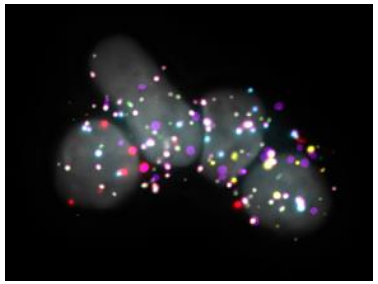
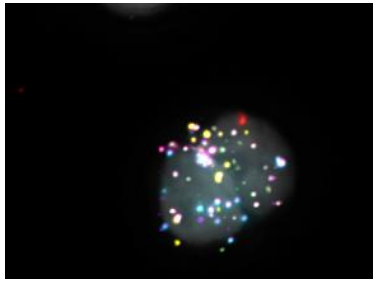
Medical University of Graz

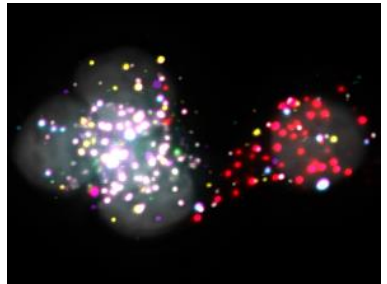
UNIV. COMPREHENSIVE CANCER CENTER Krebszentrum GRAZ
Medizinische Universität & LKH-Univ. Klinikum

CBmed
BIOMARKER RESEARCH
ENABLING APPLIED PRECISION MEDICINE

DECODING PROSTATE CANCER RESISTANCE: WHY HIGH-QUALITY SAMPLES AND STANDARDS MATTER (WITH A LITTLE HELP FROM MACHINE LEARNING)



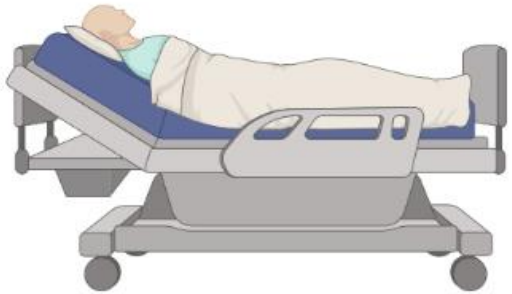




Research Prof. Priv.-Doz. Amin El-Heliebi, PhD
Gottfried Schatz Research Center
Division of Cell Biology, Histology & Embryology
Medical University of Graz, Austria
E-Mail: amin.elheliebi@medunigraz.at
26.11.2024

What has happened?

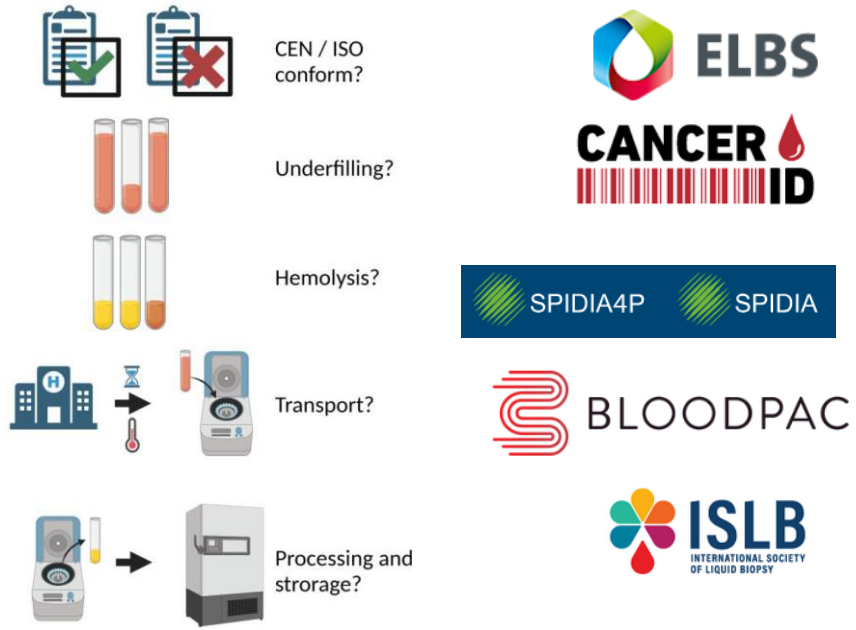
Clinic



Liquid biopsy

Lab

- What happened to the blood tube?
- How long was it lying around?
- Was the temperature ok?
- Or is it patient dependent?
- Or does it depend on the kind of blood collection tube?
- Can I still use it for liquid biopsies?



Created in BioRender. El-Heliebi, A. (2024)
Generated by OpenAI's DALL-E via ChatGPT, created by El-Heliebi, 2024

amin.elheliebi@medunigraz.at

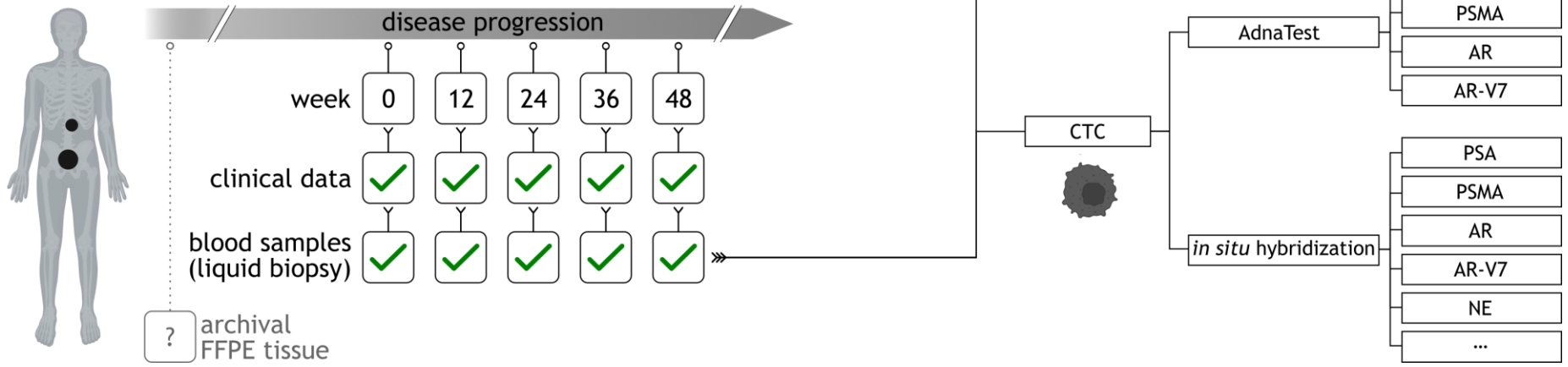
Advanced prostate cancer, liquid biopsy study design

Hypotheses and Aim

- Multiple resistance mechanisms can be detected by liquid biopsies and are dynamically changing during treatment.
- Comprehensive multi analyte liquid biopsy approach, using ctDNA and CTCs, to understand and **predict drug resistance.**
- All blood collection tubes shall follow the ISO and CEN/TS standards.

