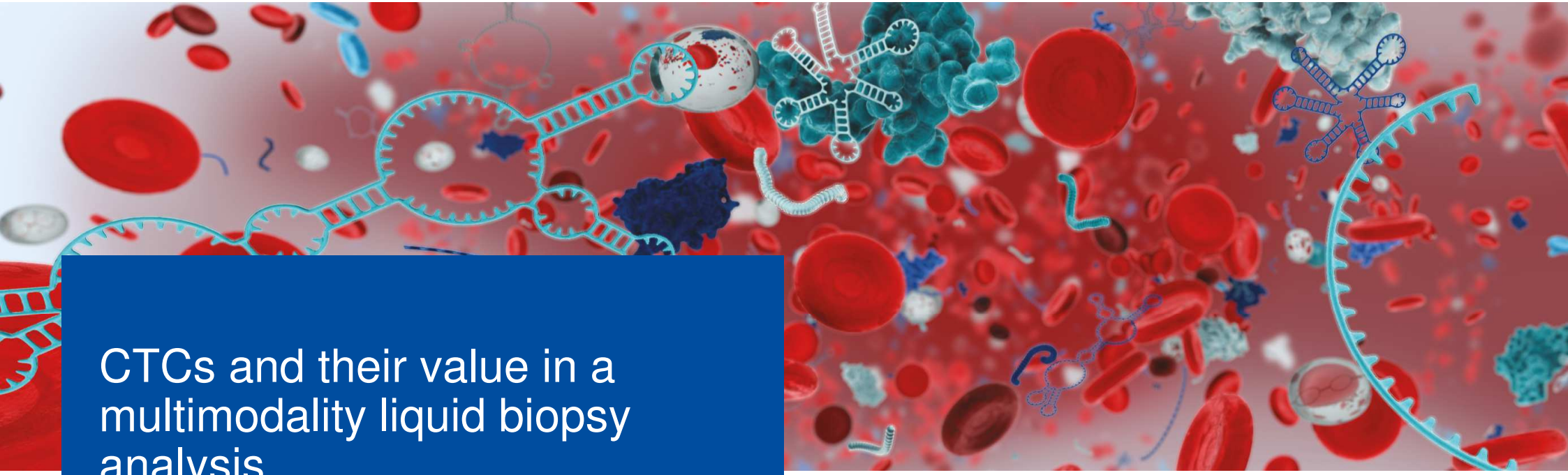
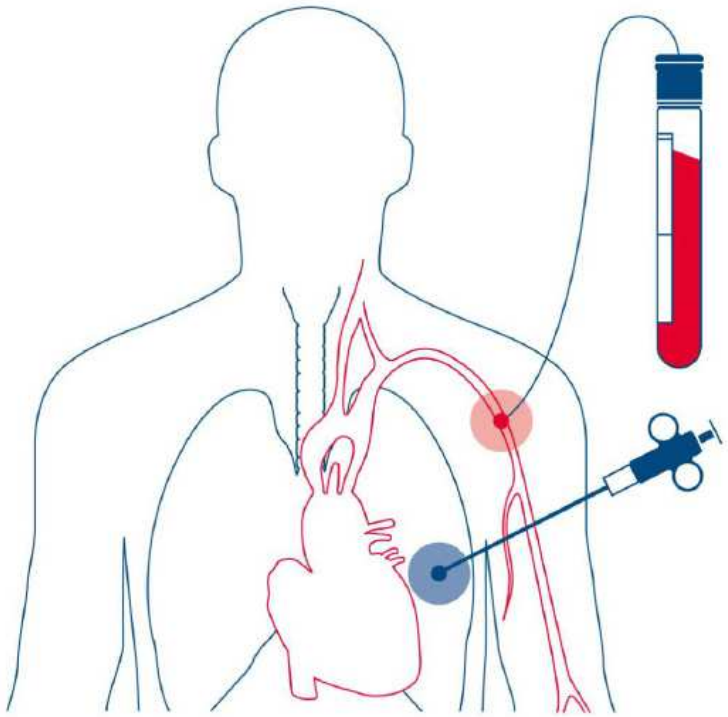


CTCs and their value in a multimodality liquid biopsy analysis

Siegfried Hauch, Ph.D
.
Director CTC Research and Development
QIAGEN GmbH

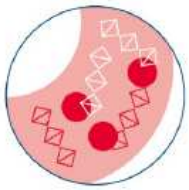
What is liquid biopsy?

A minimally invasive technology for detecting molecular signs of cancer and other diseases



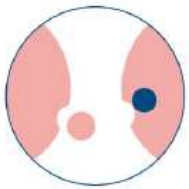
Circulating tumor cells (CTCs)

Tumor cells detached from a tumor into the bloodstream carrying genetic information (RNA and DNA)



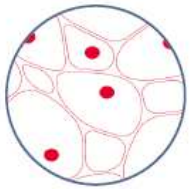
Circulating, cell-free nucleic acids

Fragmented DNA (and RNA) from dead cells, circulating in the bloodstream (can contain cancer-related mutations)



Exosomes

Tiny microvesicles actively shed into body fluids that transport RNA (and possibly DNA) between cells

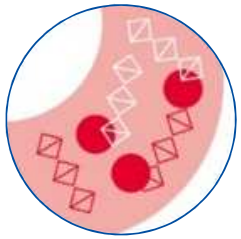


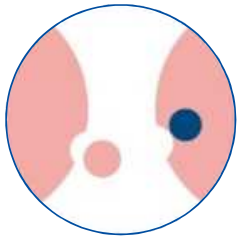
Tissue samples

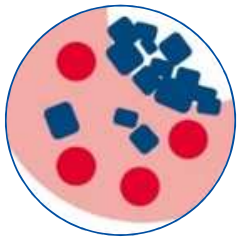
FFPE tissue samples of tumor extracted from a subject's body through an invasive procedure

Key questions in liquid biopsy

Until now, most studies focus on one liquid biopsy analyte only







- Do we miss (or even waste) genomic and transcriptomic information by considering only one analyte?
- How would the picture change in a holistic multimodal analysis?

The ELIMA study

ELIMA: **E**valuation of Multiple **L**iquid Biopsy Analytes **I**n **M**etastatic Breast Cancer Patients **A**ll from One Blood Sample

Scope:

- Develop an optimized workflow for isolation and analysis of multiple parameters: CTCs, EVs and cfDNA from one blood sample
- Achieve one condensed workflow to minimize the blood volume needed and sample-to-sample bias while getting the complete transcriptomic and genomic information
- Identify potentially predictive and prognostic biomarkers for scientific insights

Sampling:

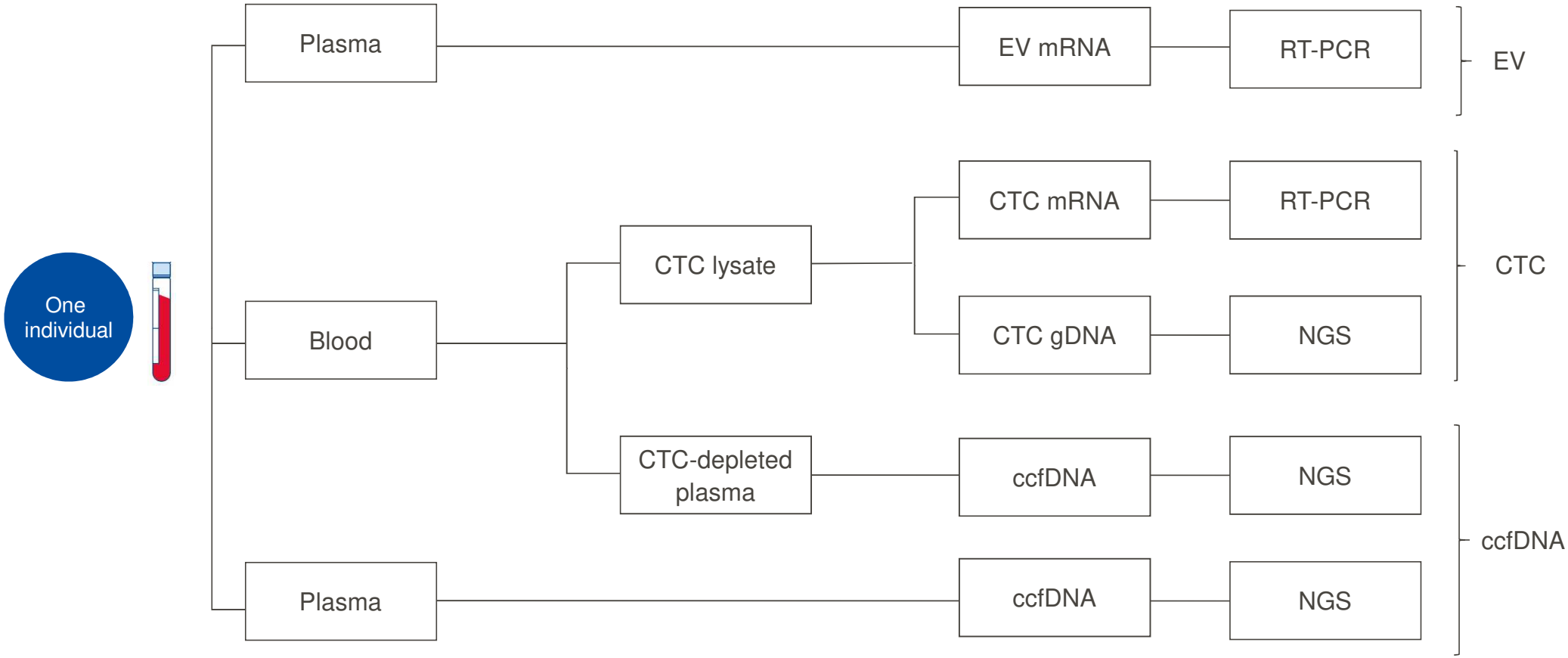
- Blood samples from individuals with metastatic breast cancer and *HR+ HER2–* primary tumors
- EDTA blood from 35 individuals
- Blood sample at 3 time points
  - T0 (progressive disease)
  - + T1 (consecutive staging)
  - + T2 (consecutive staging)

