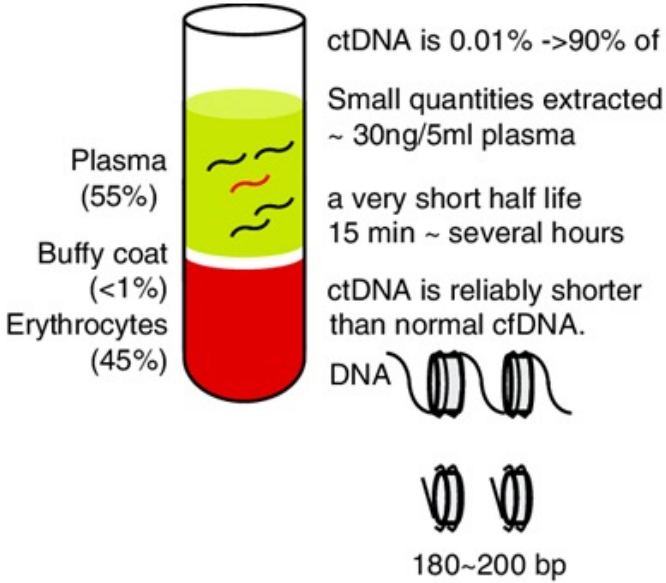
Liquid biopsy - evaluation of ctDNA



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**

Yamamoto H., Watanabe Y., Itoh F., Cell-free DNA in Biomarkers in Cancer Therapy Liquid Biopsy Comes of Age, Hideaki Shimada (editor), © Springer Nature Singapore Pte Ltd. 2019, (pp.11-24)

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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

Adapted from: O'Sullivan HM, Feber A, Popat S. Minimal Residual Disease Monitoring in Radically Treated Non-Small Cell Lung Cancer: Challenges and Future Directions. Onco Targets Ther. 2023 Apr 7;16:249-259. doi: 10.2147/OTT.S322242. PMID: 37056631; PMCID: PMC10089274.

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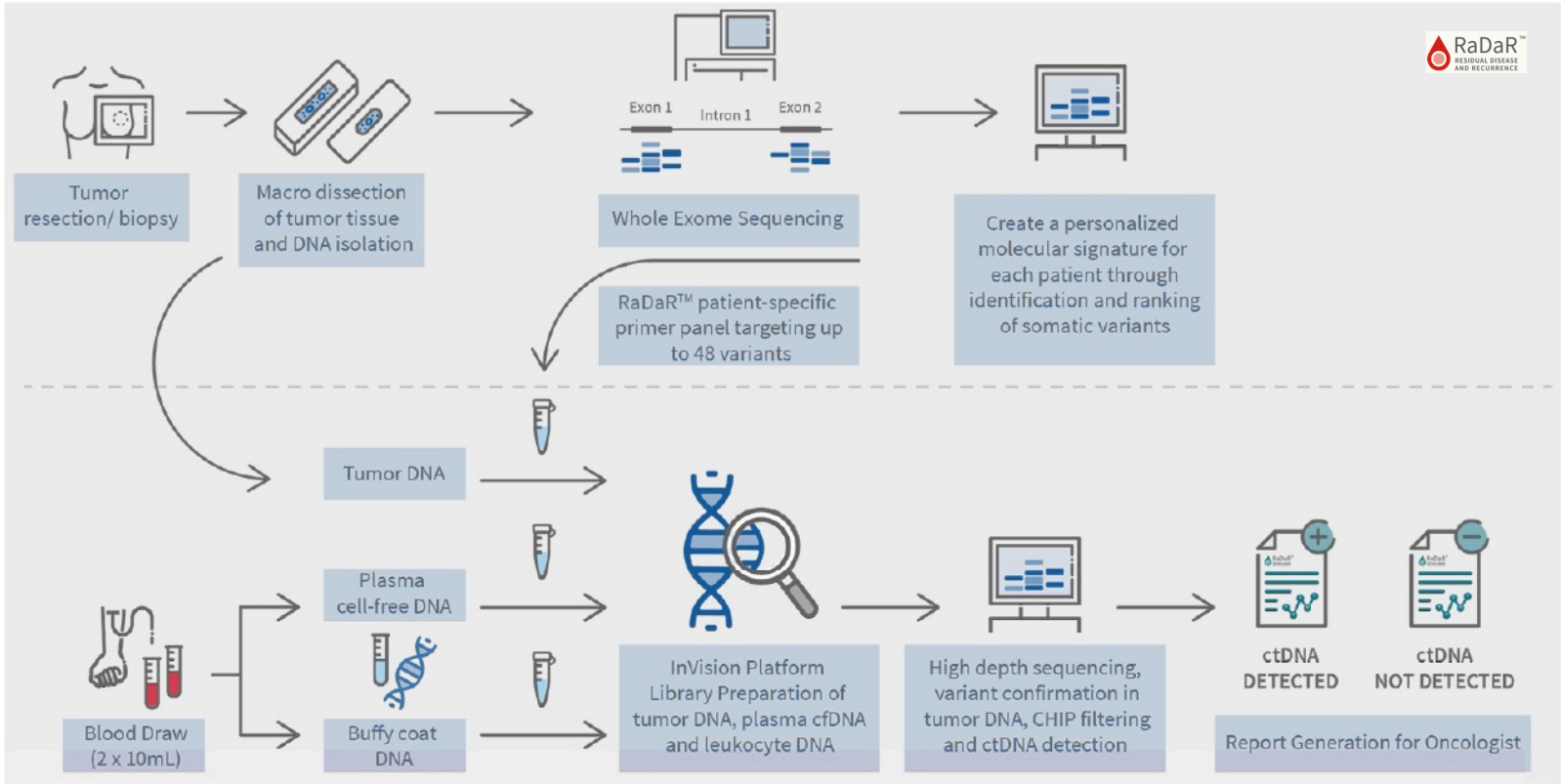
Tumor - informed workflow