Longitudinal cohort study

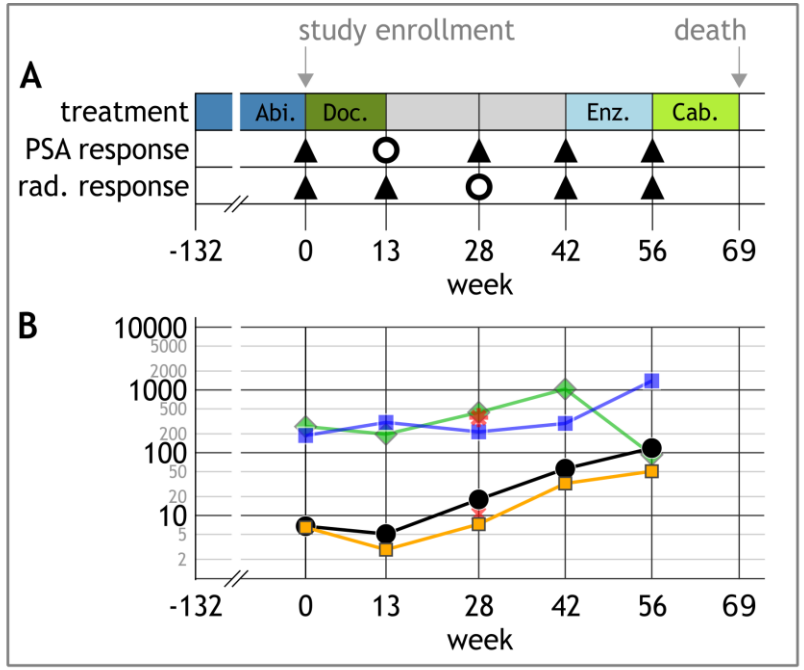
- metastatic PC patients
- progressive disease, change of systemic therapy
- 25 patients, 140 visits



Figures created with BioRender.com

amin.elheliebi@medunigraz.at

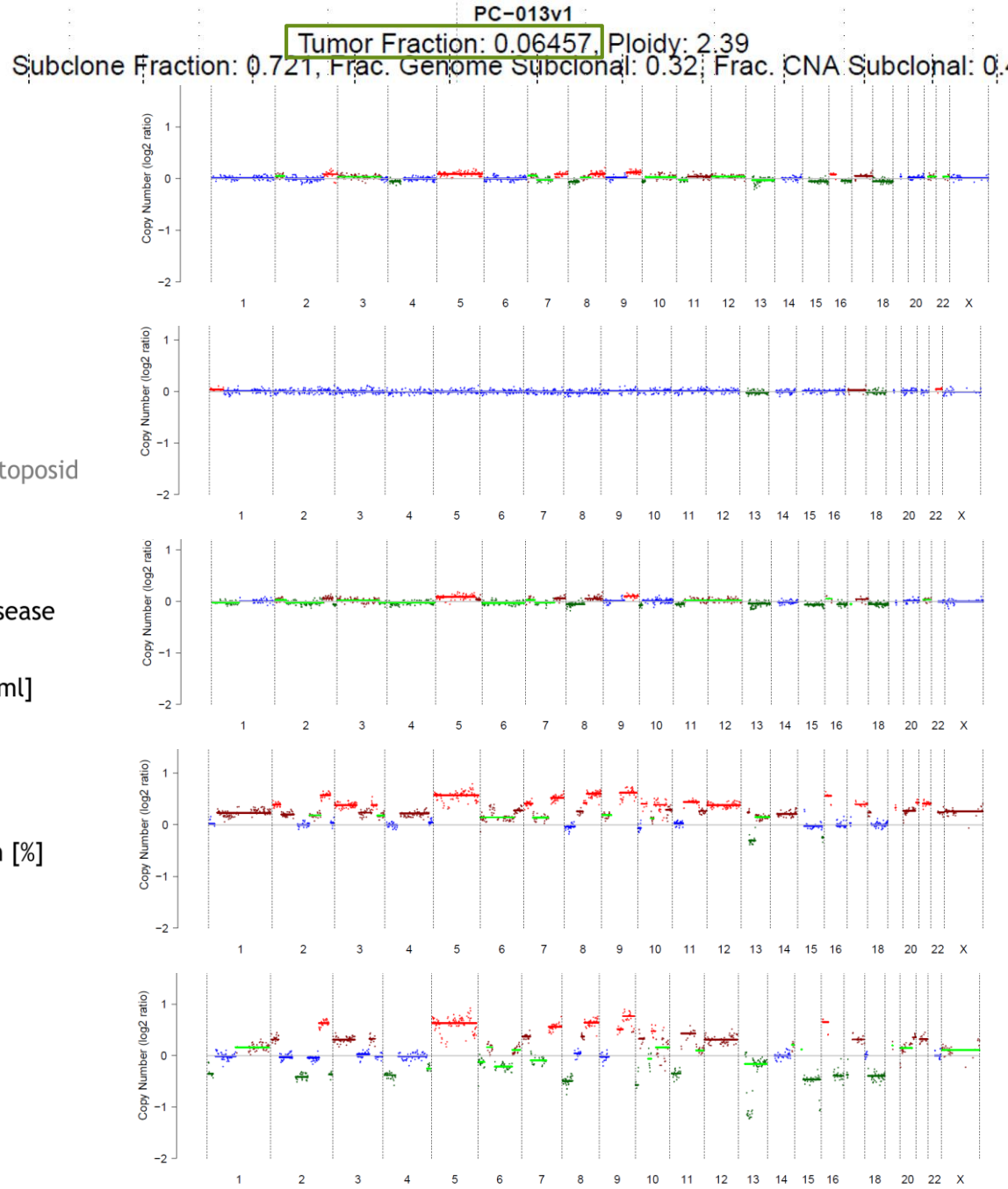
Longitudinal monitoring of drug resistance: PC-013



- Abiraterone
- Enzalutamide
- Docetaxel
- Cabazitaxel
- Carboplatin/Etoposid
- none
- *not available*

- stable disease
- progressive disease

- PSA total [ng/ml]
- LDH [U/l]
- AP [U/l]
- NSE [ng/ml]
- CgA [ng/ml]
- ctDNA fraction [%]