Parallel analysis of:

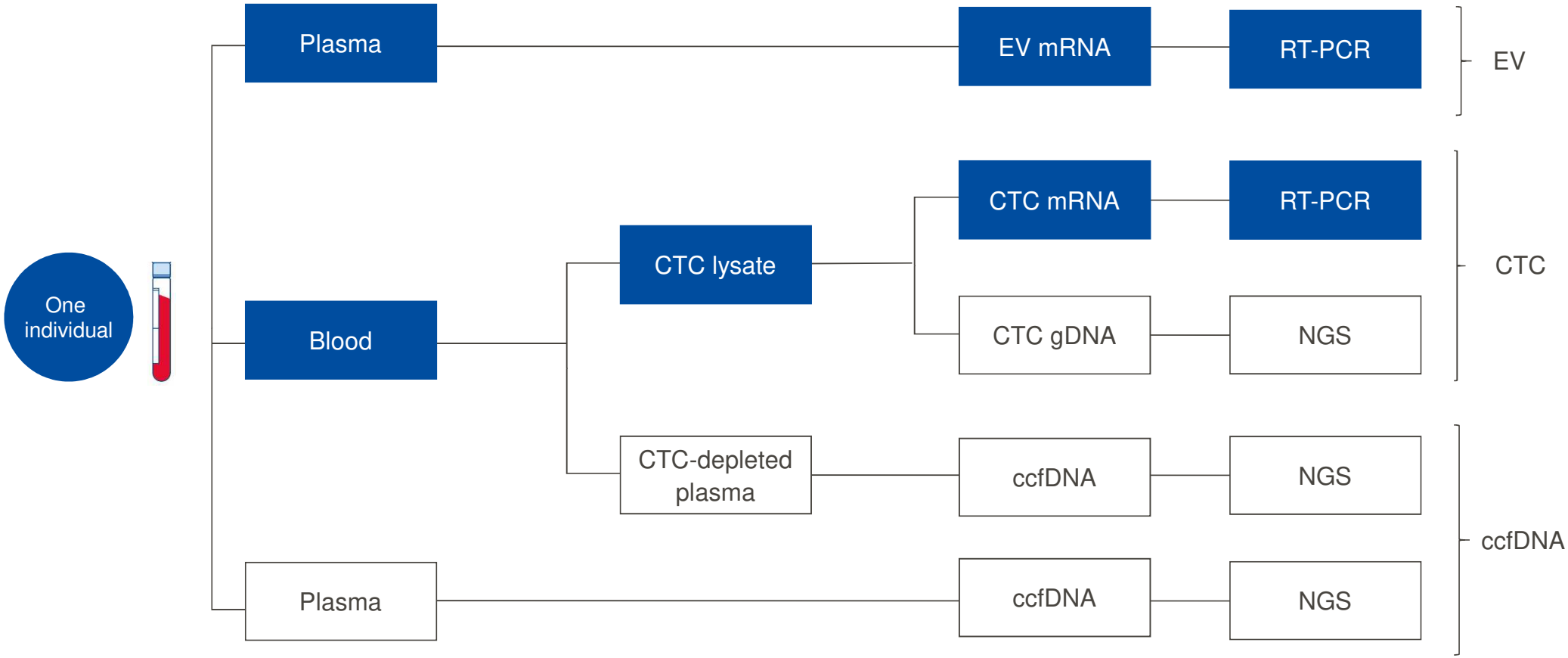
- mRNA from EVs
- mRNA from CTCs
- gDNA from CTCs
- ccfDNA from plasma
- ccfDNA from CTC-depleted plasma

Keup C. et al. (2018) Establishment of a workflow for the analysis of mRNA and gDNA from circulating tumor cells, extracellular vesicles and cell-free DNA from the same blood sample to mirror the genomic and transcriptomic complexity in metstatic breast cancer subjects AACR 2018

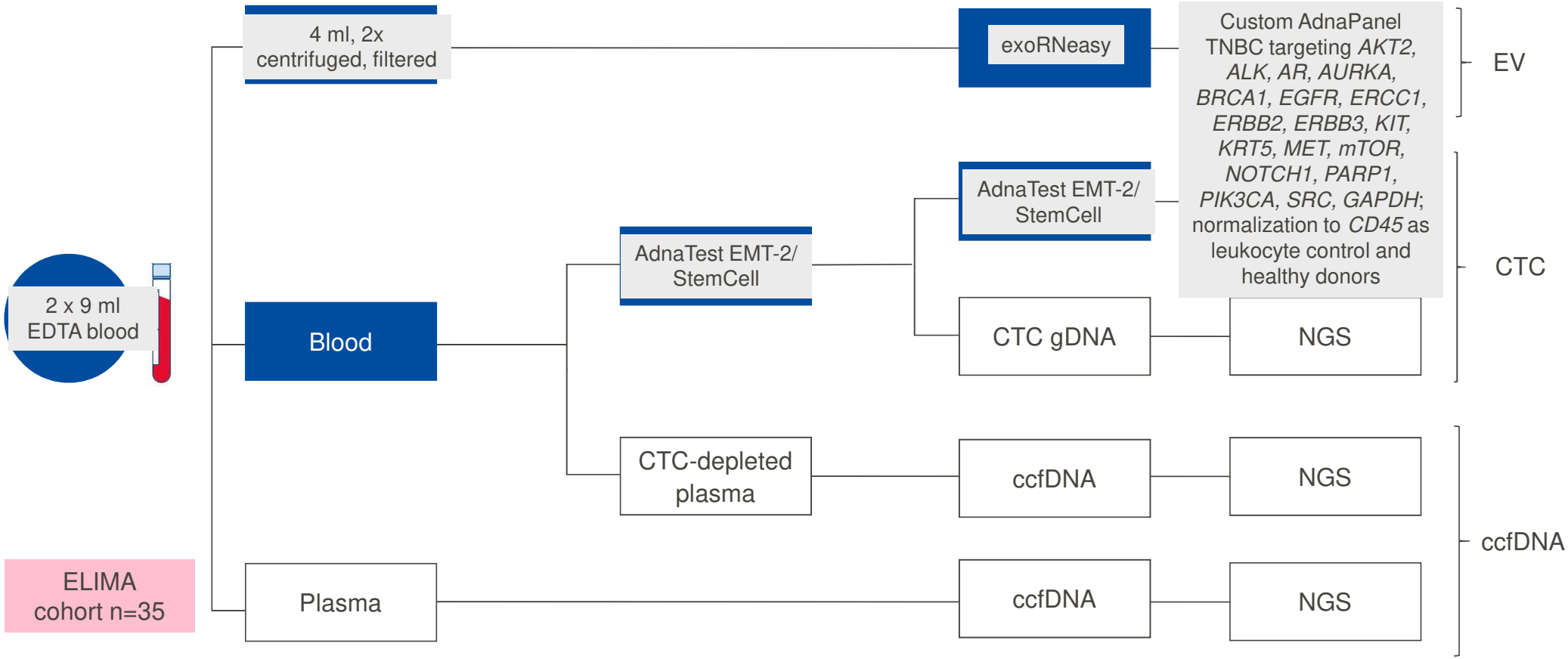
Study plan


One individual
Plasma
Blood
Plasma
CTC lysate
CTC-depleted plasma
EV mRNA
CTC mRNA
CTC gDNA
ccfDNA
ccfDNA
RT-PCR
RT-PCR
NGS
NGS
NGS
EV
CTC
ccfDNA

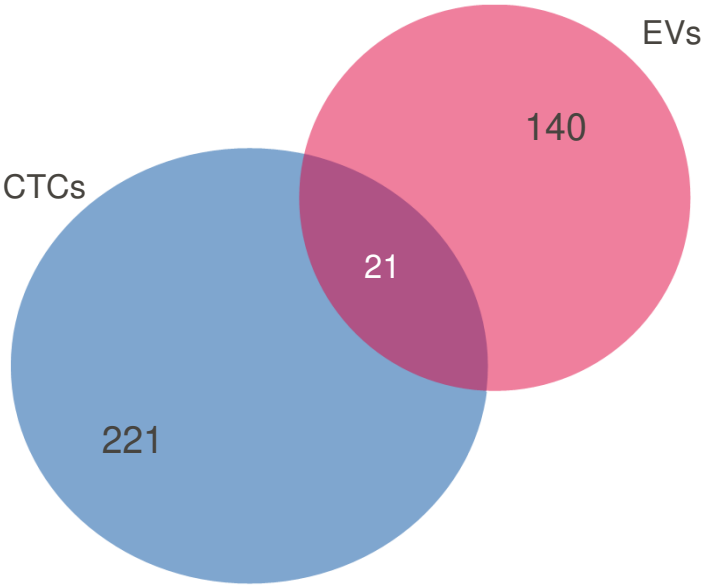
Study arm 1: RNA profiles of CTCs and extracellular vesicles