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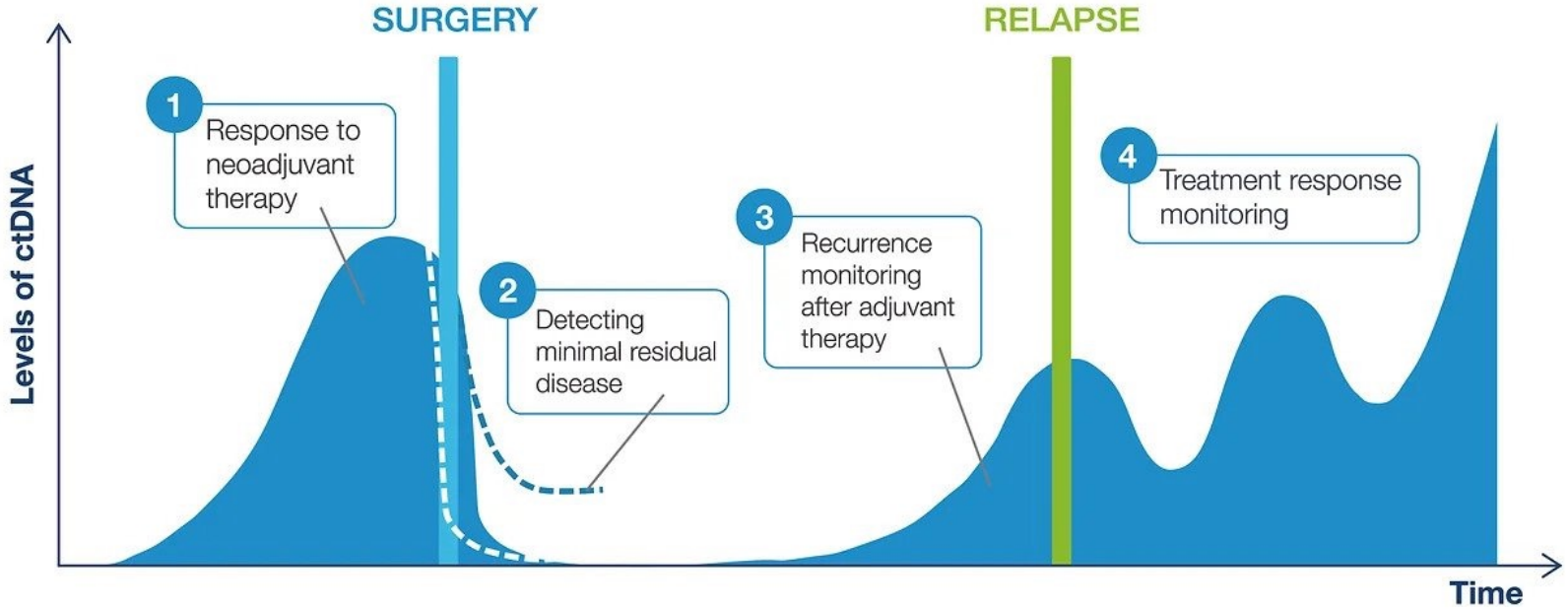
Clinical applications of ctDNA testing for MRD assessment



natera

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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 %

Adapted from: O'Sullivan HM, Feber A, Popat S. Minimal Residual Disease Monitoring in Radically Treated Non-Small Cell Lung Cancer: Challenges and Future Directions. Onco Targets Ther. 2023 Apr 7;16:249-259. doi: 10.2147/OTT.S322242. PMID: 37056631; PMCID: PMC10089274.