Week 0

Week 13

Week 28

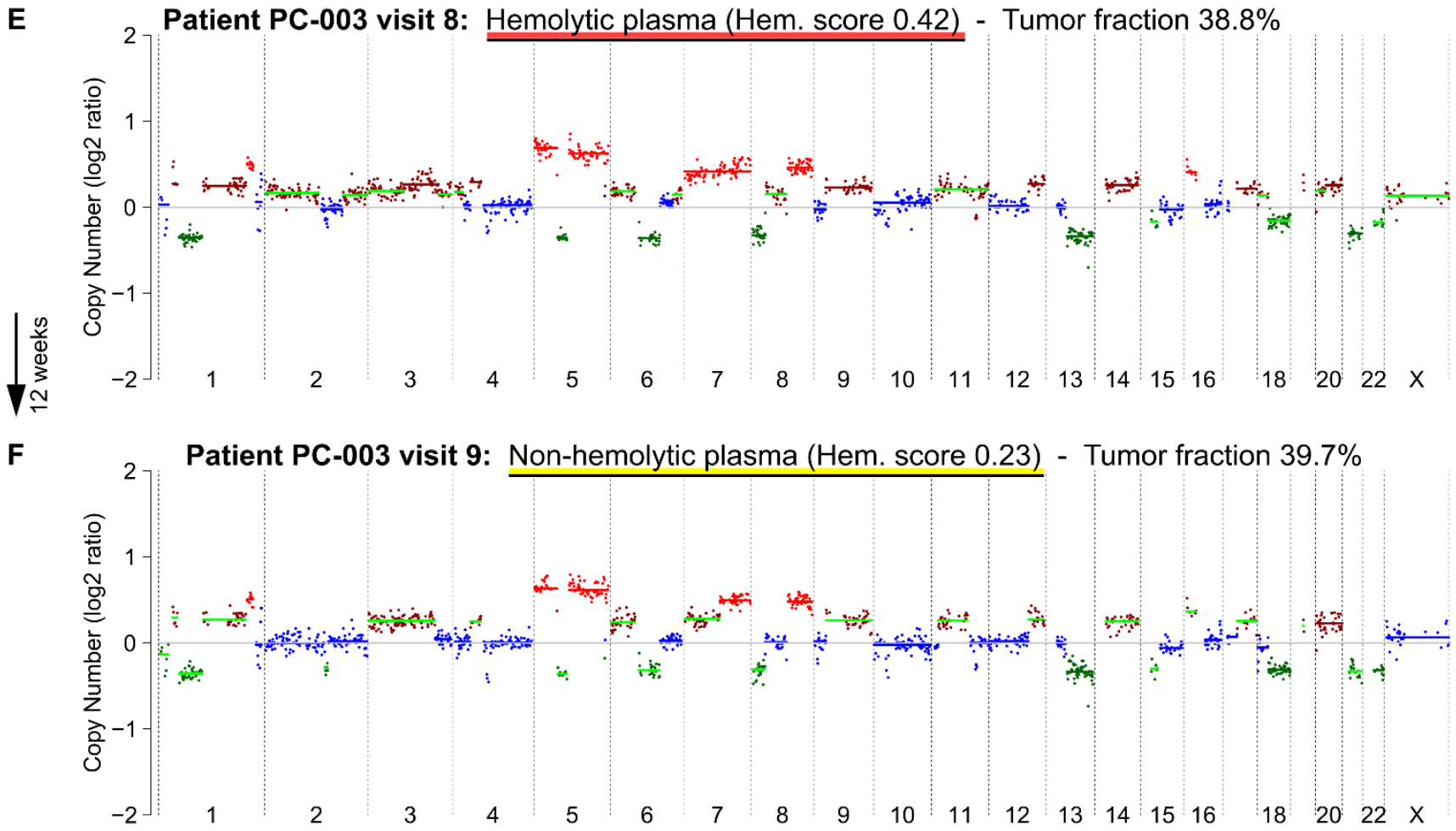
Week 42

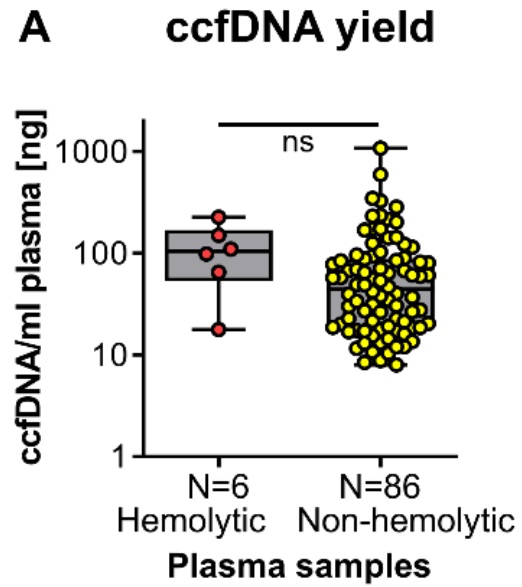
Week 56

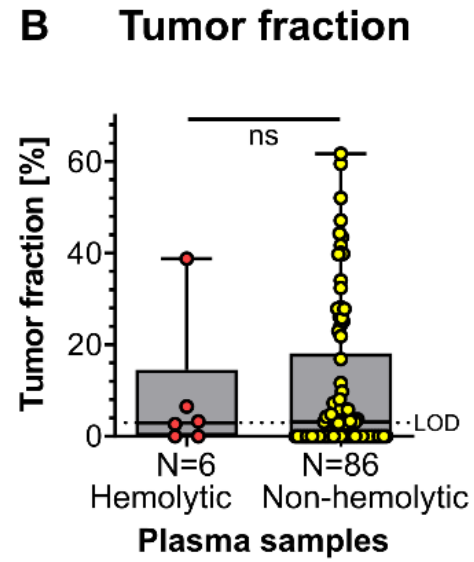
Medical University of Graz

amin.elheliebi@medunigraz.at

Patient PC-003







Even challenging hemolytic patient samples can produce high-quality ctDNA data

Bonstingl et al, Clin Chem 2024

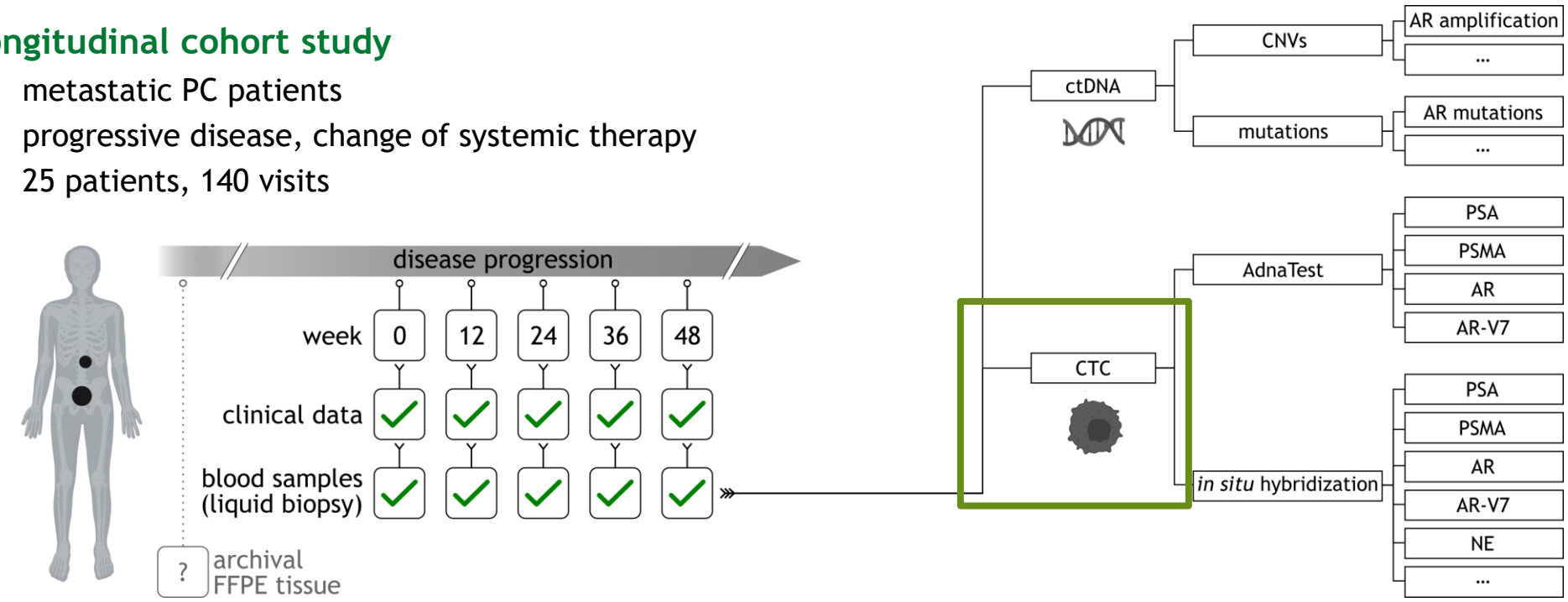
Advanced prostate cancer, liquid biopsy study design