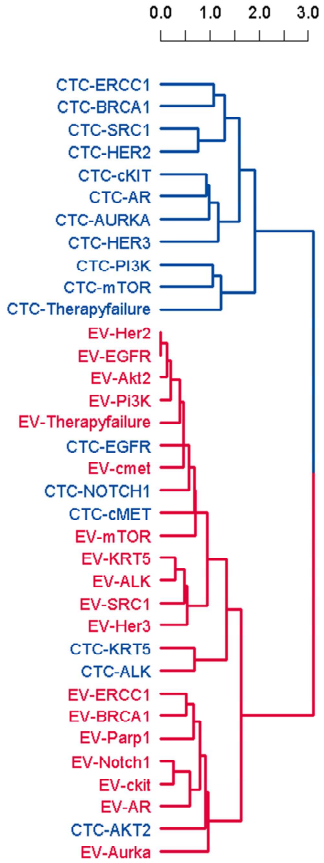


Study arm 1: RNA profiles of CTCs and extracellular vesicles



CTC- and EV-derived mRNAs profiles differ significantly

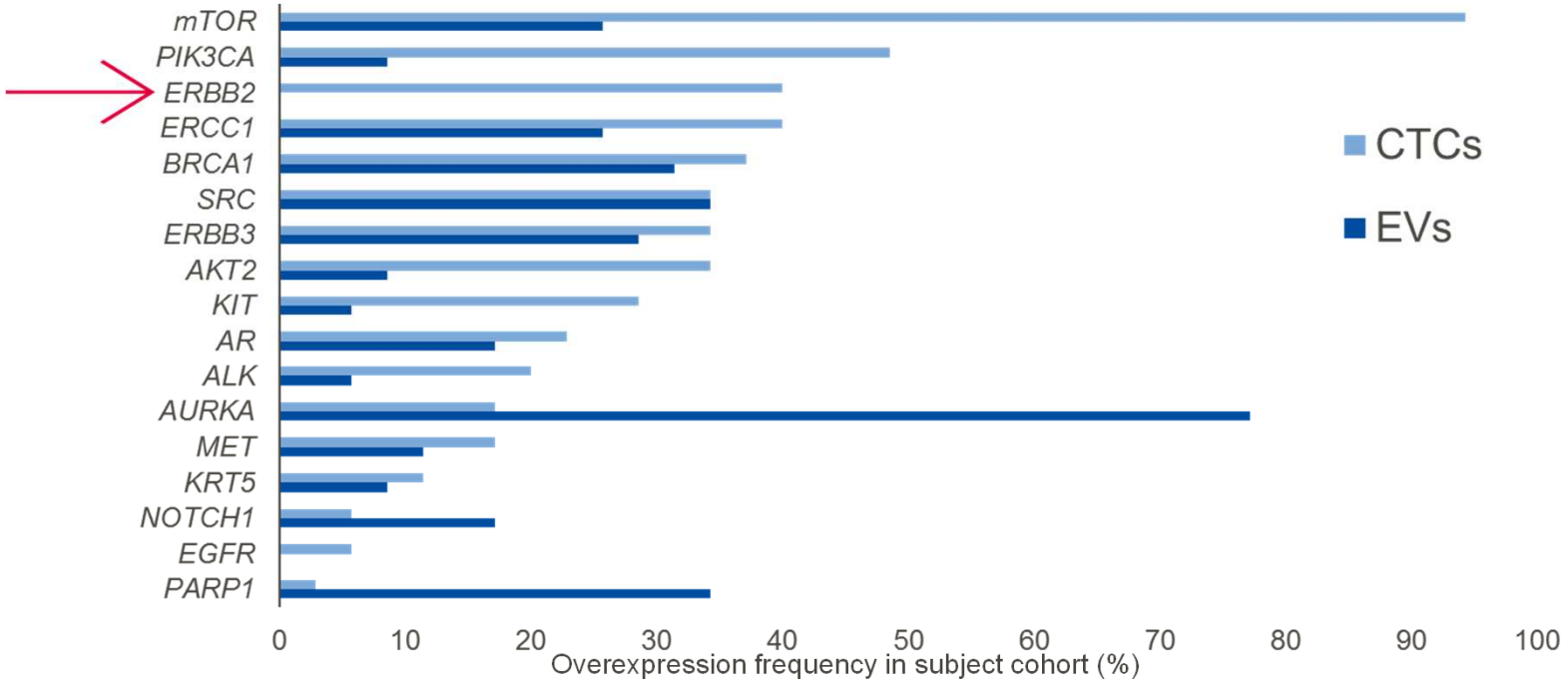




- Transcripts cluster primarily by their origin
- CTCs and EVs share only a small number of overexpression signals

Keup C. et al. (2018) RNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Clin. Chem. 64, 1054.

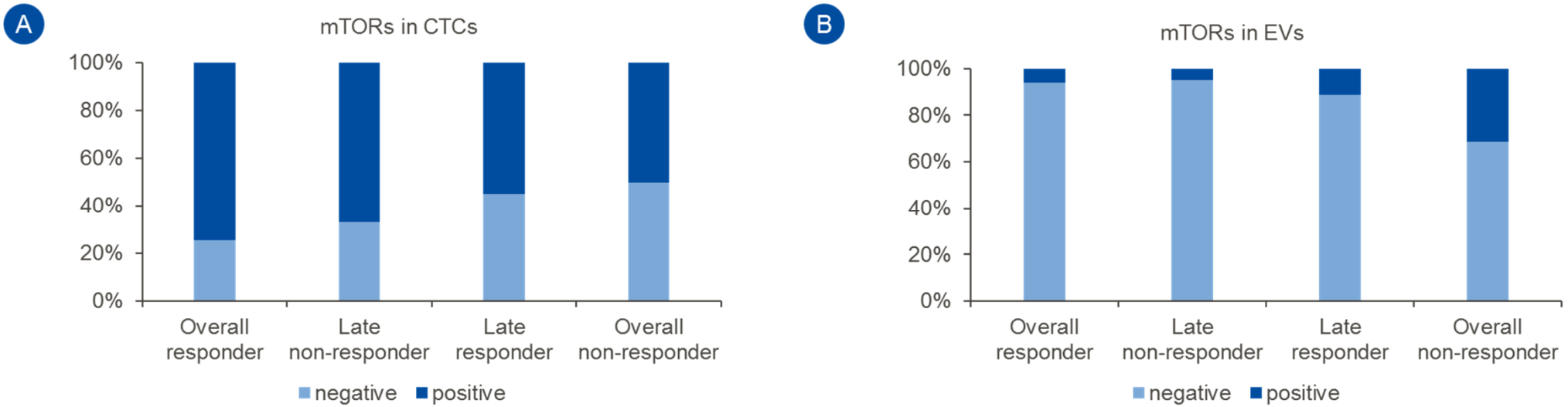
Differences between mRNA profiles from CTCs and EVs



- All subjects in this study were initially staged negative for HER2 (ERBB2) protein expression on primary tumor tissue
- *ERBB2 (Her2)* transcript overexpressing CTCs were found in 40% of all subjects, pointing towards de novo *Her2* positivity

Keup C. et al. (2018) RNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Clin. Chem. 64, 1054.

mRNA profiles from CTCs and EVs show different effects

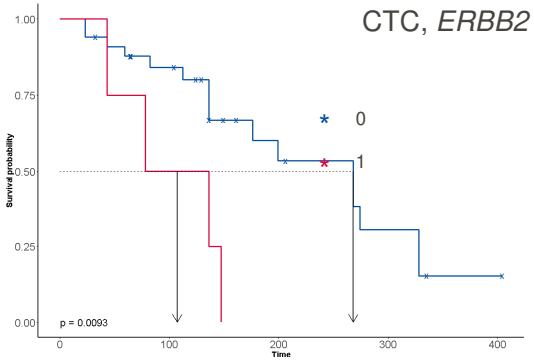


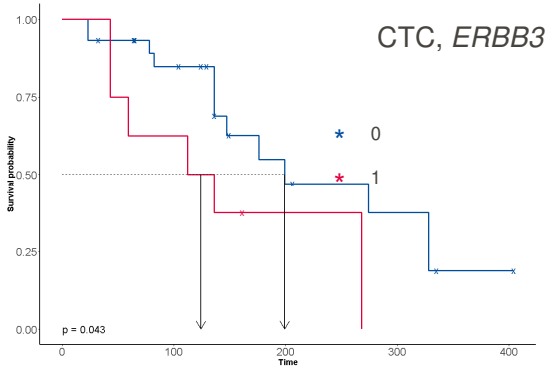
CTC and EV mRNA profiles show substantial differences, correlating with potential therapy outcomes:

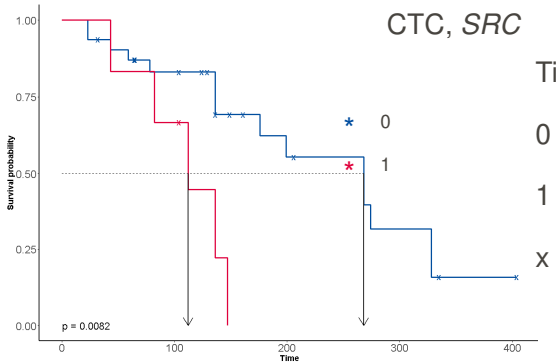
- Overexpression of mTOR in CTCs is related to therapy responsiveness (A)
- mTOR signals in EVs are related to therapy failure (B)

Keup C. et al. (2018) RNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Clin. Chem. 64, 1054.

