The role of circulating biomarkers in colorectal cancer

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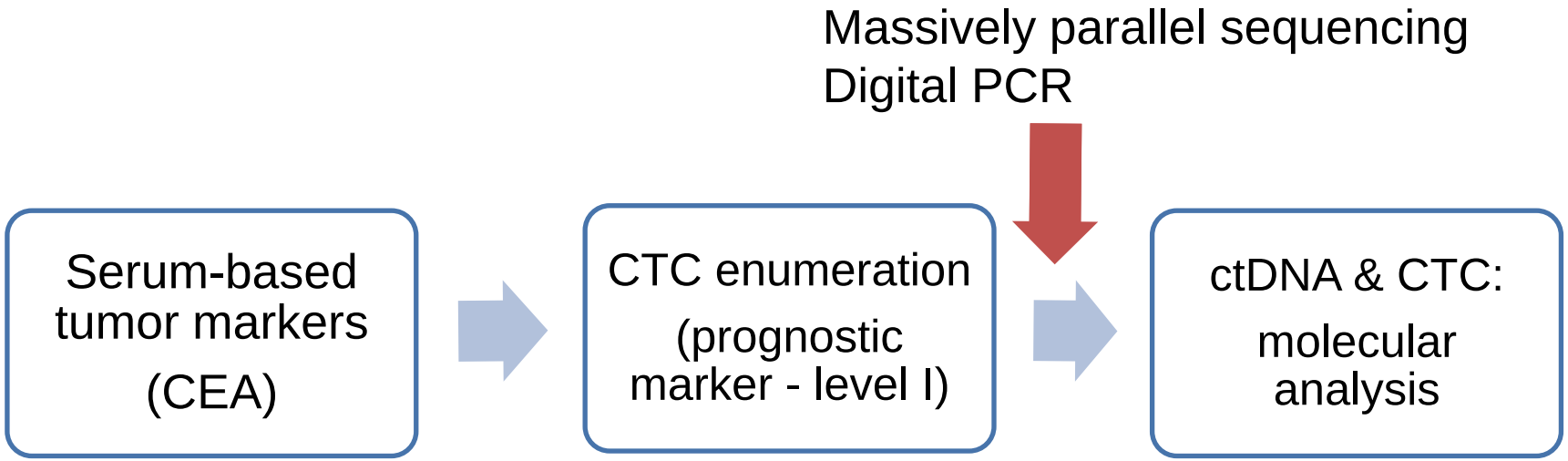
I have no conflicts of interest to declare

Outline

- Circulating biomarkers
- Circulating tumor DNA (ctDNA)
- Circulating tumor cells (CTCs)
- Challenges: intra-tumor genetic heterogeneity

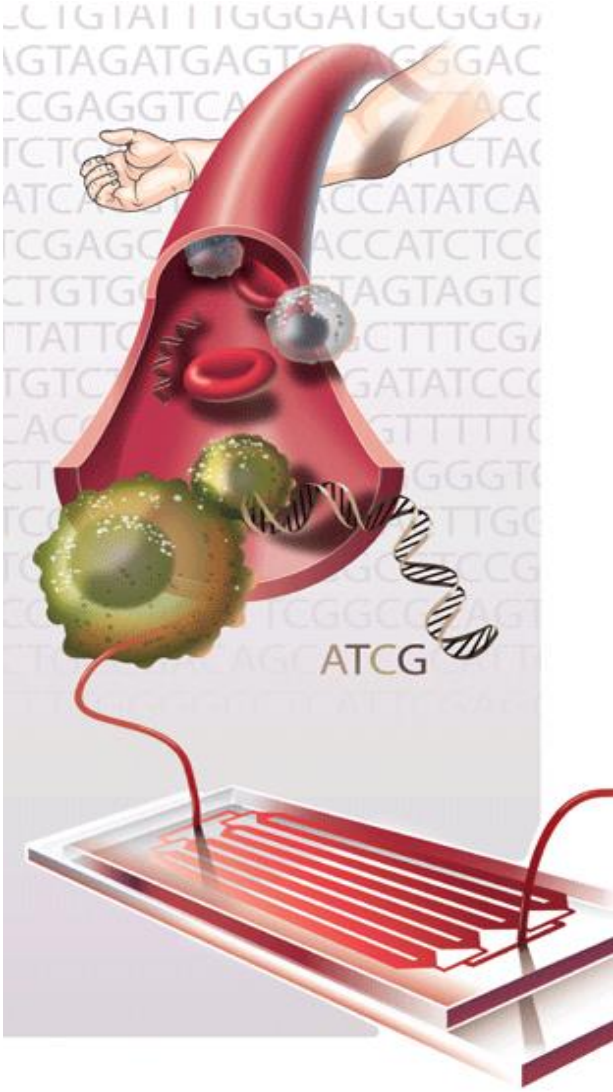
Circulating biomarkers

Circulating biomarkers



Potential uses of circulating biomarkers

- Prognosis
- Prediction of targeted therapy response
- Monitoring (minimal residual) disease
- Tracking secondary (‘acquired’) resistance
- Assessing intra-tumor heterogeneity