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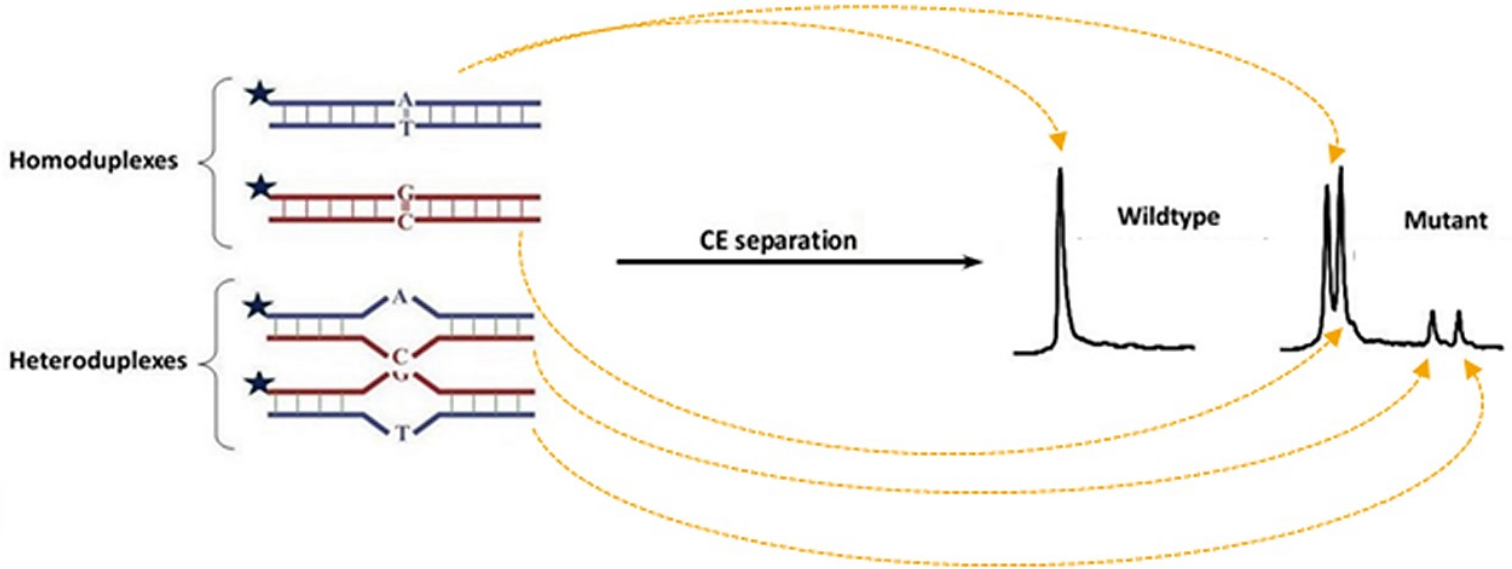
Denaturing Capillary Electrophoresis (DCE)



Sanger DNA sequencer

Levy M, Benesova L, Lipska L, Belsanova B, Minarikova P, Veprekova G, Zavoral M, Minarik M. Utility of cell-free tumour DNA for post-surgical follow-up of colorectal cancer patients. Anticancer Res. 2012 May;32(5):1621-6. PMID: 22593440.

oncoMonitor assay - a two-tier method

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

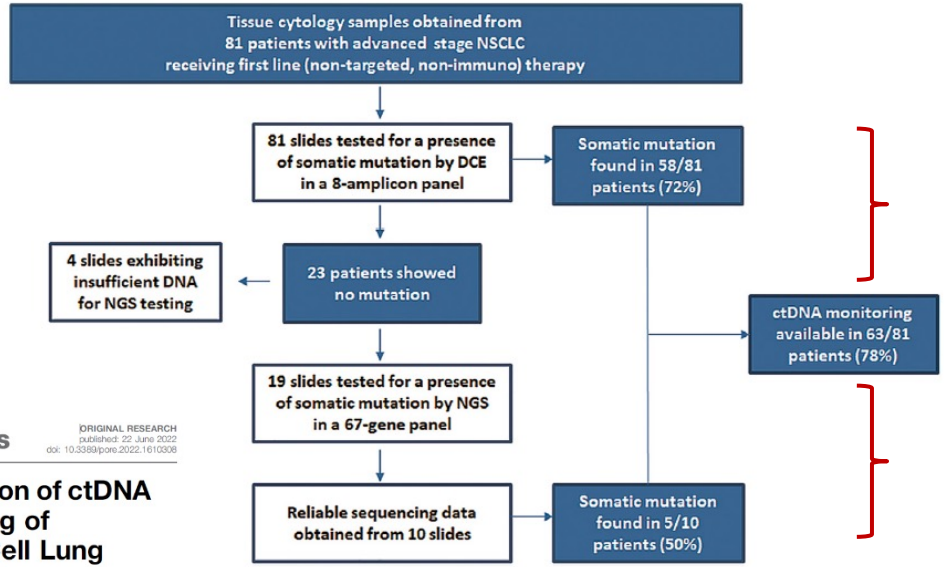
Monitoring of Early Changes of Circulating Tumor DNA in the Plasma of Rectal Cancer Patients Receiving Neoadjuvant Concomitant Chemoradiotherapy: Evaluation for Prognosis and Prediction of Therapeutic Response

Filip Pazdirek[1], Marek Minarik[1,2], Lucie Benesova[3], Tereza Halkova[3], Barbora Belsanova[3], Milan Macek[4], Lubomir Stepanek[5] and Jiri Hoch[1]*

Pathology & Oncology Research | frontiers
ORIGINAL RESEARCH
published: 22 June 2022
doi: 10.3389/pore.2022.1610308

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 Benesova[1], Renata Ptackova[1], Tereza Halkova[1], Anastasiya Semyakina[1], Martin Svaton[2], Ondrej Fiala[3,4], Milos Pesek[2] and Marek Minarik[5,6]**



small DCE panel
(10 – 20 hotspots)

large NGS panel
(128 genes)

AmoyDx

Elphogene
oncoMonitor

A tumor-informed liquid biopsy

(Capillary Electrophoresis heteroduplex assay)