Survival analysis of EV AURKA positive samples





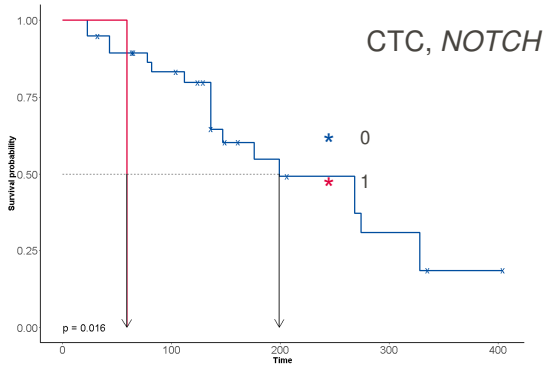


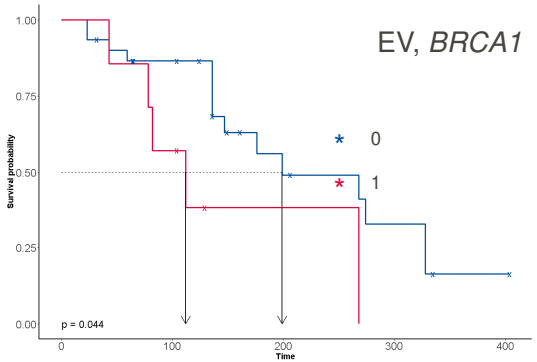
Time = months

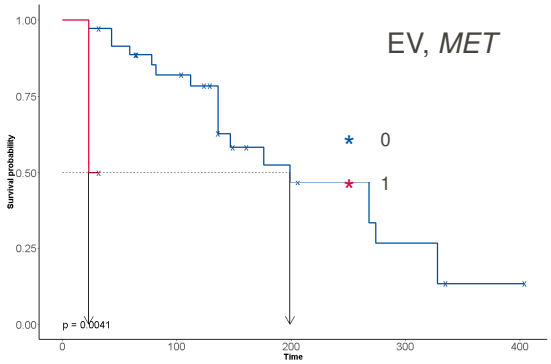
0 = negative

1 = positive

x = censored



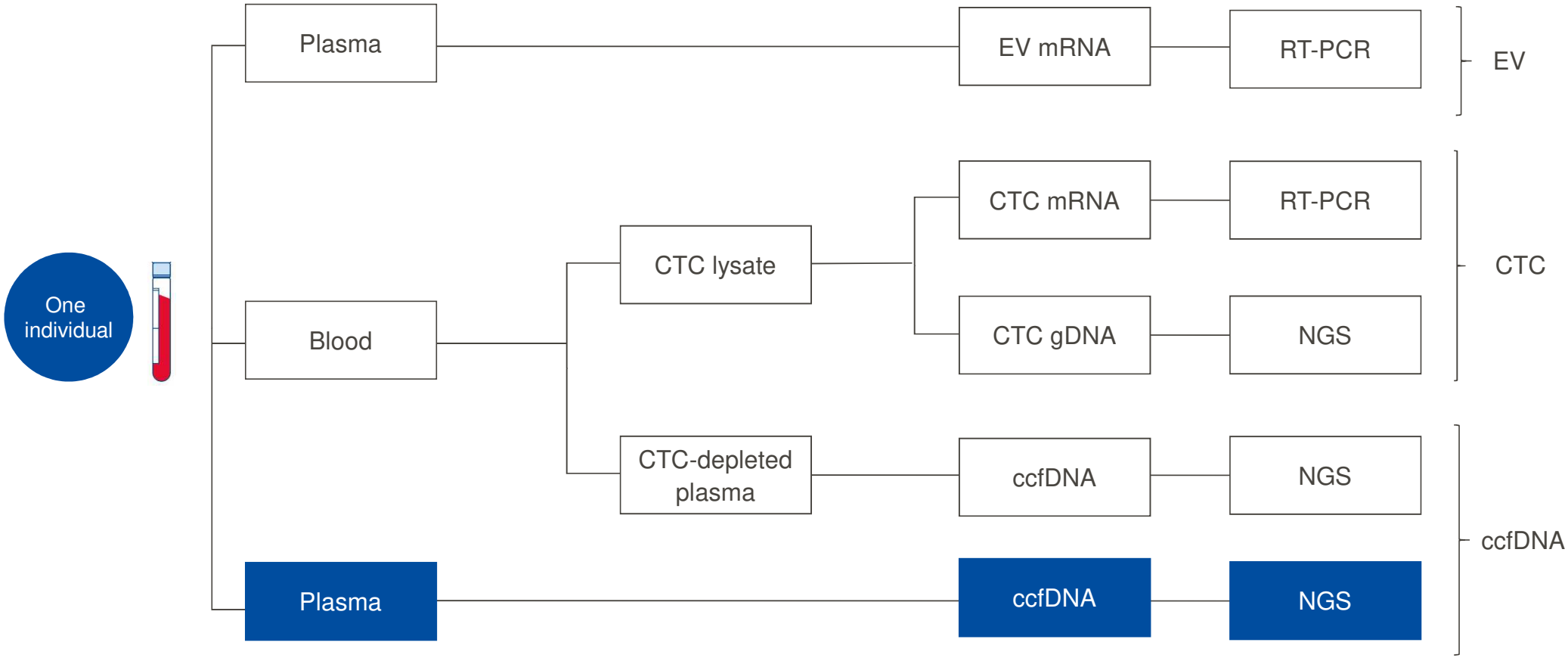




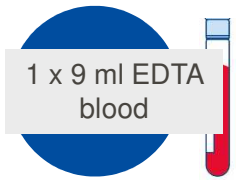
Statistical analysis of samples with Aurora A Kinase gene overexpression in EVs:

- *ERBB2, ERBB3, SRC* or *NOTCH1* overexpression in CTCs and *BRCA1* or *MET* overexpression in EVs are negatively correlated with survival probability
- These genes have a high impact on cancer research, underlining a potential interaction between the tumor and CTCs and EVs in tumor proliferation & metastasis

Study arm 2: Establish a cell-free DNA NGS workflow



Study arm 2: Establish a cell-free DNA NGS workflow



1 x 9 ml EDTA blood

Independent *HR+ HER2-* cohort n=44

Plasma

Blood

4 ml, 2x centrifuged

CTC lysate

CTC-depleted plasma

EV mRNA

CTC mRNA

CTC gDNA

ccfDNA

RT-PCR

RT-PCR

NGS

QIAamp MinElute ccfDNA Kit

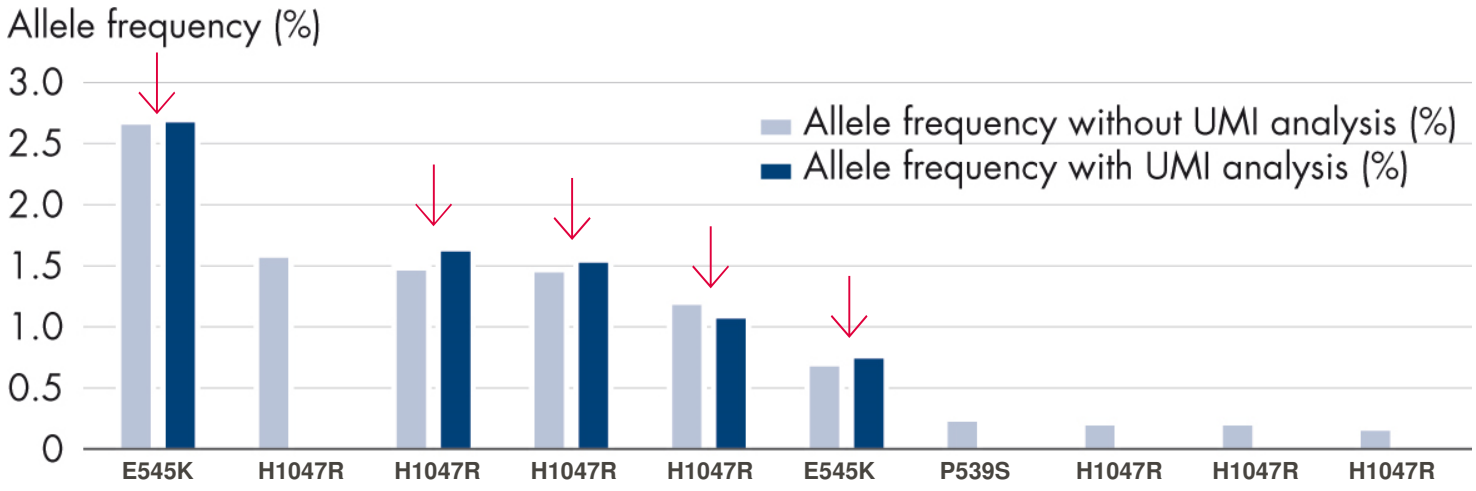
QIAseq Targeted DNA Custom Panel targeting *AKT1, AR, BRCA1, BRCA2, EGFR, ERBB2, ERBB3, ERCC4, ESR1, KRAS, FGFR1, MUC16, PIK3CA, PIK3R1, PTEN, PTGFR and TGFB1*; NextSeq runs with mean coverage of around 16,000x

EV

CTC

ccfDNA

Impact of DNA input and unique molecular indices (UMIs)



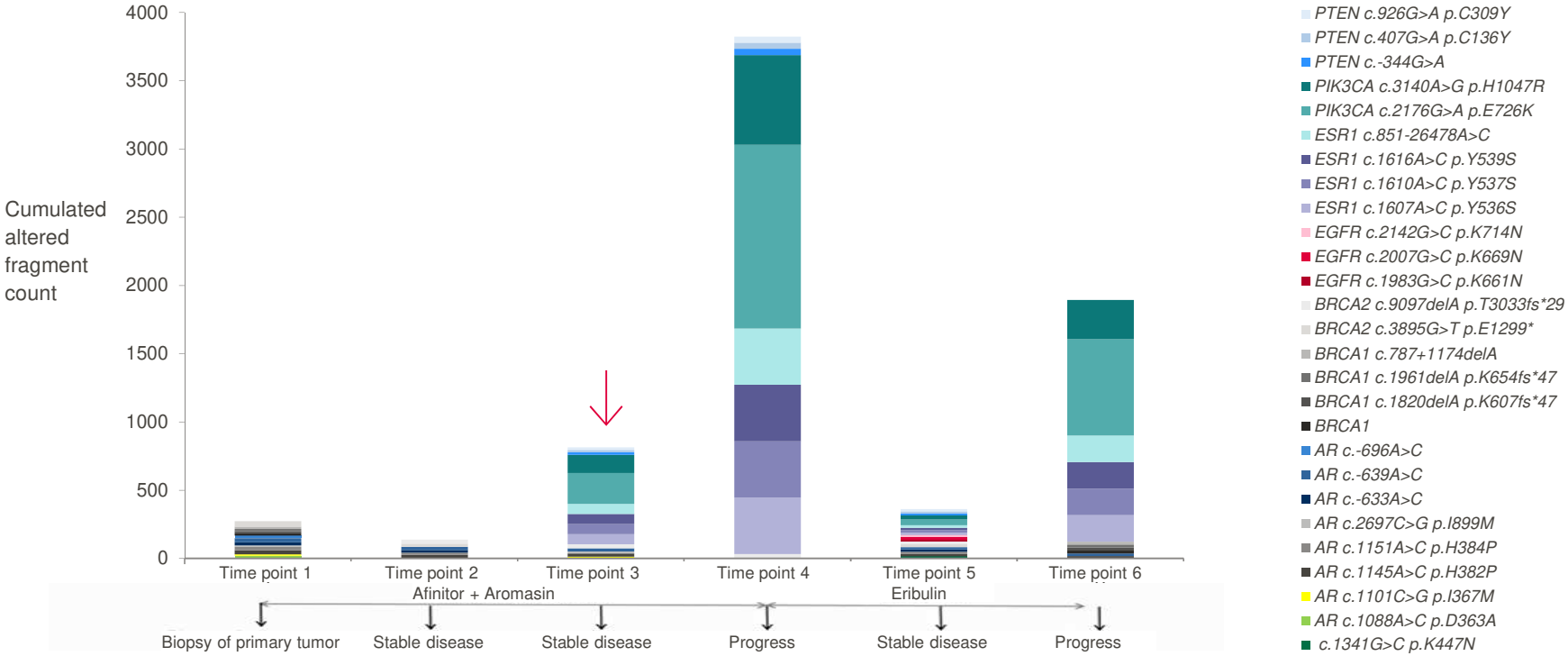
	Case 22	Case 6	Case 53	Case 31	Case 3	Case 15	Case 46	Case 42	Case 5	Case 40
Allele frequency without UMI analysis (%)	2.66	1.56	1.46	1.45	1.18	0.68	0.22	0.20	0.20	0.15
Allele frequency with UMI analysis (%)	2.67	0	1.62	1.52	1.06	0.72	0	0	0	0
cfDNA input (ng)	60	7	12	60	60	60	29	6	28	2
Read fragments per UMI	2.0	14.1	17.2	7.7	4.5	2.8	10.9	11.7	18.8	24
UMI coverage	6403	230	888	684	4499	6351	2066	1662	152	449
Mean coverage x 1000	15.3	3.2	19.5	5.3	26.9	21.5	28.7	26.8	2.8	14.7