Hypotheses and Aim

- Multiple resistance mechanisms can be detected by liquid biopsies and are dynamically changing during treatment.
- Comprehensive multi analyte liquid biopsy approach, using ctDNA and CTCs, to understand and predict drug resistance.
- All blood collection tubes shall follow the ISO and CEN/TS standards

Longitudinal cohort study

- metastatic PC patients
- progressive disease, change of systemic therapy
- 25 patients, 140 visits

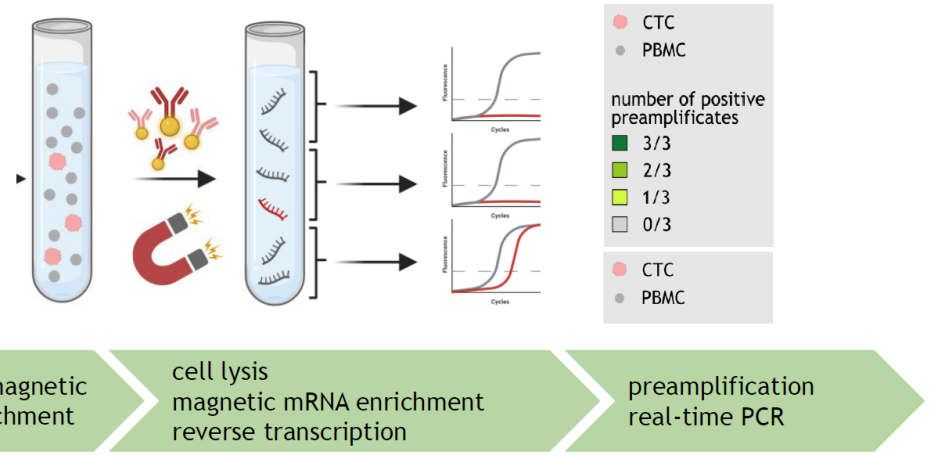


Figures created with BioRender.com

amin.elheliebi@medunigraz.at

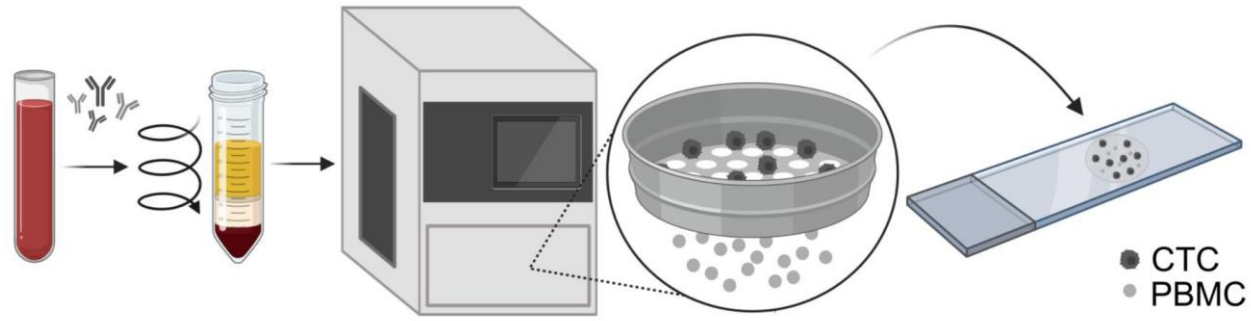
CTC enrichment

AdnaTest ProstateCancerPanel AR-V7 (QIAGEN)



immunomagnetic CTC enrichment → cell lysis, magnetic mRNA enrichment, reverse transcription → preamplification, real-time PCR

Bonstingl et al., Biomark Res 2024

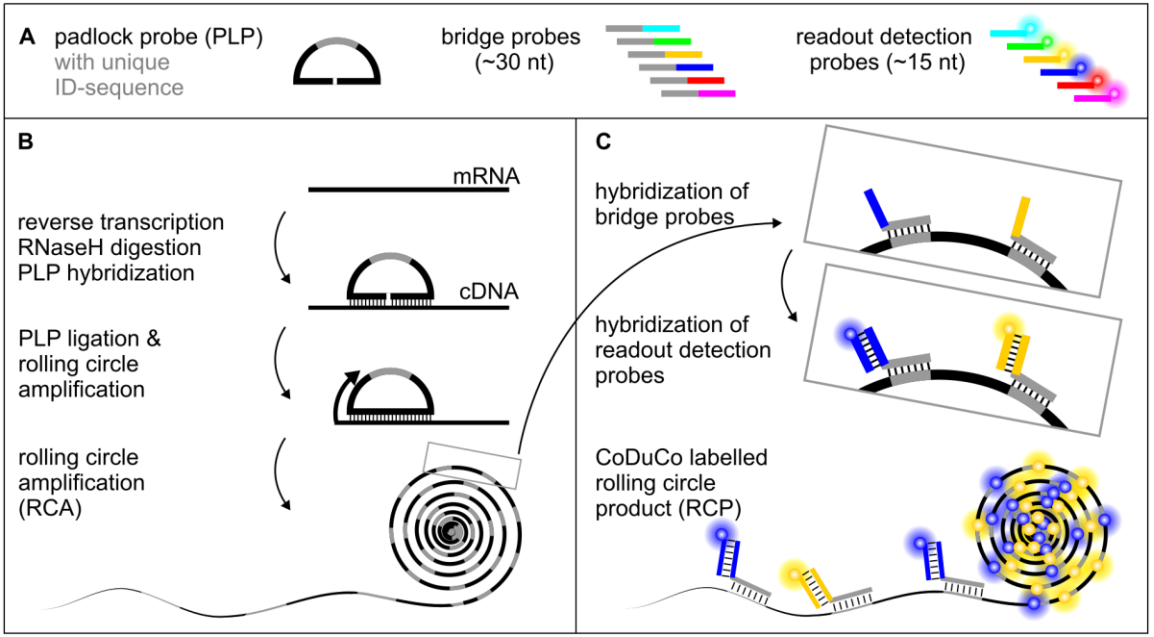


depletion of leukocytes and erythrocytes → density gradient centrifugation → gravity flow filtration (High-Density Micro-porous HDM chip) → cytocentrifugation and in situ analysis