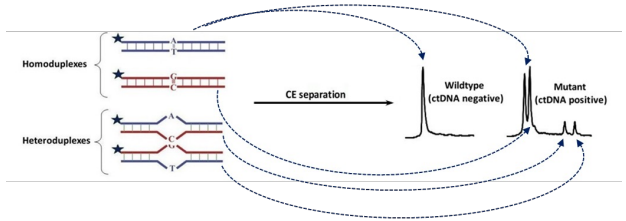
AmoyDx ddCap® Comprehensive NGS panel
Tissue or plasma (128 genes)

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	BCL2L11	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	TPS3[1]
KDM5C	KDR	KIT	KRAS	MAP2K1	MAPK1	MET	MLH1	MRE11	MSH2	MSH6	DPYD	UGT1A1
MTOR	MYC	NF1	NF2	NOTCH1	NRAS	NRG1	NTRK1	NTRK2	NTRK3	PALB2	DYNC2H1	UMPS
PAX8	PDCD1	PDGFRA	PGR	PIK3CA	PIK3R1	PMS2	POLD1	POLE	PSMD4	PTCH1	ERCC1	XPC
PTEN	RAF1	RASA1	RASAL1	RB1	RET	RICTOR	RIT1	ROS1	RSF1	SF3B1	GSTP1	XRCC1
SMAD4	SMARCA4	SMO	STK11	TERT	TOP2A	TP53[1]	TSC1	TSC2	TSHR	VHL	MTHFR	

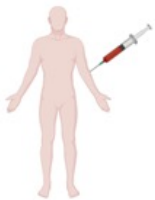
SNV, InDel ● SNV, InDels, CNV ● SNV, InDel, Fusion ● SNV, InDel, Fusion, CNV ● Fusion ●

1: TP53: SNVs, InDels and polymorphisms

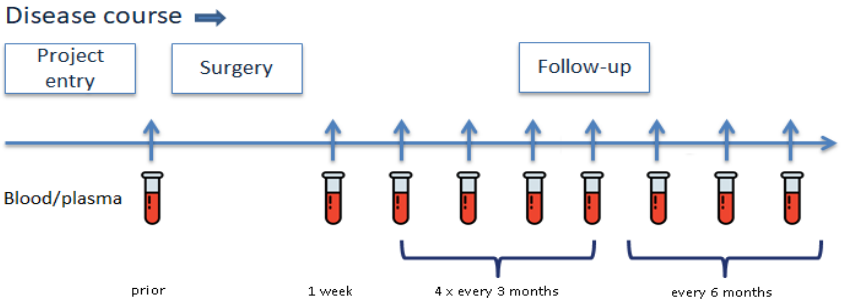
oncoMonitor (denaturing CE)



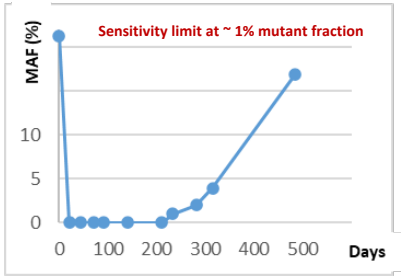
1. Tissue/plasma mutation profiling by NGS
2. Simple PCR design on any mutants
3. ctDNA detection by partially-denaturing CE



Cancer patient



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



Early detection of recurrence

Benesova L, Ptackova R, Halkova T, Semyakina A, Svaton M, Fiala O, Pesek M and Minarik M (2022) 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. 28:1610308.

NGS panel (tissue and plasma)

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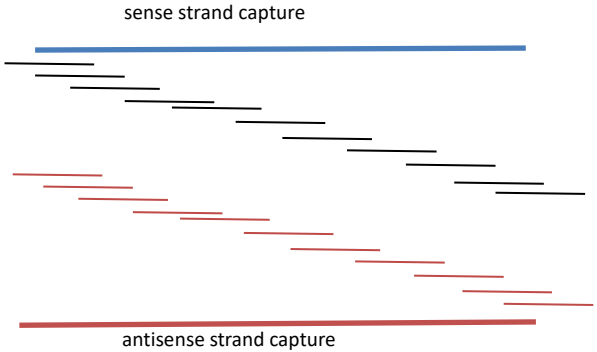
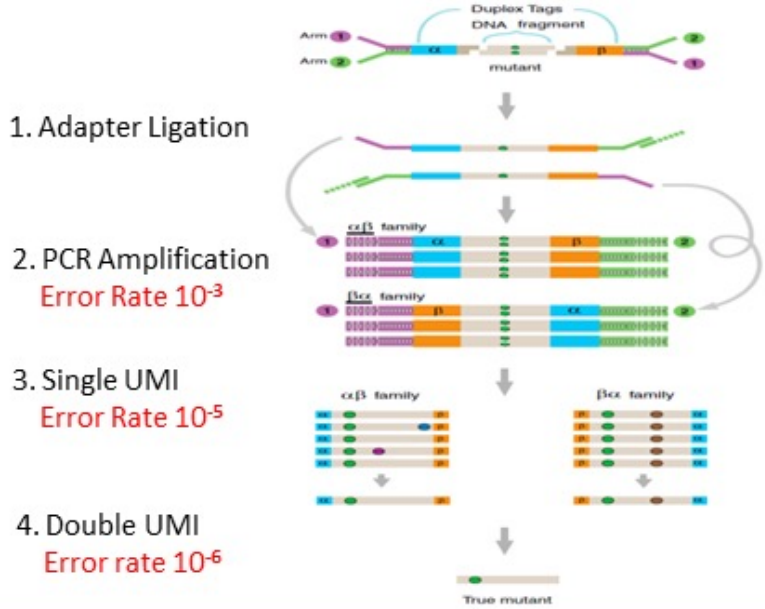
AmoyDx

ddCap® Comprehensive Panel

Dual: probes targeting double strands



Digital: Unique molecular identifiers



Total turnaround time

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

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NGS panel (tissue and plasma)