- UMIs increase specificity; 50% of variants were confirmed by UMI analysis
- High cfDNA input, high UMI coverage and low ratio of read fragments per UMI are crucial for UMI detection

Keup C. et al. (2019) Targeted deep sequencing revealed variants in cell-free DNA of hormone receptor-positive metastatic breast cancer patients. Cellular and Molecular Life Sciences

Impact of cfDNA variants

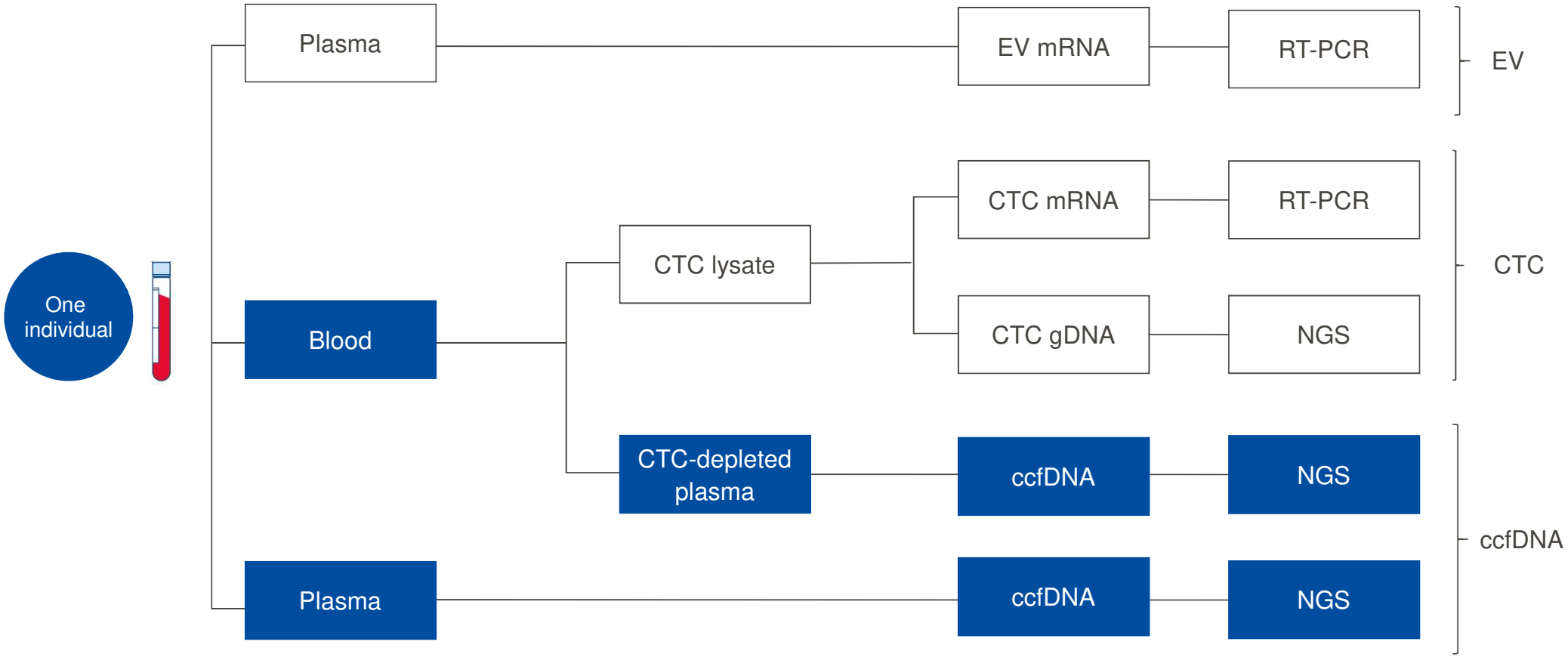
Longitudinal monitoring of case 39: Only pathogenic and likely-pathogenic mutants plotted



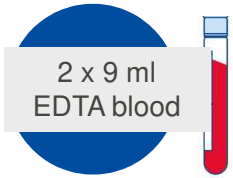
Pathogenic cfDNA variants vary over the course of the disease and may reflect the evolution of a mutation profile due to different interventions

Keup C. et al. (2019) Targeted deep sequencing revealed variants in cell-free DNA of hormone receptor-positive metastatic breast cancer patients. Cellular and Molecular Life Sciences

Study arm 3: Comparison of ccfDNA from naive or CTC-depleted plasma



Study arm 3: Comparison of ccfDNA from naive or CTC-depleted plasma



2 x 9 ml
EDTA blood

Independent
HR+ HER2-
cohort n=17

Plasma

EV mRNA

RT-PCR

EV

Blood

CTC lysate

CTC mRNA

RT-PCR

CTC gDNA

NGS

CTC

CTC-depleted
plasma

4 ml, 2x centrifuged

Plasma

ccfDNA

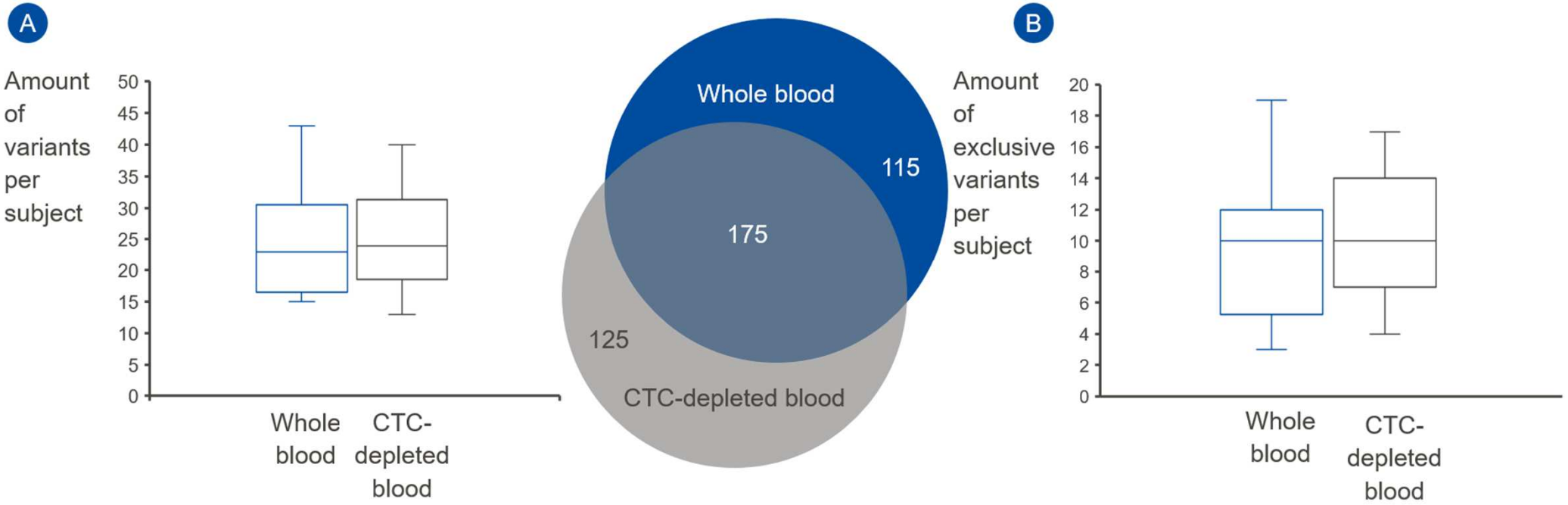
QIAamp
MinElute ccfDNA
Kit

ccfDNA

QIAseq Targeted DNA
Custom Panel targeting
AKT1, AR, BRCA1,
BRCA2, EGFR, ERBB2,
ERBB3, ERCC4, ESR1,
KRAS, FGFR1, MUC16,
PIK3CA, PIK3R1, PTEN,
PTGFR and *TGFB1*;
NextSeq® runs with
mean coverage of
around 16,000x