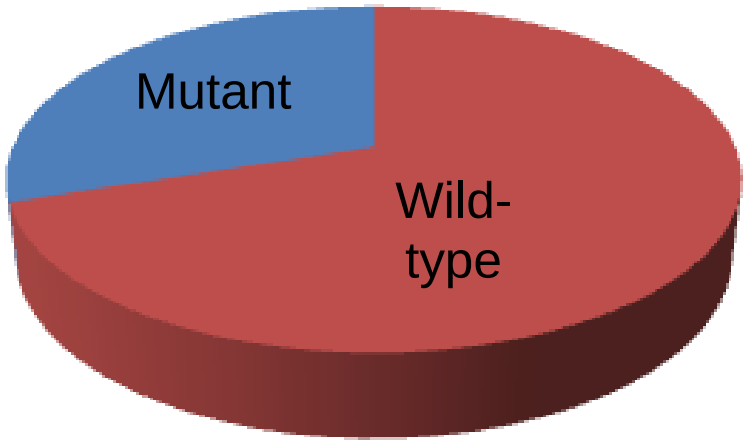


Bidard et al, Sci Trans Med 2013

Circulating tumor DNA

Circulating DNA

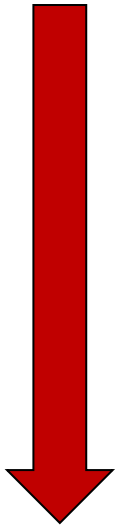
- Fragmented DNA (140 - 170 base pairs) in plasma or serum
- Sources of DNA release: necrosis, apoptosis.
- Not cancer specific
  - Exercises, trauma, surgery



Cancer: small % of circulating DNA is tumor-derived

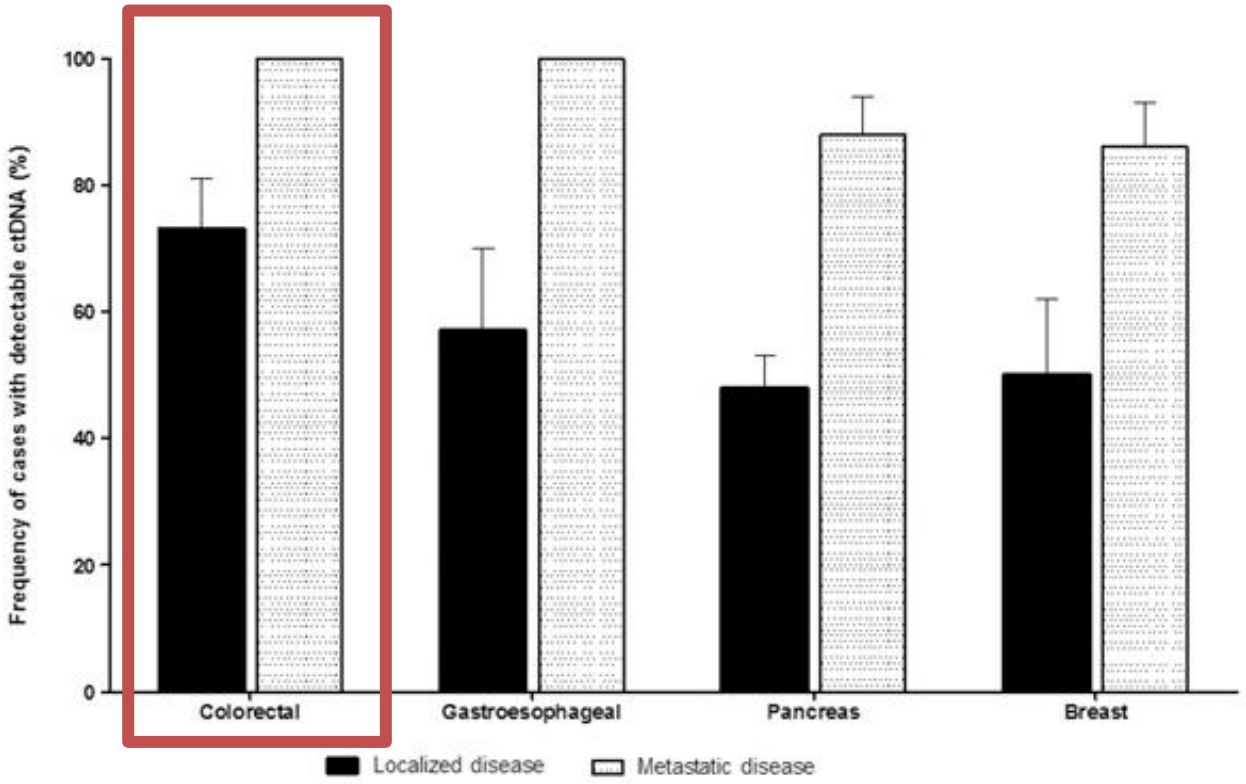
Circulating DNA: methods

Technique	Sensitivity
Sanger sequencing	>10%
Massively Parallel Sequencing	1 - 2%
Quantitative PCR	1%
BEAMing / Digital PCR	< 0.01%