AmoyDx

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
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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

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Amoy NGS Data Analysis Server (ANDAS)

Bioptická laboratoř
CYTOLOGIE | BIOPSIE | MOLEKULÁRNÍ GENETIKA

illumina SEQUENCE HUB

illumina® NovaSeq 6000

The next era in sequencing starts now

NextSeq™ 500

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Colorectal cancer

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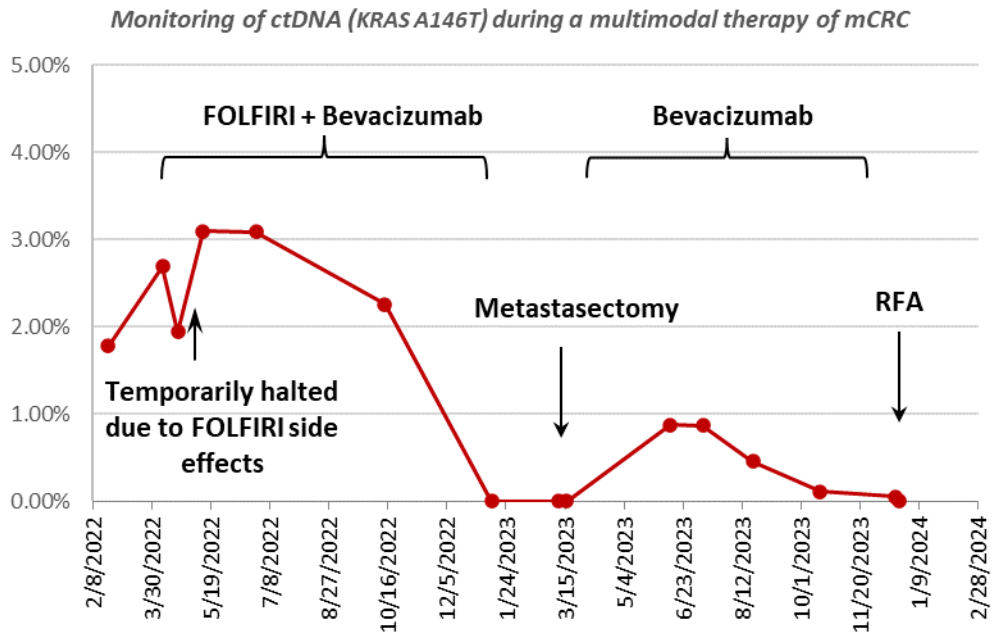
Colorectal cancer
(metastatic disease)

Female patient (*1967)

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

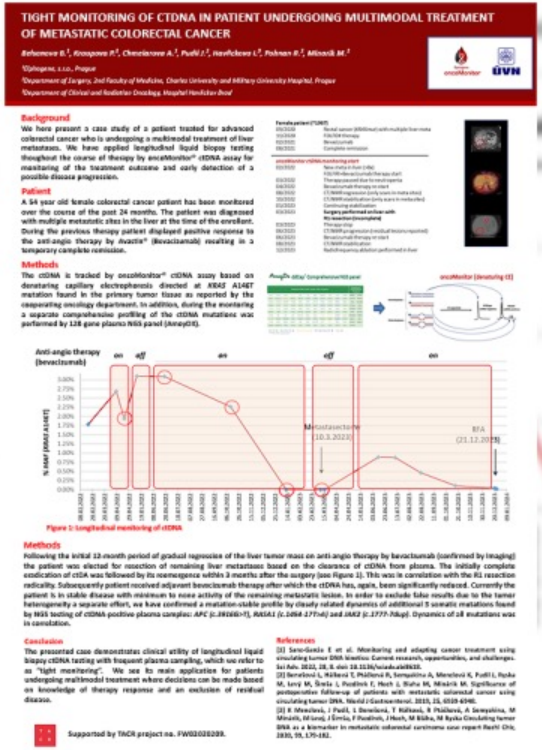
oncoMonitor ctDNA monitoring start

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



Belsanova B., Kroupova P., Chmelarova A., Pudil J., Havlickova I., Pohnan R., Minarik M. Tight-monitoring of ctDNA in patient undergoing multimodal treatment of metastatic colorectal cancer (in preparation)