ccfDNA

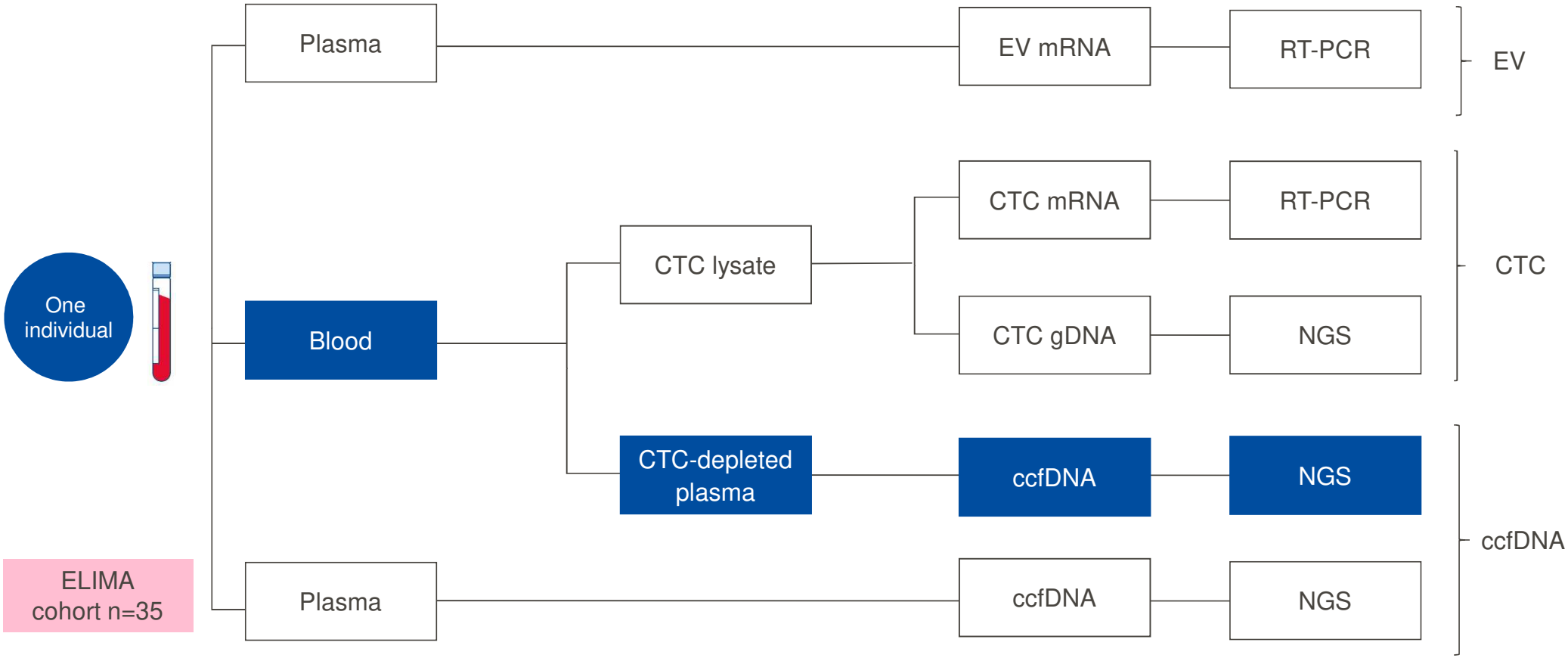
Comparison of ccfDNA from naive or CTC-depleted plasma



No significant difference between the two ccfDNA sources

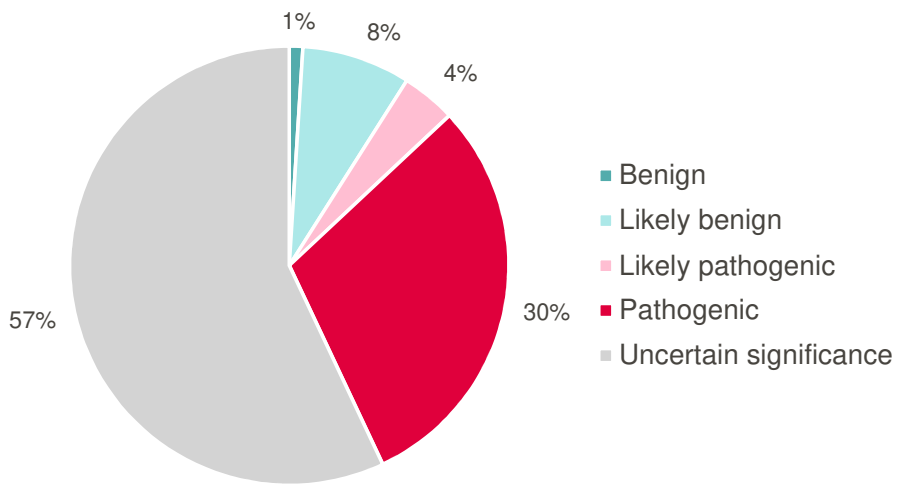
Keup C. et al. (2019) Cell-free DNA variant sequencing using ctc-depleted blood for comprehensive liquid biopsy testing in metastatic breast cancer. Cancers 11(2), 238

Study arm 4: Analysis of ccfDNA variants from CTC-depleted plasma

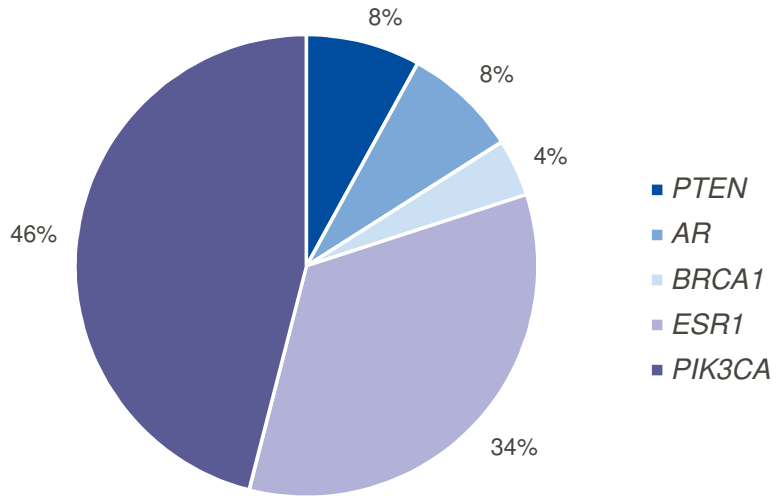


ccfDNA variants in plasma from CTC-depleted blood

Classification (n=76)



Gene location of pathogenic or likely pathogenic variants (n=26)

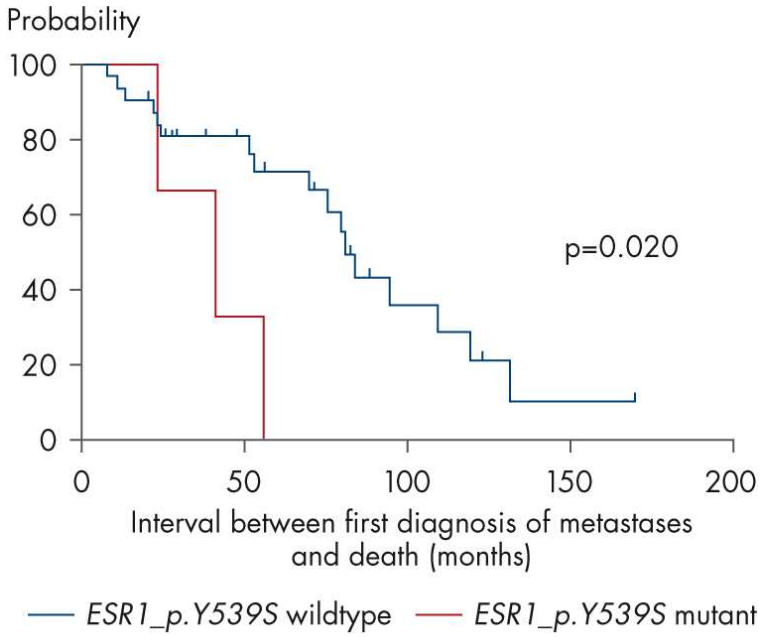


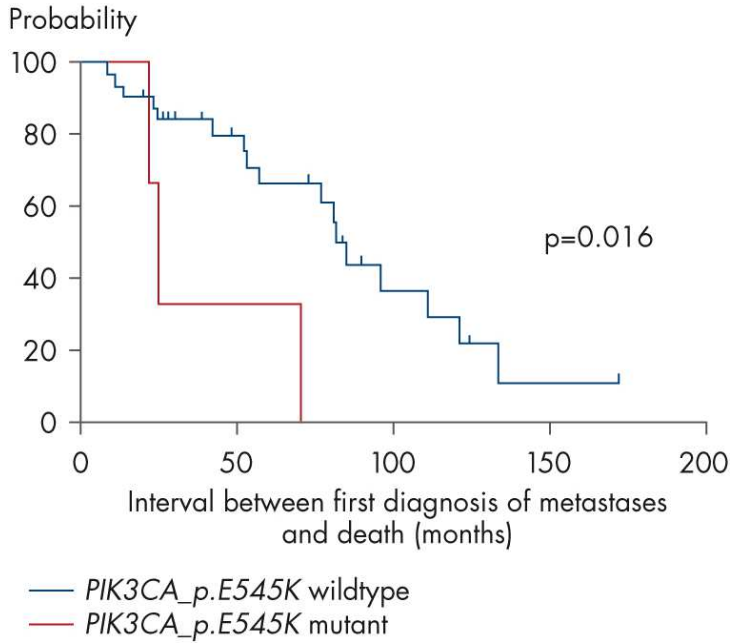
One quarter of all detected variants are known pathogenic variants

Among them, most variants occurred in *AR, PIK3CA* and *ESR1*

Keup C. et al. (2019) Establishment of a workflow for the analysis of mRNA and gDNA from circulating tumor cells, extracellular vesicles and cell-free DNA from the same blood sample to mirror the genomic and transcriptomic complexity in metastatic breast cancer subjects. Poster presented at AACR, 2019.