Circulating tumor DNA in CRC

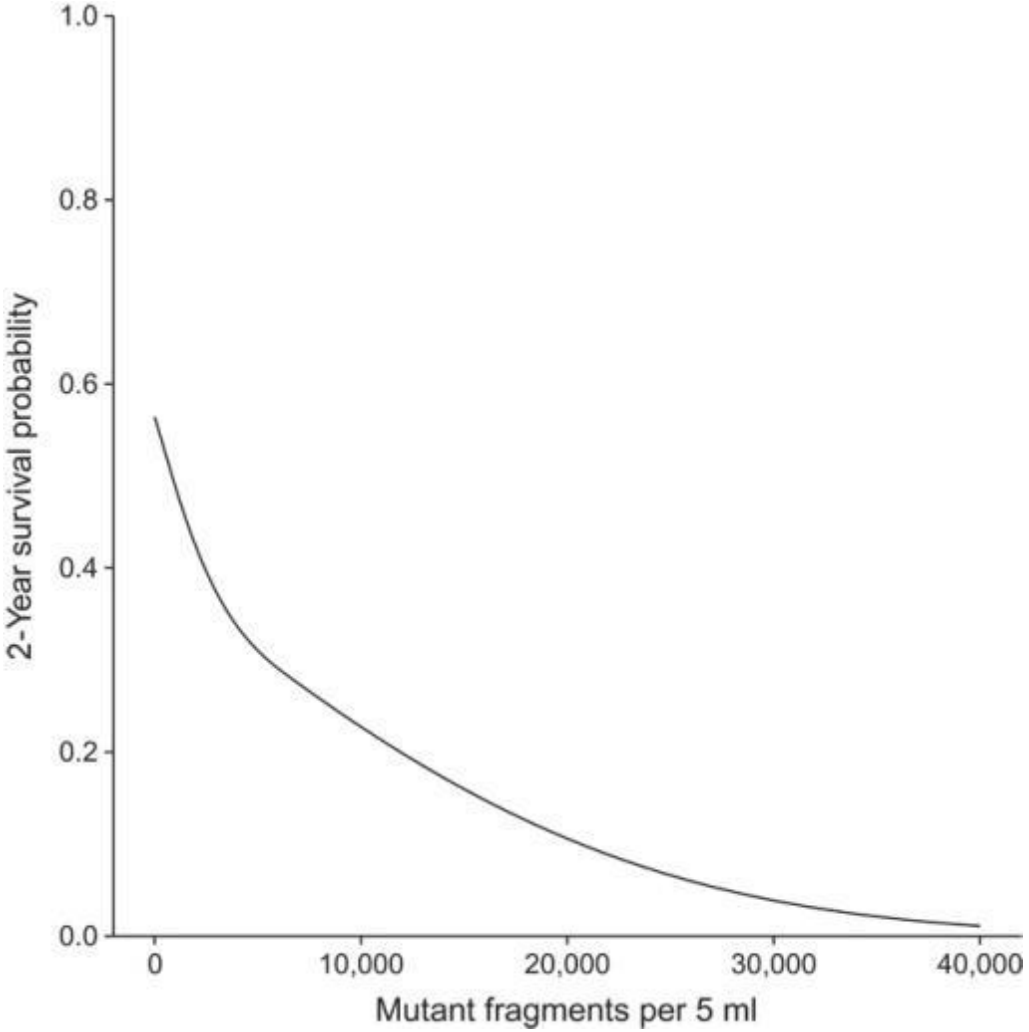
100% Metastatic CRC / 73% localized CRC



Bettegowda et al., Sci Transl Med 2014

Prognosis

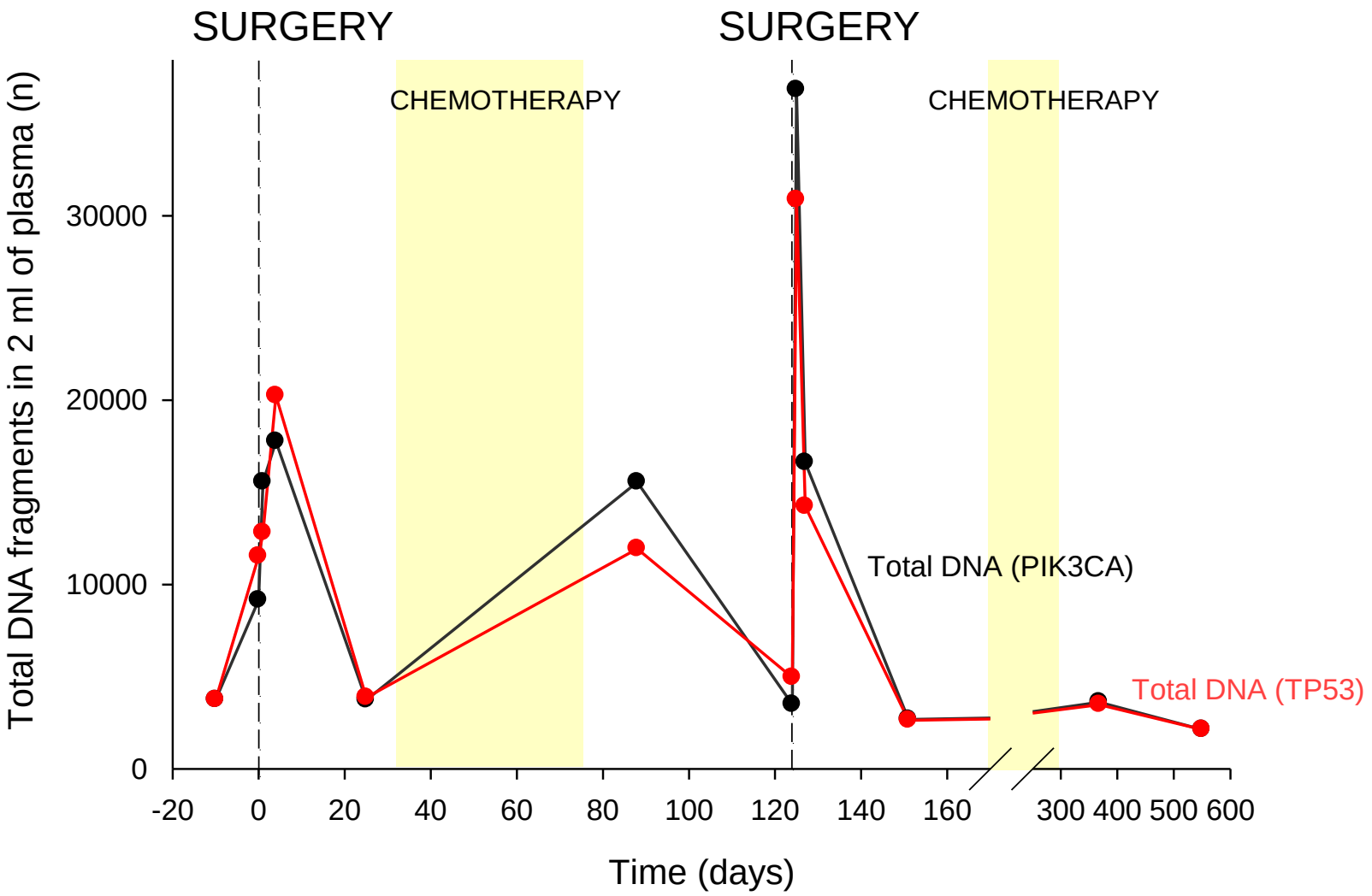
Metastatic CRC patients with higher levels of ctDNA have a worse prognosis