PLEASE COME SEE OUR POSTER



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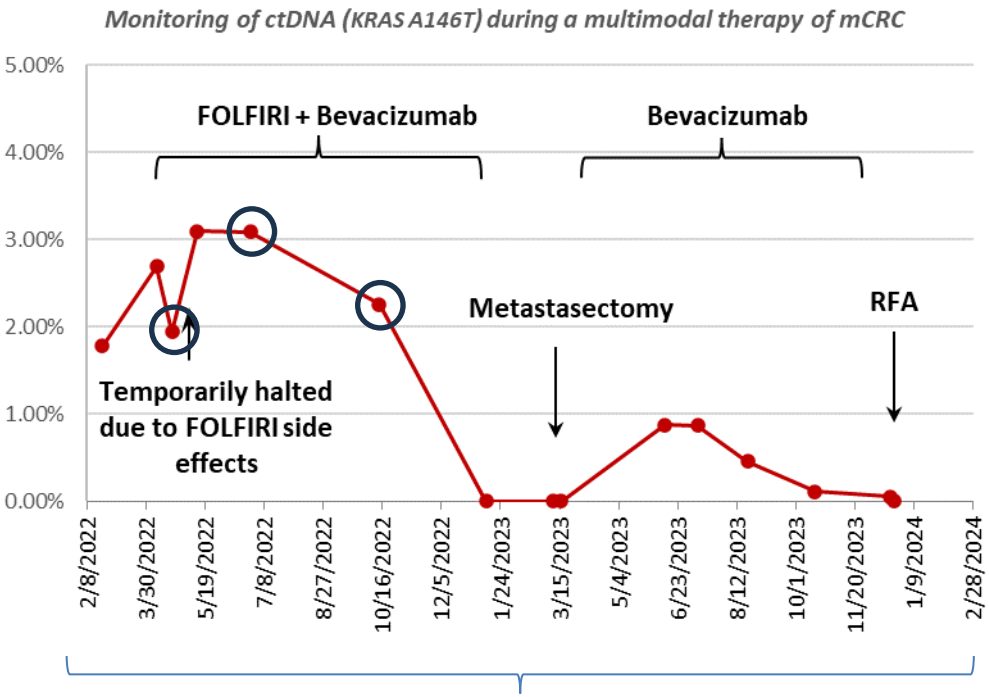
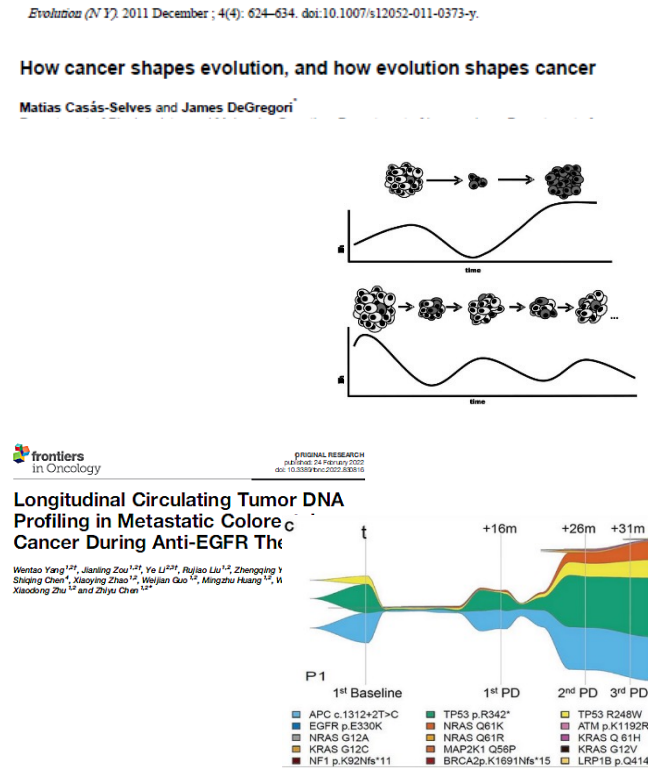
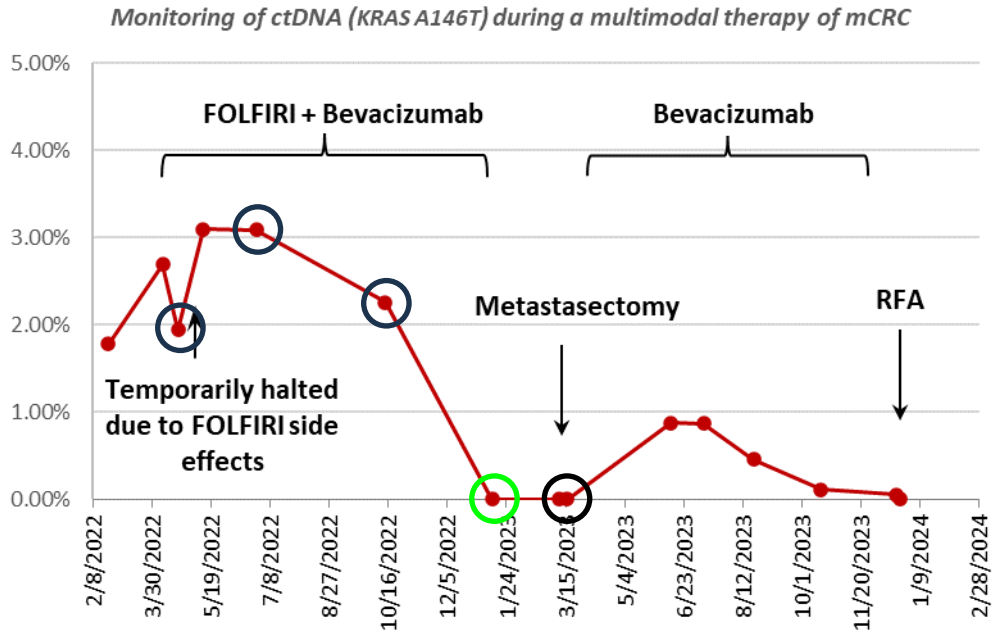
Evolution of mutation profile over time





Evolution of plasma mutation profile



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?