cfDNA variants in plasma from CTC-depleted blood

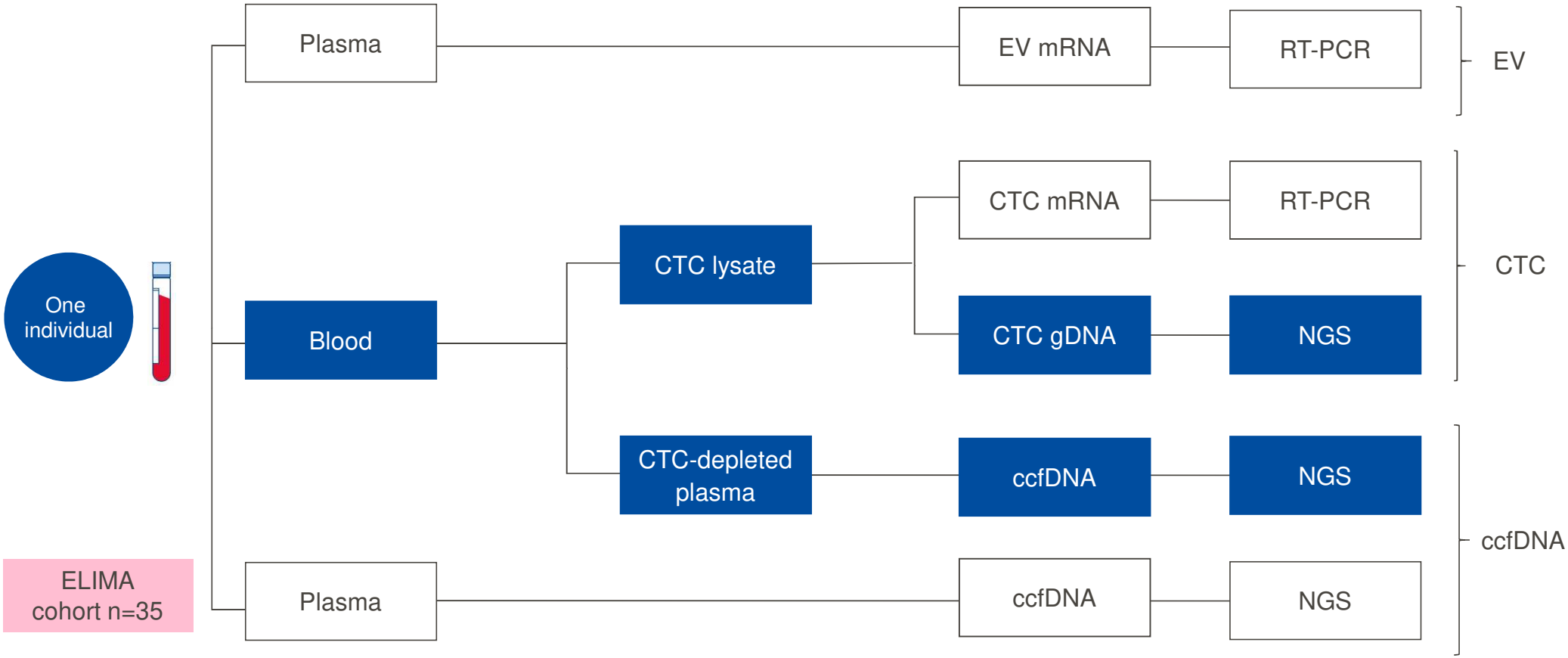




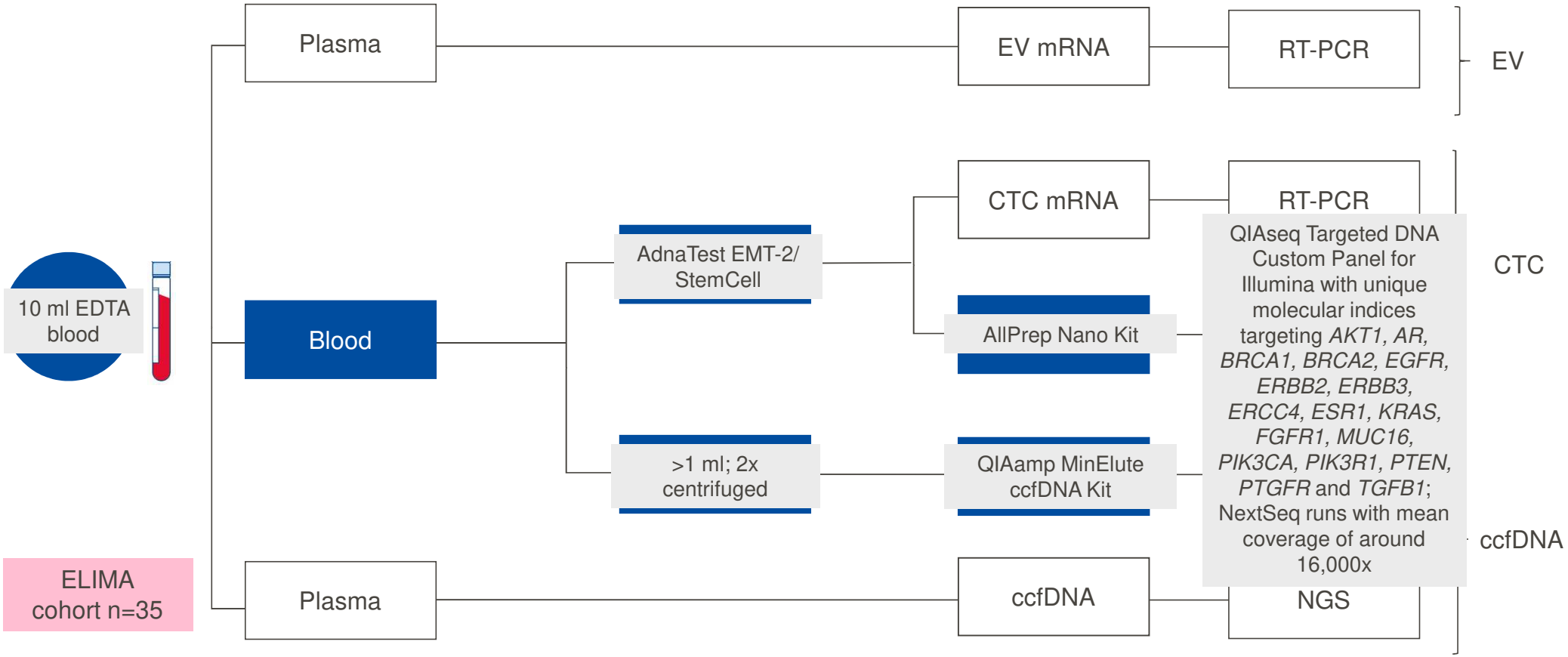
Mutations in *ESR1* and *PIK3CA* correlate with a lower survival probability

Keup C. et al. (2019) Establishment of a workflow for the analysis of mRNA and gDNA from circulating tumor cells, extracellular vesicles and cell-free DNA from the same blood sample to mirror the genomic and transcriptomic complexity in metastatic breast cancer subject. Poster presented at AACR, 2019.

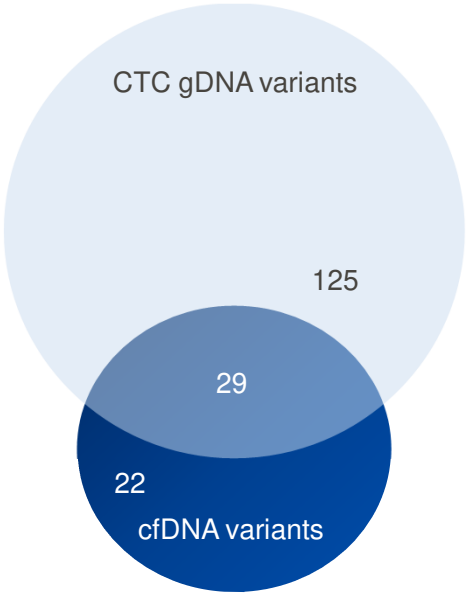
Study arm 5: Comparison of variants from ccfDNA and CTC gDNA

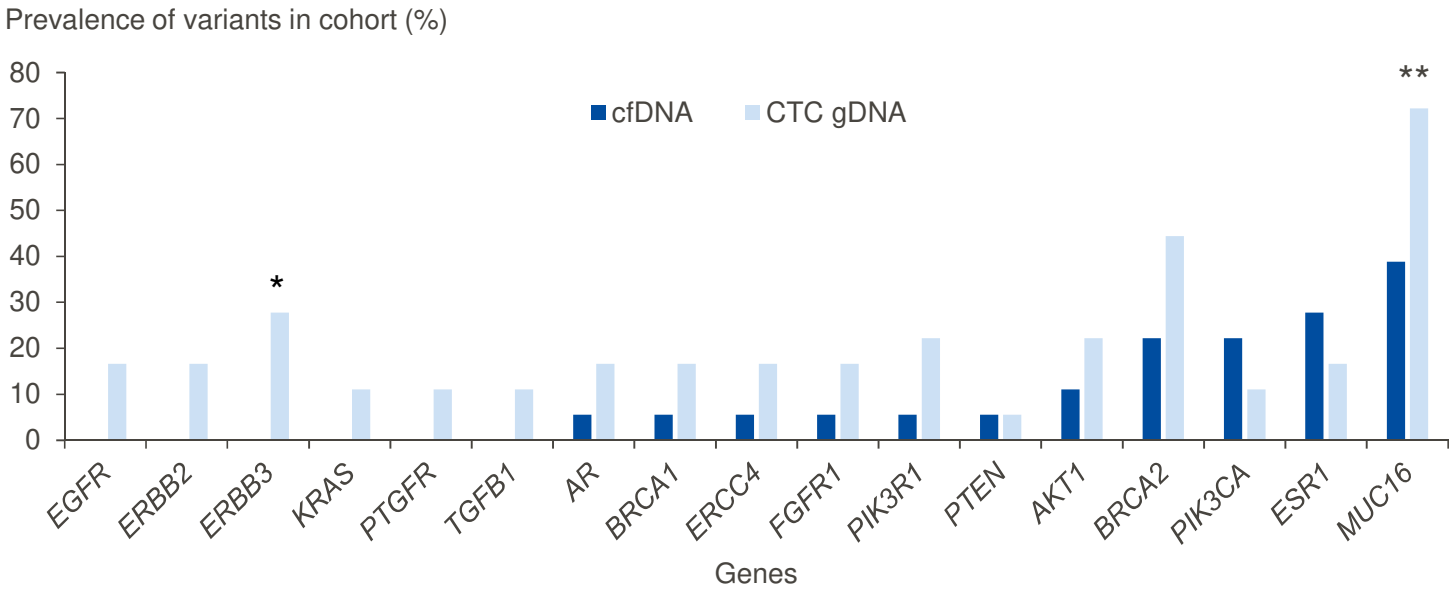


Study arm 5: Comparison of variants from ccfDNA and CTC gDNA



Prevalence of variants and concordance between CTC gDNA and cfDNA



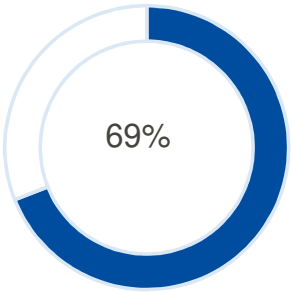


- The large majority of variants was detected uniquely in one fraction. Half of the cfDNA variants were also found in the CTC gDNA fraction
- *ESR1* and *PIK3CA* variants are more prominent in cfDNA, while *ERBB2* variants were only detected in CTC gDNA

Keup, C. et al. Multimodal targeted deep sequencing of circulating tumor cells and matched cell-free DNA provides additive value in metastatic breast cancer patients (submitted to Nucleic Acid Research); Posters presented at ACTC 2019 and SABC 2019.