Mr. Jong Kil, Lee / PhD
Kim et al., Anal Biochem. 2013; Lee et al., Oncol. Lett. 2017

Combinatorial Dual-Color (CoDuCo) staining

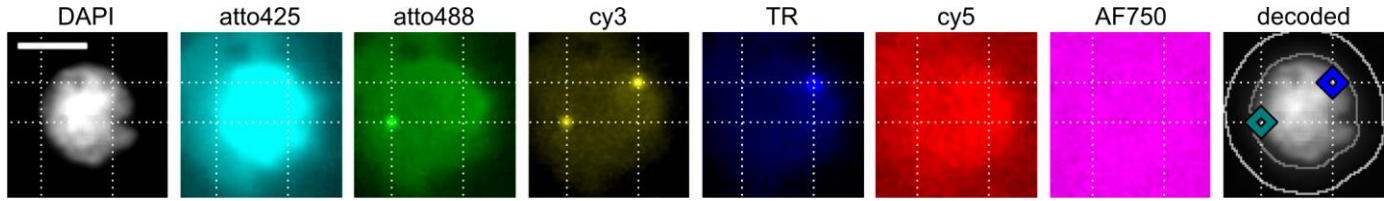
El-Heliebi et al., Clin. Chem. 2018
Hofmann et al., Cancers 2020
Welsch et al., Clin Exp Metastasis 2024
Bonstingl et al., Biomark Res 2024



	decoded marker		color code channel 1	color code channel 2
hematopoietic (PBMCs)	PTPRC, ITGAM, FCGR3, CD4, ITGB2 (hem.)	=	cy3	TR
mesenchymal	VIM	=	atto488	cy3
epithelial (CTCs)	KRT8, KRT18, KRT19 (KRT)	=	atto425	cy5
	EPCAM	=	atto488	AF750
PC-specific (CTCs)	PSA	=	atto425	AF750
	PSMA	=	cy3	cy5
	AR-FL	=	atto488	cy5
	AR-V7	=	cy3	AF750
NE-associated	SYP, CHGA, NCAM1 (NE)	=	cy5	AF750
	SLFN11	=	atto425	atto488
	DLL3	=	atto425	cy3

>> New mRNA targets can be designed

>> Works very well with CytoGen
>> Compatible to Parsortix, CellSearch (ferrofluid!), Gilupi



Bonstingl et al. Biomarker Res 2024

Two colors hybridized for each target of interest

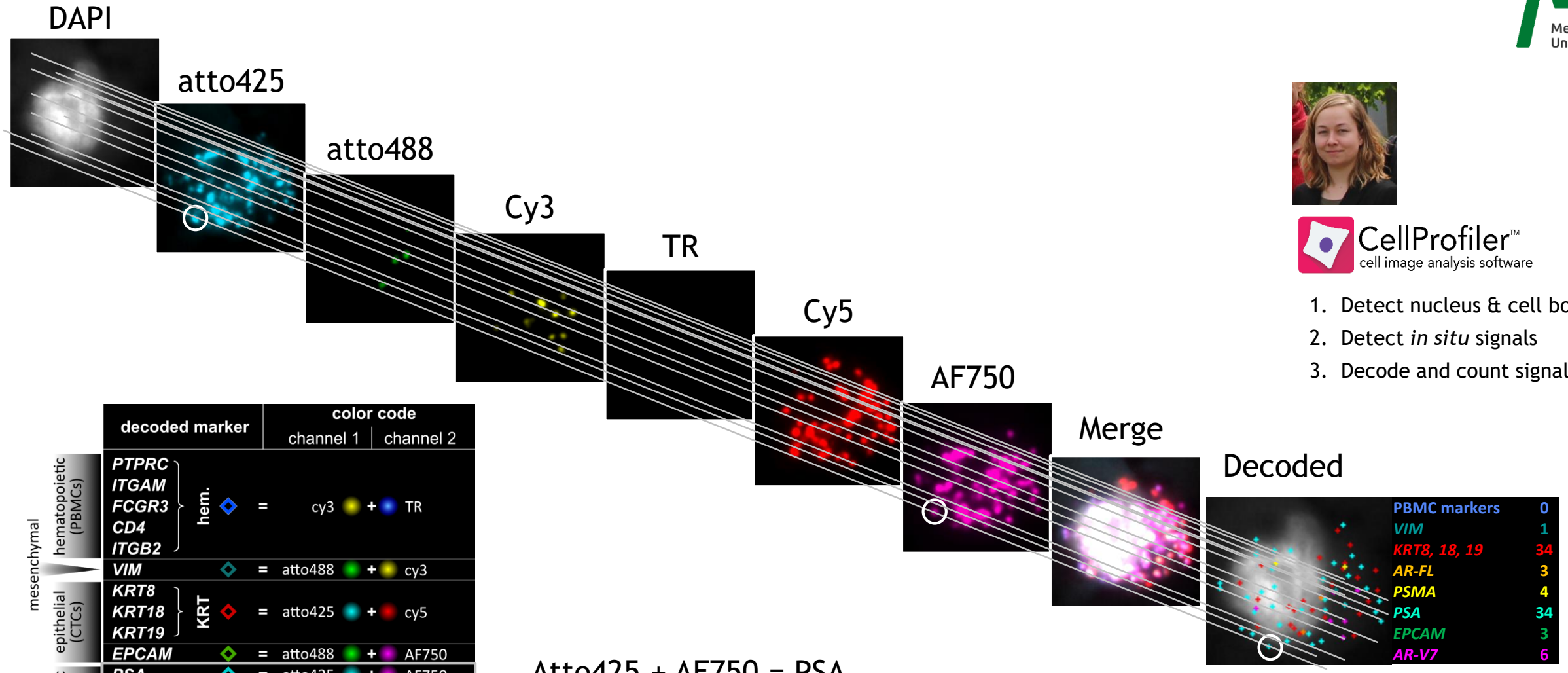
6 colors combined in pairs
>> 15 unique color codes $\binom{n}{k} = 15$

>> distinguish up to 15 markers

Figures created with BioRender.com

amin.elheliebi@medunigraz.at

Decoding: Workflow for *in situ* padlock probe analysis of CTCs



CellProfiler™ cell image analysis software

1. Detect nucleus & cell border
2. Detect *in situ* signals
3. Decode and count signals

	decoded marker		color code: channel 1	channel 2
hematopoietic (PBMCs)	PTPRC, ITGAM, FCGR3, CD4, ITGB2	hem.	cy3	TR
mesenchymal	VIM		atto488	cy3
epithelial (CTCs)	KRT8, KRT18, KRT19	KRT	atto425	cy5
	EPCAM		atto488	AF750
PC-specific (CTCs)	PSA		atto425	AF750
	PSMA		cy3	cy5
	AR-FL		atto488	cy5
	AR-V7		cy3	AF750
NE-associated	SYP, CHGA, NCAM1	NE	cy5	AF750
	SLFN11		atto425	atto488
	DLL3		atto425	cy3
			classification >>	