Bettegowda et al., Sci Transl Med 2014

Monitoring disease

Monitoring disease

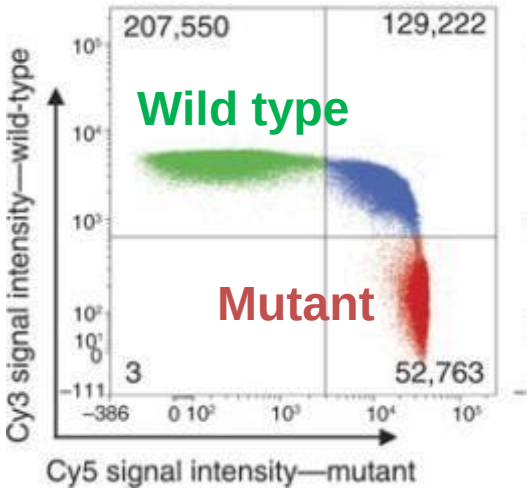


Diehl et al Nature Medicine, 2008
Courtesy Dr. Luis Diaz

Monitoring disease

BEAMing

Before Surgery
Day 0



13.4 %

% Mutant APC

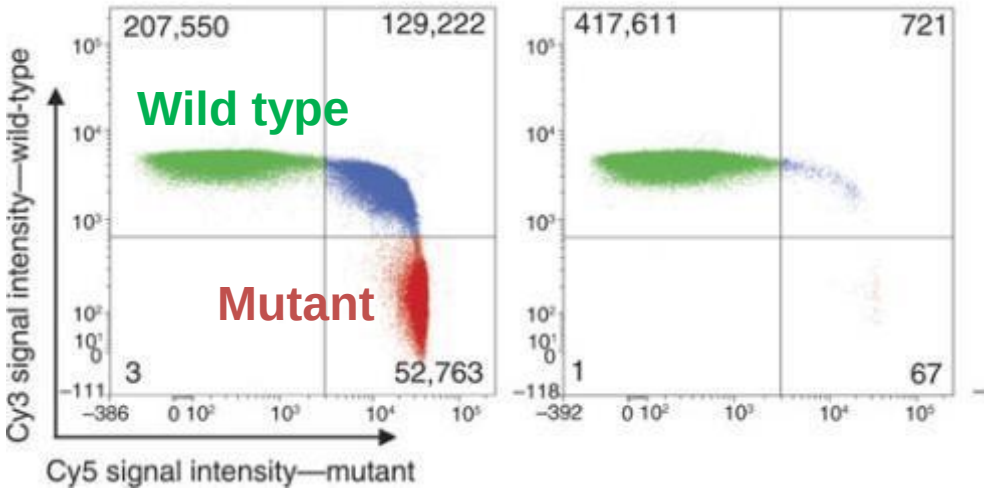
Diehl et al Nature Medicine, 2008

Monitoring disease

BEAMing

Before Surgery
Day 0

After Surgery
Day 1



13.4 %

0.015 %

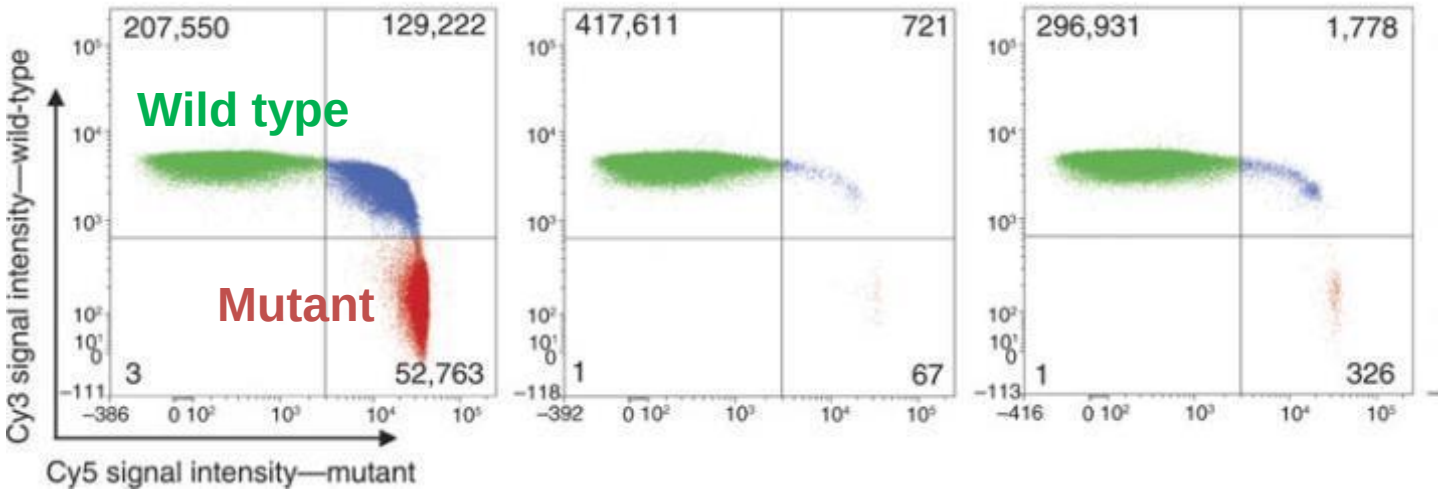