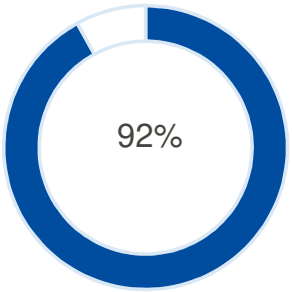
The value of multimodality liquid biopsy testing

EV mRNA



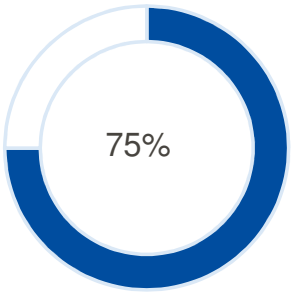
Positive cases (n=69) of all analyzed cases (n=100)

CTC mRNA



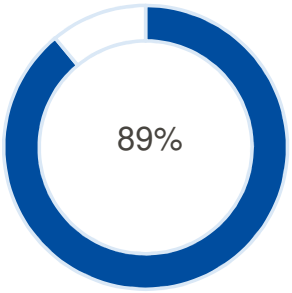
Positive cases (n=97) of all analyzed cases (n=105)

cfDNA



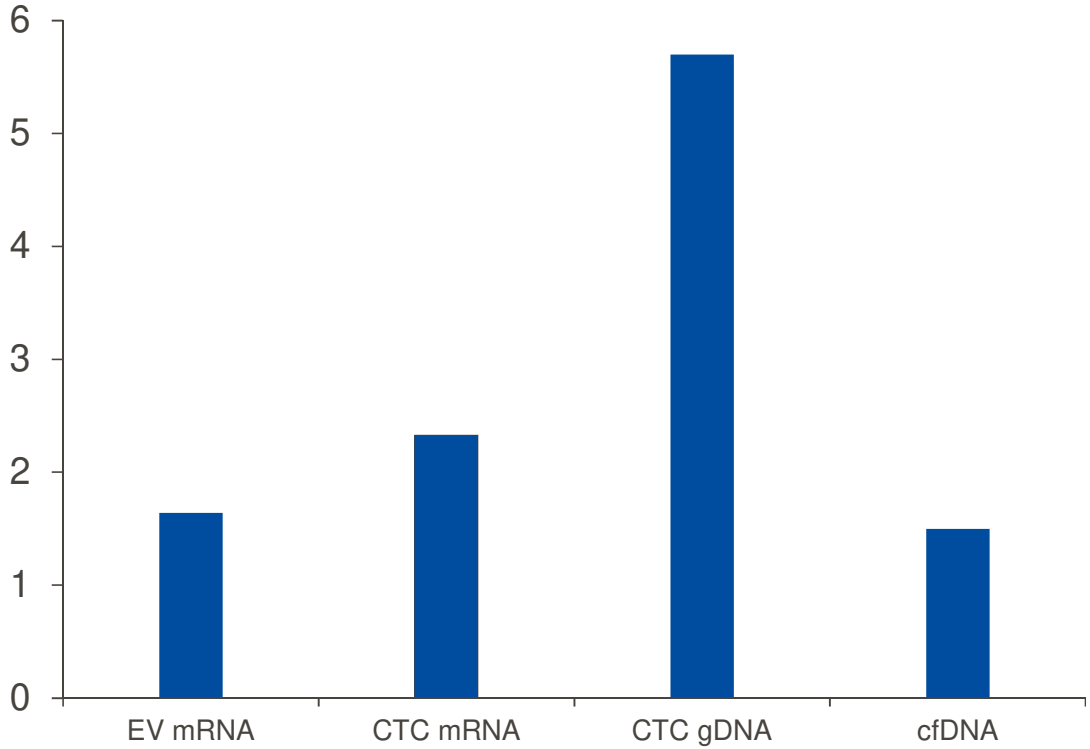
Positive cases (n=73) of all analyzed cases (n=97)

CTC gDNA



Positive cases (n=25) of all analyzed cases (n=28)

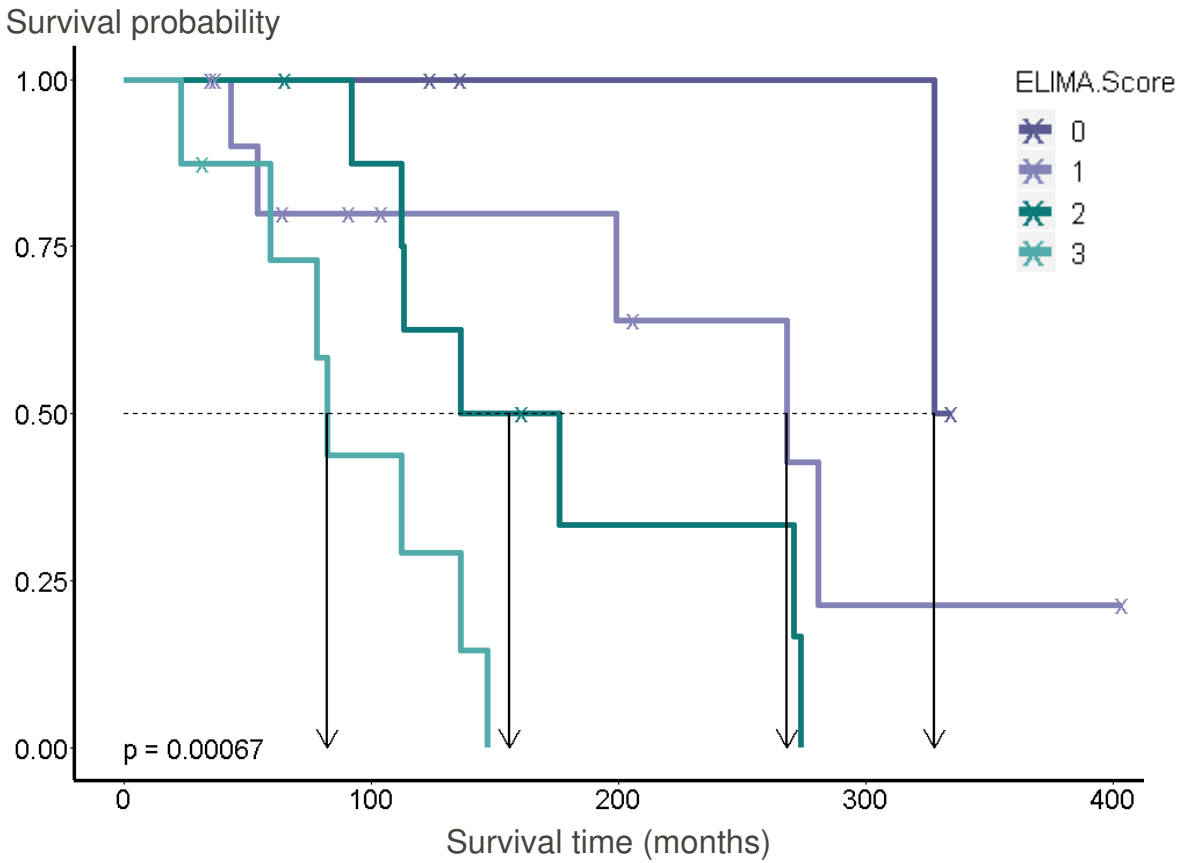
Mean amount of signals/variants per sample



Keup C. et al. Posters presented at ACTC 2019: The ELIMA study: Evaluation of multiple Liquid biopsy analytes including CTCs, EVs and cfDNA In Metastatic breast cancer patients All from one blood sample

The value of multimodality liquid biopsy testing

Analyte	Number of cases with actionable signals/variants
cfDNA	17
CTC gDNA	17
EV mRNA	14
CTC mRNA	18
Any of the four	26



Keup C. et al. Posters presented at ACTC 2019: The ELIMA study: Evaluation of multiple Liquid biopsy analytes including CTCs, EVs and cfDNA In Metastatic breast cancer patients All from one blood sample

Conclusions: Towards a comprehensive understanding of cancer

1. mRNA profiles from EVs and CTC differ and together give a more comprehensive picture
2. CTC-depleted plasma is suitable for cell-free DNA NGS sequencing – a separate ccfDNA analysis workflow from naive plasma is not required
3. Mutational profiles from ccfDNA and gDNA from CTCs differ significantly and together give a more comprehensive picture
4. Multiple analyte extraction and analysis from one blood sample is now possible (previously 3 x 9 ml), hence, avoiding sample bias
5. Multiparametric comparison of all four analytes gets us closer to a comprehensive understanding of cancer

Acknowledgements

University Hospital Essen

Corinna Keup, Ph.D.

Prof. Sabine Kasimir-Bauer, Ph.D.

QIAGEN

Siegfried Hauch, Ph.D.

Markus Storbeck, Ph.D.

Constanze Kindler, Ph.D.

Michael Otte

Peter Hahn, Ph.D.

Markus Sprenger-Haussels, Ph.D.

Thank you for your attention.

siegfried.hauch@qiagen.com

conquercancer.qiagen.com

Legal disclaimer

QIAGEN products shown here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.