Atto425 + AF750 = PSA

Bonstingl et al., Biomark Res 2024

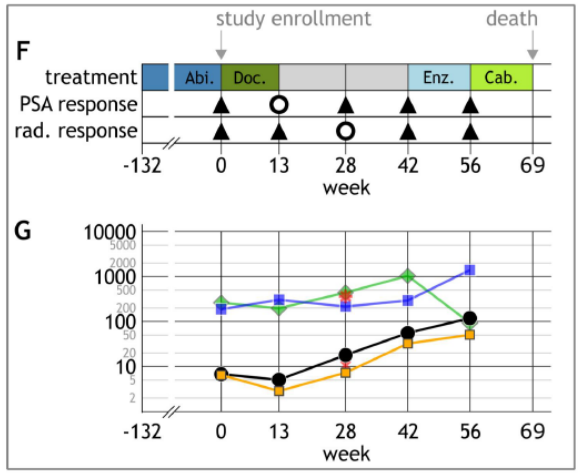
Image analysis

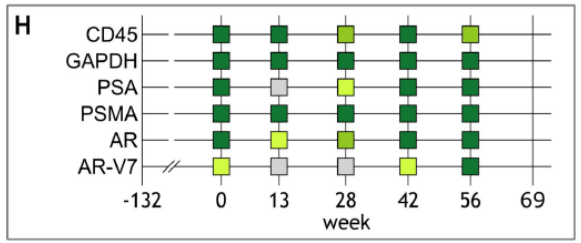
- Signal detection, decoding, and feature extraction

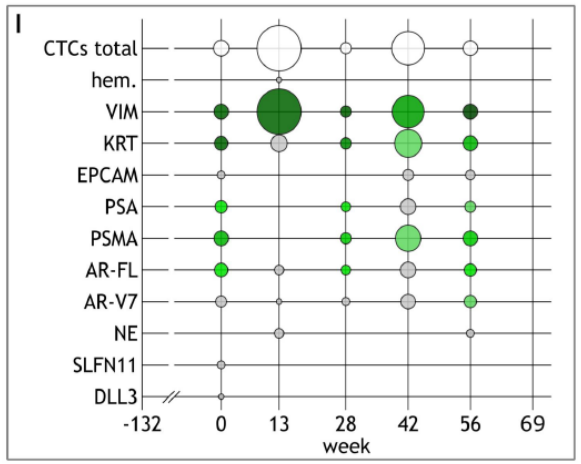
Classification of cells

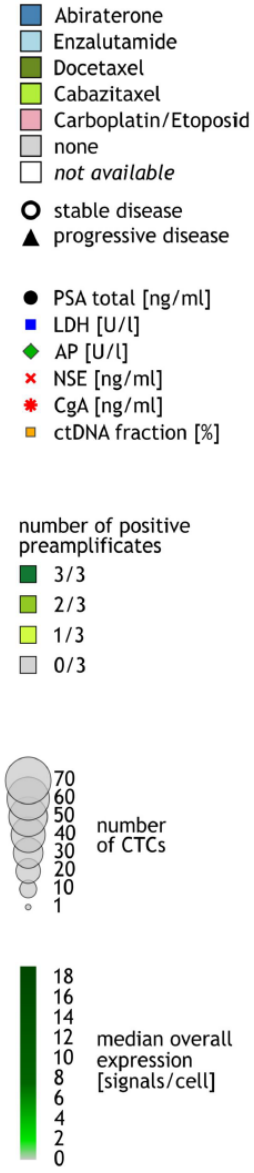
- Supervised machine learning-assisted classification

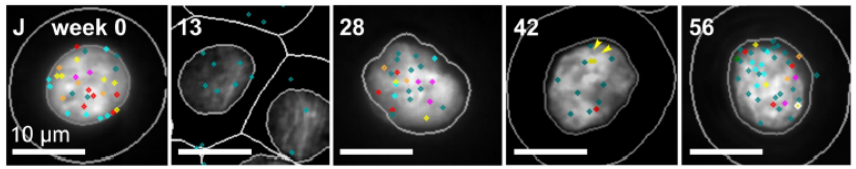
Patient 13





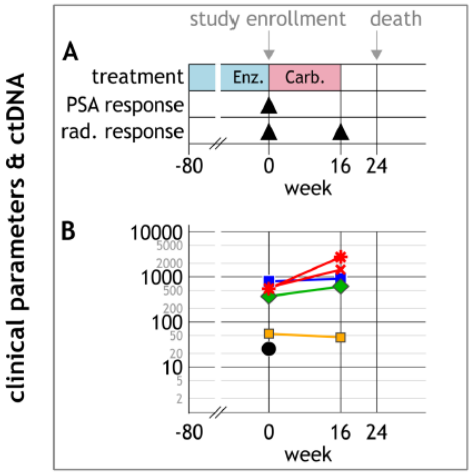


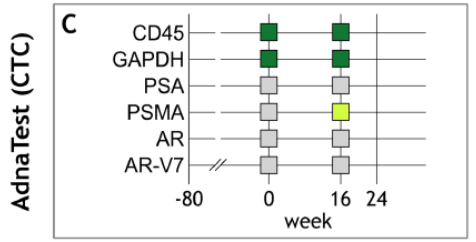


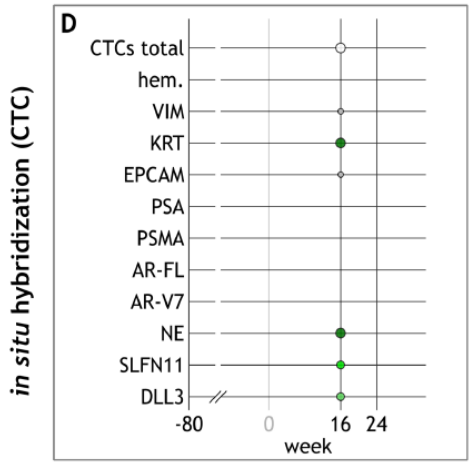


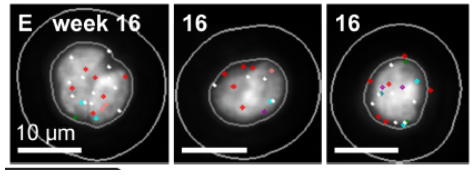
Patient 13 showed progression under antihormonal treatment. Switch to taxan-based chemotherapy reduced ctDNA and CTCs, but was discontinued due to toxicity. ctDNA and CTCs increased again and showed high EMT plasticity, ultimately leading to therapy resistance. **Most important liquid biopsy finding: CTC expression data identified PSMA as druggable target.**