% Mutant APC

Diehl et al Nature Medicine, 2008

Monitoring disease

BEAMing

Before Surgery Day 0	After Surgery Day 1	CT scan negative After Surgery Day 42



13.4 %	0.015 %	0.11 %

% Mutant APC

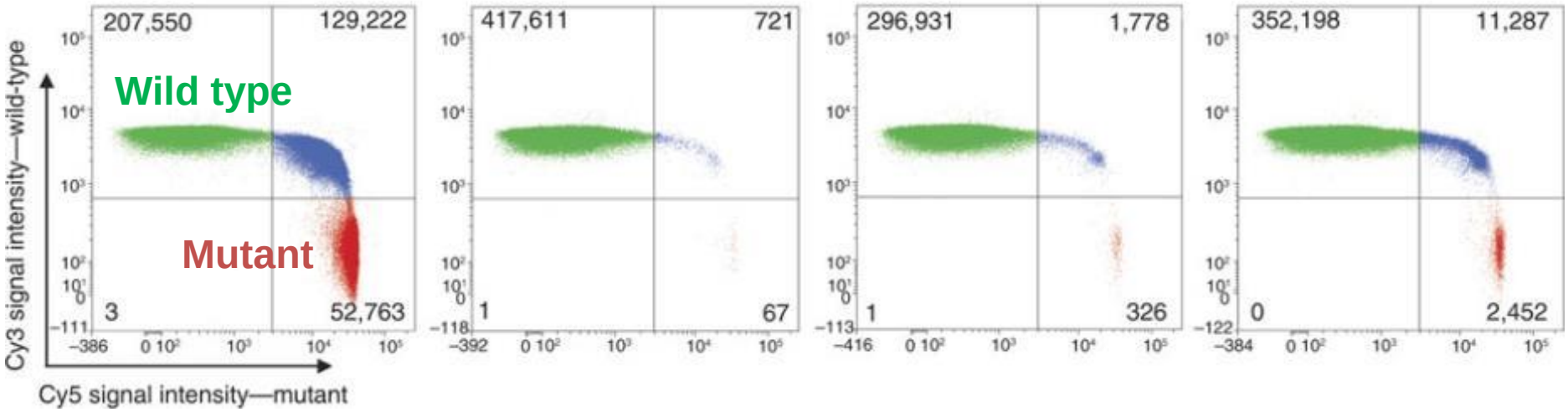
Diehl et al Nature Medicine, 2008

jezra@jezrakaye.com

Monitoring disease

BEAMing

Before Surgery Day 0	After Surgery Day 1	CT scan negative After Surgery Day 42	CT scan **positive** After Surgery Day 244