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Lung cancer

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Lung cancer
(metastatic disease)

FAKULTNÍ NEMOCNICE PLZEŇ
Genomac

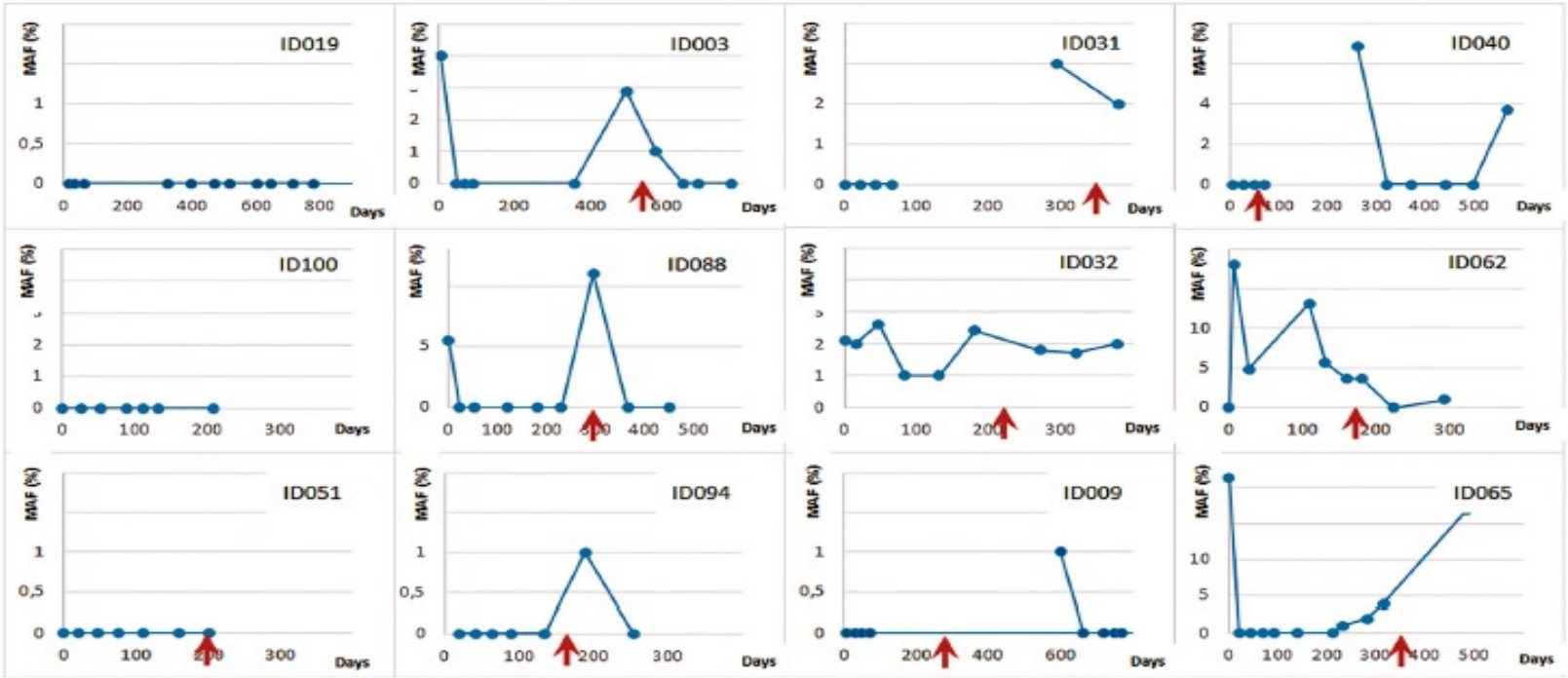


FIGURE 5 | DCE longitudinal MRD monitoring for advanced NSCLC patients undergoing chemotherapy (MAF—% of mutated minor allele fraction). The red arrows denote clinically confirmed disease progression.