Patient 15



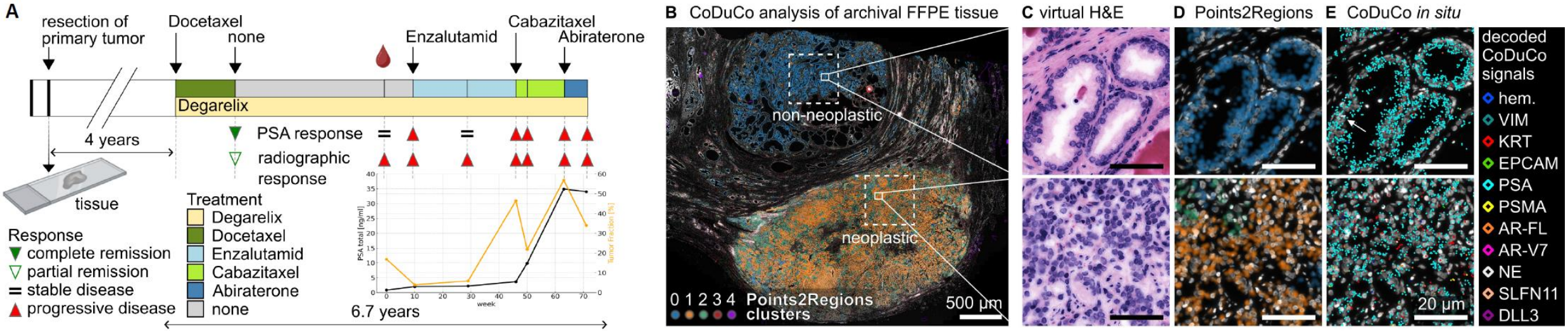


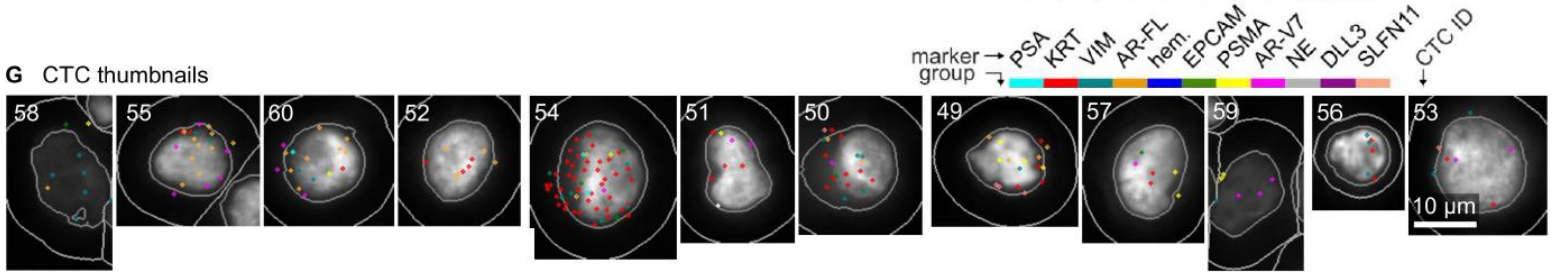


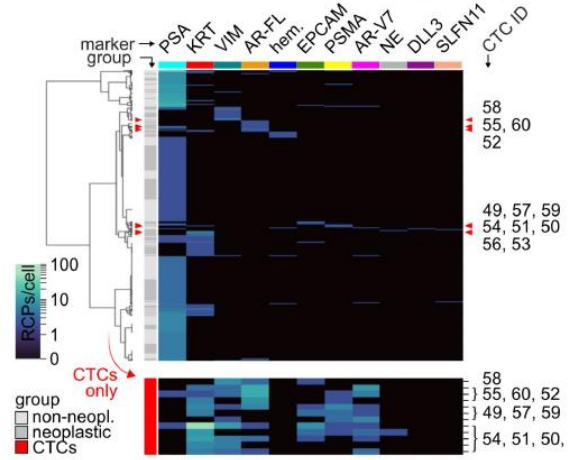


Patient 15 had progression with low PSA levels, indicating neuroendocrine transdifferentiation. CTC analysis revealed neuroendocrine CTCs. **Most important liquid biopsy finding: CTC expression data identified DLL3 and SLFN11 as druggable targets.**