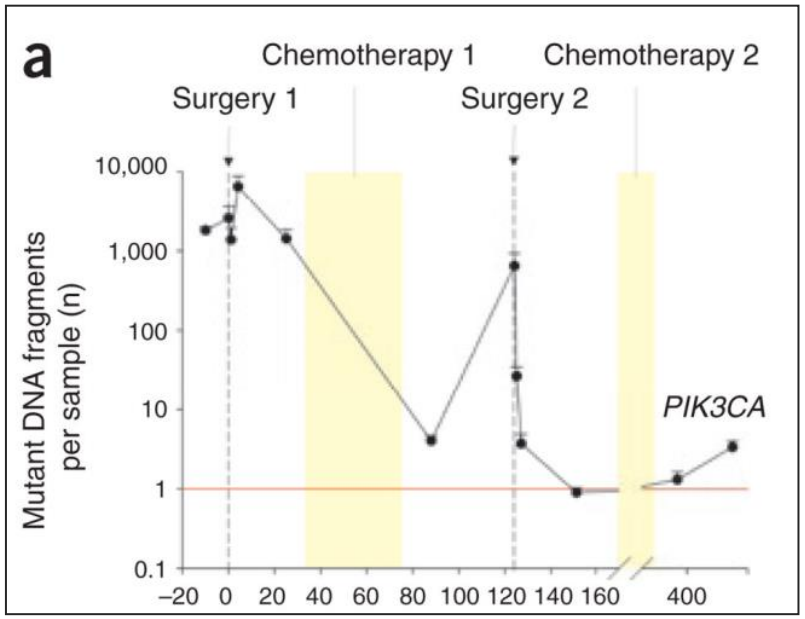
13.4 %	0.015 %	0.11 %	0.66 %

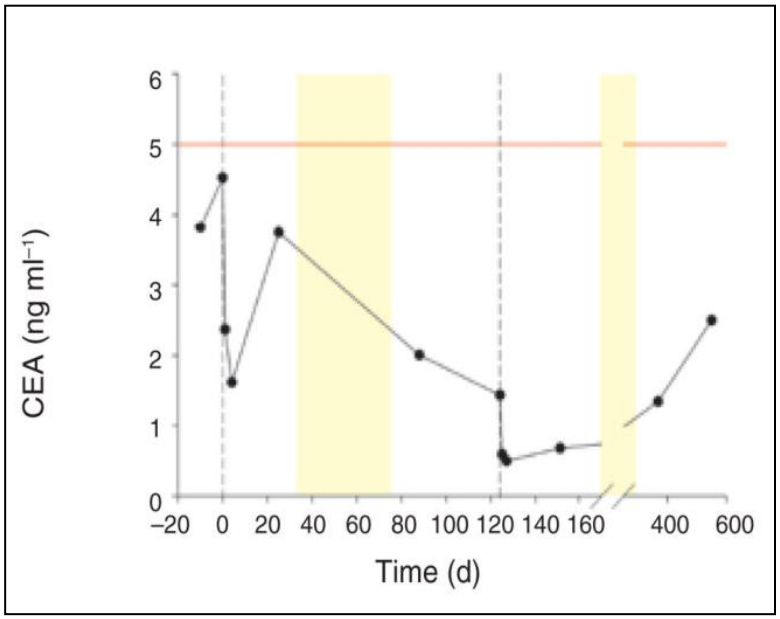
% Mutant APC

Diehl et al Nature Medicine, 2008

Monitoring disease

ctDNA : reliable tool to monitor metastatic CRC dynamics





Diehl et al Nature Medicine, 2008

Tracking secondary (‘acquired’) resistance

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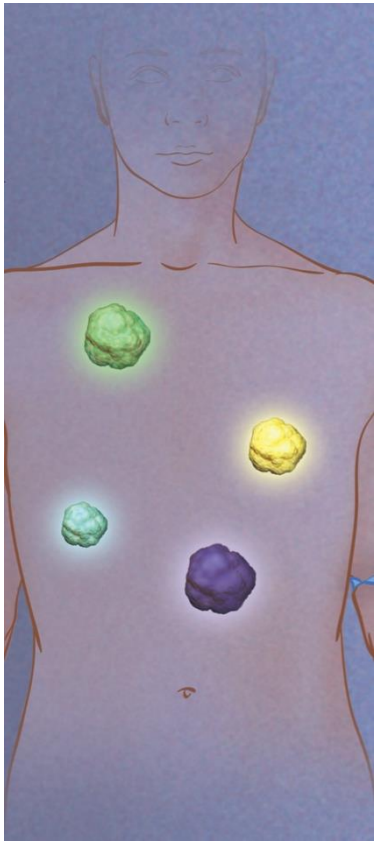
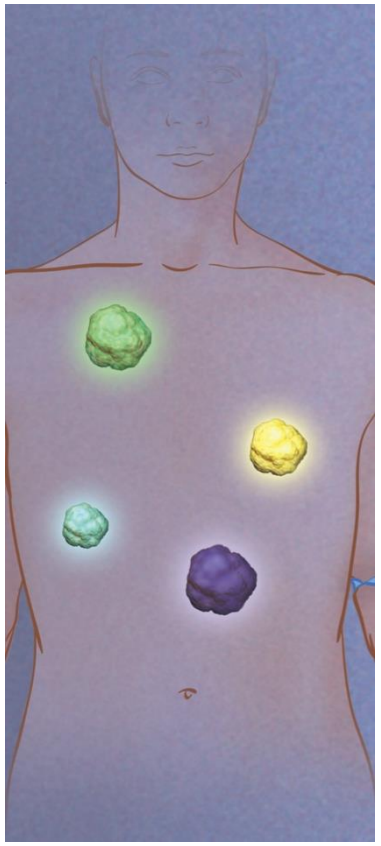