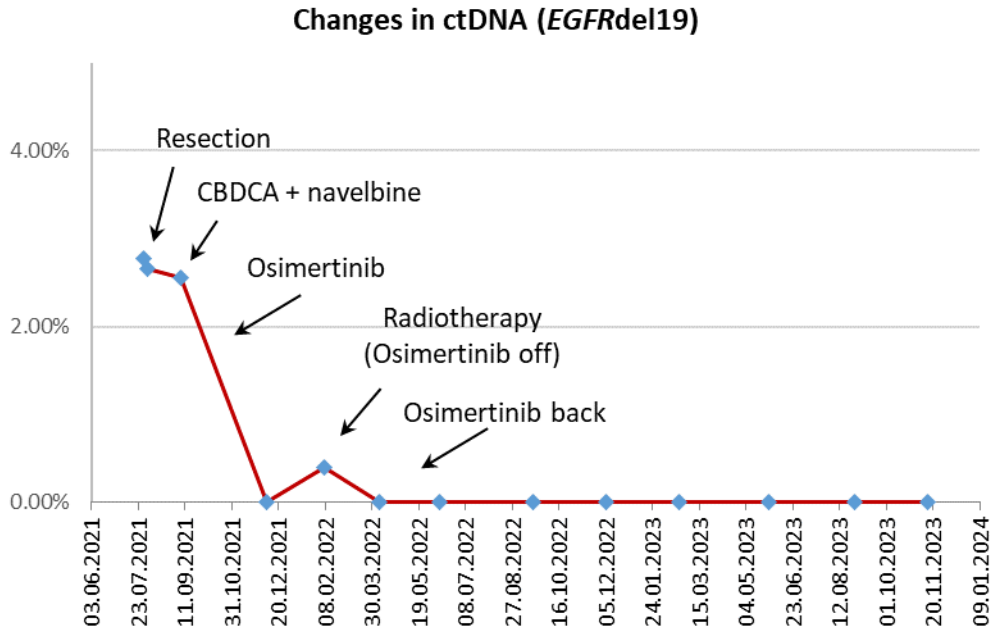
Benesova L., Ptackova R., Halkova T., Semyakina A., Svaton M., Fiala O., Pesek M., Minarik M., Detection and Quantification of ctDNA for Longitudinal Monitoring of Treatment in Non-Small Cell Lung Cancer Patients Using a Universal Mutant Detection Assay by Denaturing Capillary Electrophoresis. Pathol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

Lung cancer
(resectable disease)

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

*ctDNA (EGFR*del19, *TP53*mut)

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



Taskova A. et al. Monitoring of adjuvant therapy after lung cancer resection, (in preparation).

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Breast cancer

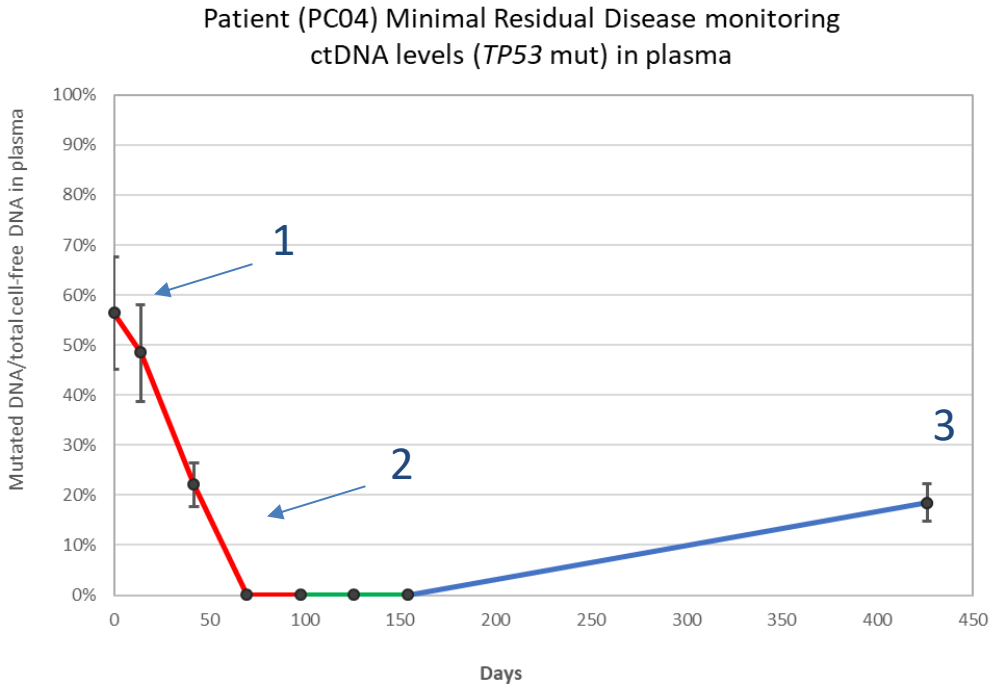
Breast cancer

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



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

Elphogene

Mgr. Petra Kroupová
Mgr. Barbora Belšánová

FN MOTOL

MUDr. Jannis Tornikidis
MUDr. Alena Bulová

Fakultní Thomayerova nemocnice

MUDr. Alice Tašková

ÚVN

Prim. Jiří Pudil doc. Radek Pohnán

FAKULTNÍ NEMOCNICE PLZEŇ

MUDr. Martin Svatoň, prof. MUDr. Miloš Pešek, CSc.

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RNDr. Martina Putzová

GeneTiCA

Mgr. František Snítilý

Genomac

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

Elphogene

2. LÉKAŘSKÁ FAKULTA UNIVERZITA KARLOVA

UNIVERZITA KARLOVA Přírodovědecká fakulta

ÚVN

Charles University

T A
Č R
Technology Agency of the Czech Republic

Projekt byl podpořen grantem TAČR FW02020209 - Systém a technologie pro předúpravu vzorku pro vyšetřování nádorů tekutou biopsií

Thank you for your attention!