Patient 14



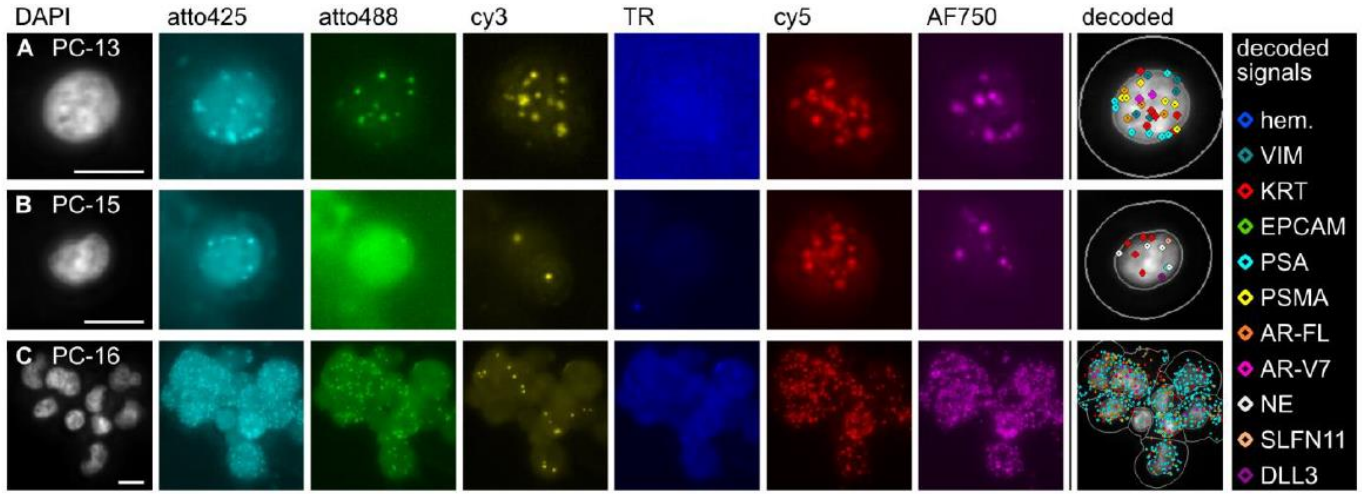


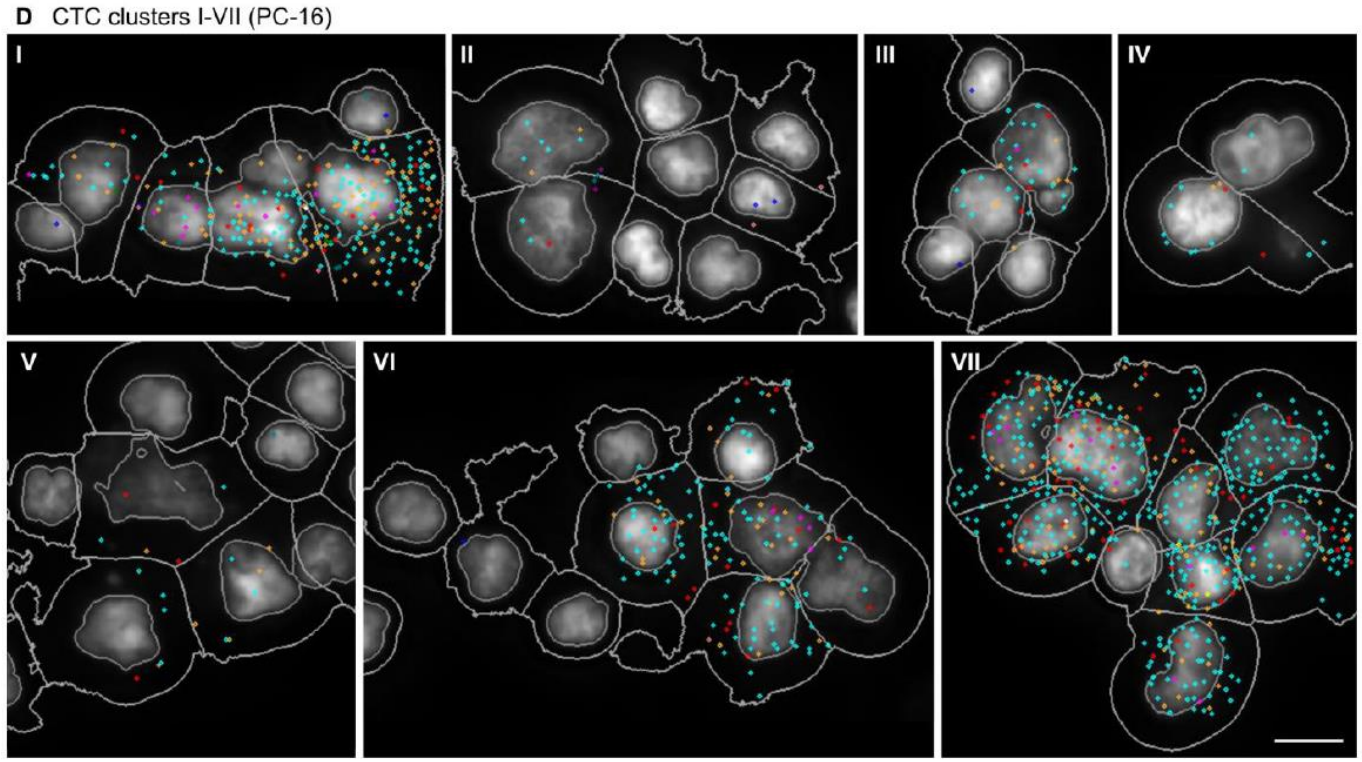


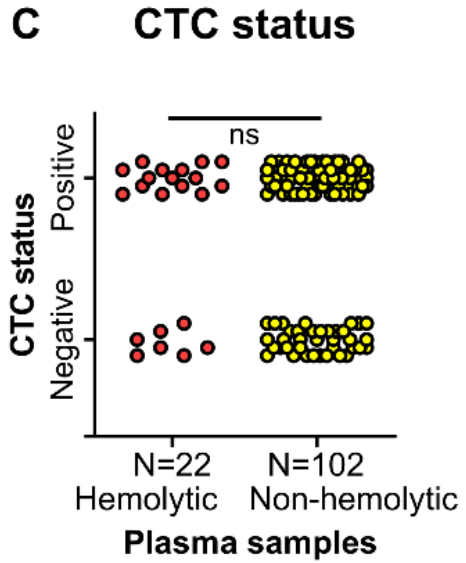
Patient 14 showed resistance to antihormonal therapy with spiking ctDNA levels. Switch to taxan-based chemotherapy led to reduction of ctDNA levels. CTC in situ mRNA analysis revealed resistance mechanisms (high AR-V7 expression) and identified PSMA as druggable target. Spatial tissue analysis revealed tumor evolution under therapeutic pressure and expansion of resistant clones.

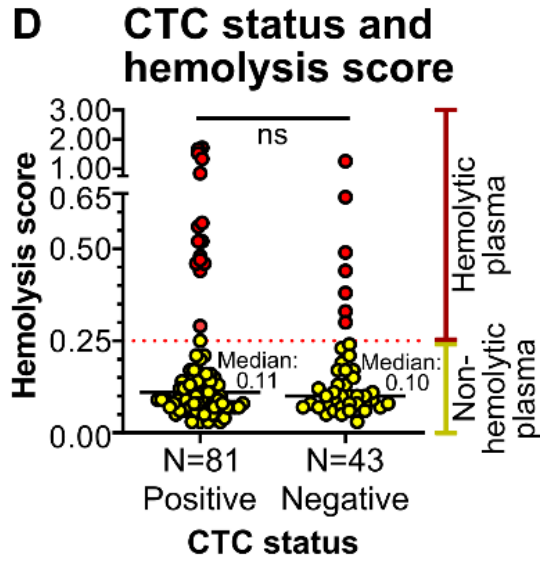
Most important liquid biopsy finding: AR-dependent resistance identified and CTC expression data indicated PSMA as druggable target.

Bonstingl et al., Biomark Res 2024









Even challenging hemolytic patient samples can produce high-quality CTC data

Bonstingl et al, Clin Chem 2024
Bonstingl et al. Biomark Res 2024

amin.elheliebi@medunigraz.at

Want to become a beta tester for in situ CTC / Tissue analysis?

- You want to see how the in situ would work on your cells?
- Become a beta tester

https://forms.gle/MEmyaPvDPe2AeESm9

What do we learn from multi-analyte analysis

Medical University of Graz

Multi-analyte liquid biopsy analysis with ISO and CEN/TS standards enabled us to…

- ✓ **Detect potential druggable target (PSMA, DLL3, SLFN11)**
- ✓ **Detect multiple co-occuring resistance mechanisms (EMT, AR-V7, Neuroendocrine transdifferntiation)**
- ✓ **Track dynamic shifts of ctDNA and CTCs**
- ✓ **High concordance and complementary data of ctDNA and CTCs with clinical data**
- ✓ **Hemolysis does not affect ccfDNA yield, ctDNA tumor fraction, CTC status (positive/negative), if you follow the standards**

Next steps

- ✓ **Search for beta tester for in situ mRNA detection in CTCs and tissue**
- ✓ **Evaluate if resistance mechanisms are concortant with ctDNA, CTC expression patterns and clinical data (paper follows soon)**

https://forms.gle/MEmyaPvDPe2AeESm9

https://forms.gle/MEmyaPvDPe2AeESm9

Medical University of Graz

Research Group El-Heliebi + Friends

CBmed BIOMARKER RESEARCH ENABLING APPLIED PRECISION MEDICINE

EVIDENT

UNIV. COMPREHENSIVE CANCER CENTER Krebszentrum GRAZ

SciLifeLab

QIAGEN

CytoGen LIQUID BIOPSY FRONTIER

GILUPi MEDICINE. PERSONALIZED.

VIENNA LAB ESTABLISHED INNOVATIONS IN DIAGNOSTICS

Karolinska Institutet Science Park AB

BIH Berlin Institute of Health @Charité

UNIVERSITY OF TWENTE. HIGH TECH HUMAN TOUCH

BioTechMed GRAZ

amin.elheliebi@medunigraz.at