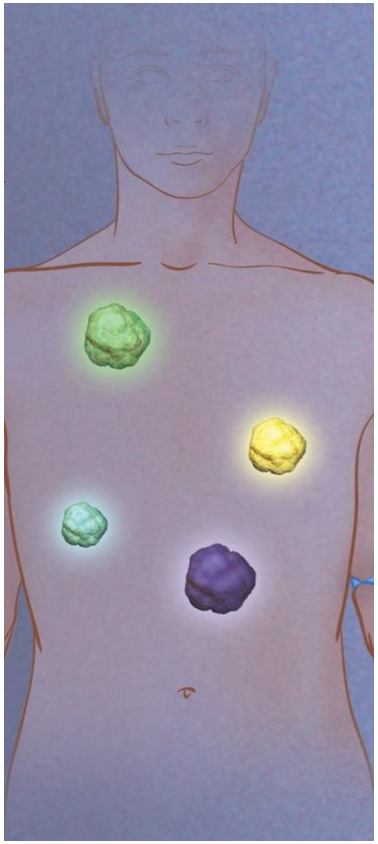
***KRAS* WT**

EGFR BLOCKADE



Courtesy Dr. Luis Diaz

Tracking secondary (‘acquired’) resistance



***KRAS* WT**

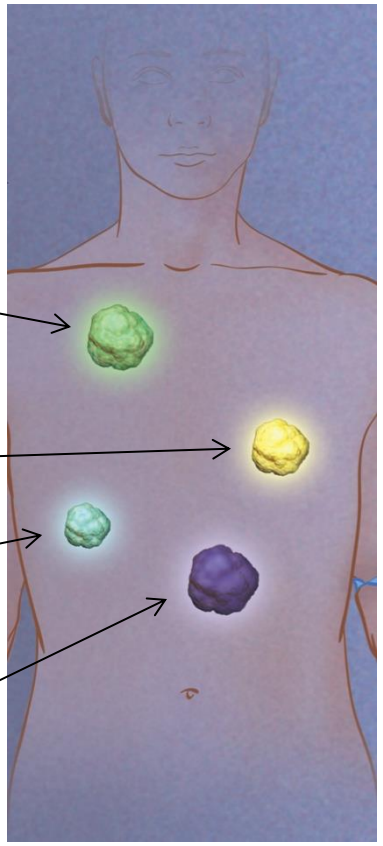
EGFR BLOCKADE

KRAS mutant

NRAS mutant

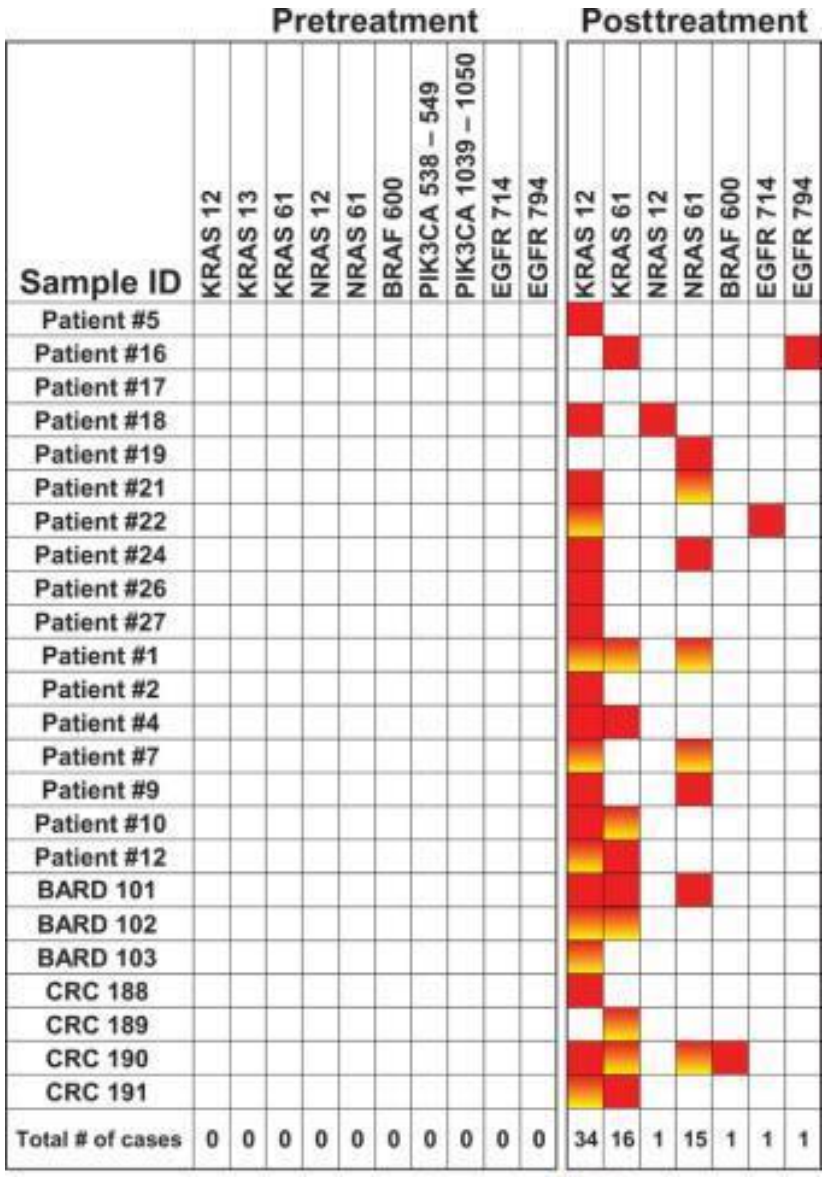
MET amplified

EGFR mutant



Courtesy Dr. Luis Diaz

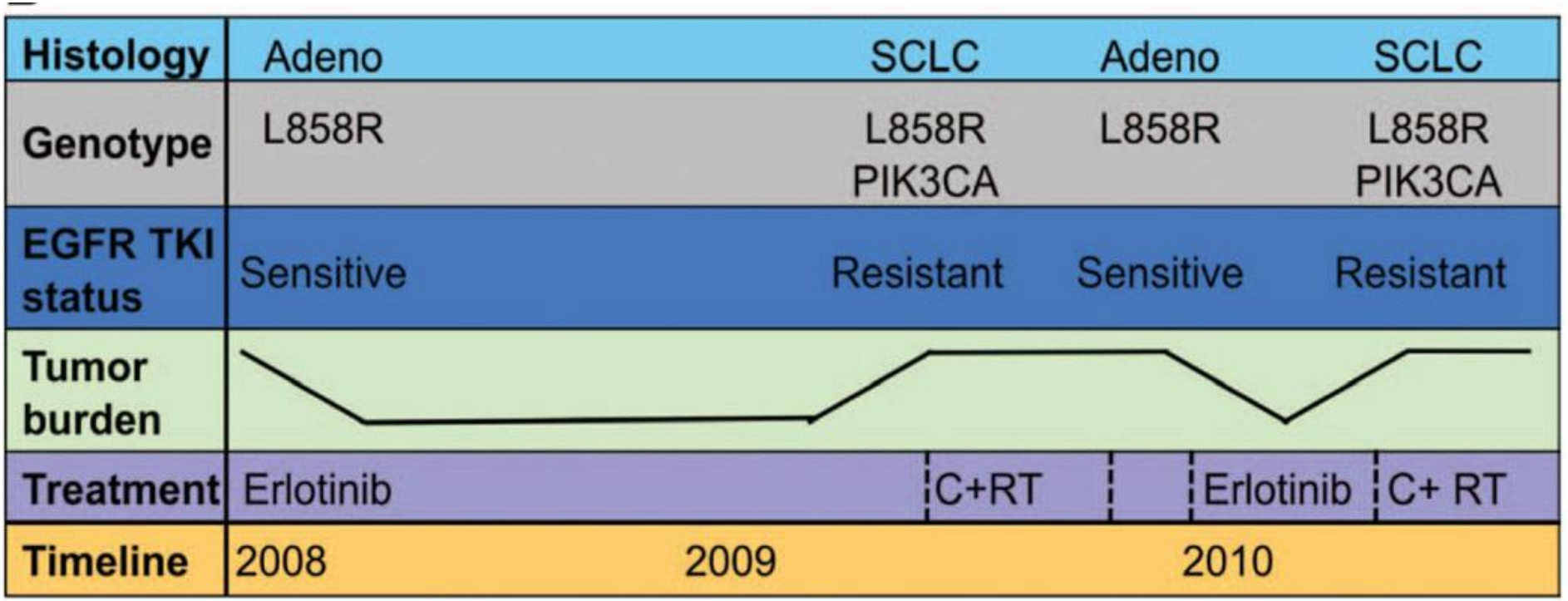
Tracking secondary (‘acquired’) resistance



Acquired resistance mutations to EGFR blockade identified ctDNA from patients with KRAS wild-type metastatic CRC.

Bettegowda et al., Sci Transl Med 2014

Tracking secondary (‘acquired’) resistance



ctDNA may identify “targetable” mechanisms of resistance

Sequist et al., Sci Transl Med 2011

Take home message

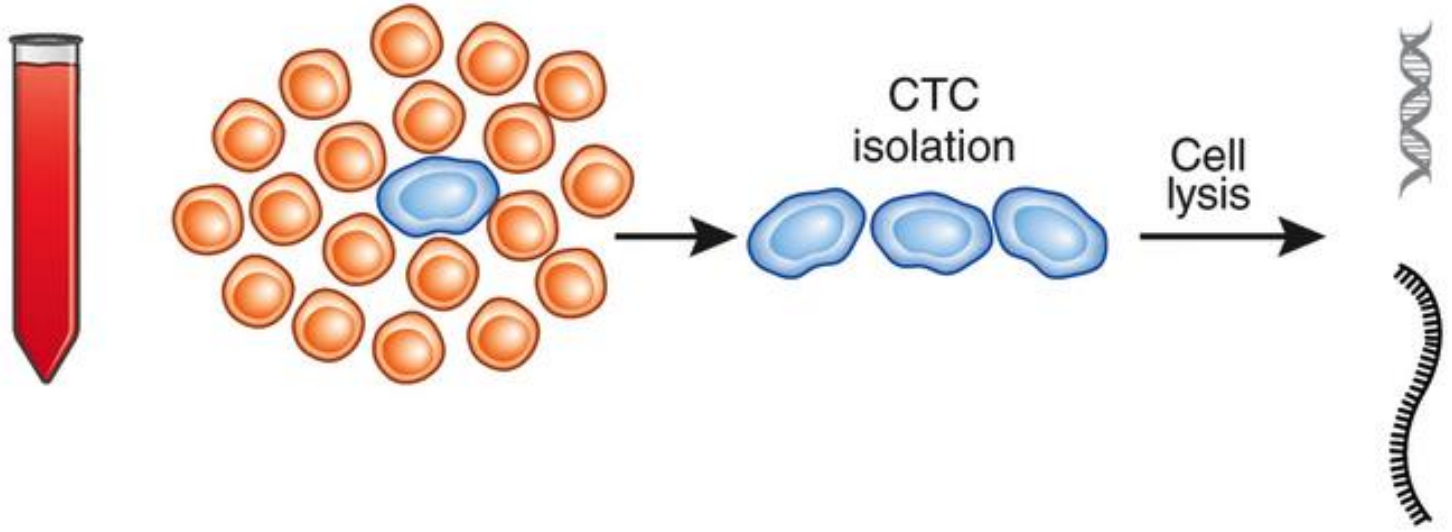
- Different sensitivity methods for capturing mutations in ctDNA
- ctDNA is a sensitive and specific biomarker that may be useful for longitudinally monitoring of CRC cancer
- ctDNA levels:
  - associated with tumor burden
  - may predict survival
  - identify potential mechanisms of resistance

Circulating tumor cells

Circulating tumor cells

- Rare cancer cells in the peripheral blood
- Role in the process of metastasis
- Lack of technologies capable to isolate CTCs in sufficient numbers
- No known universal marker:

    - Epithelial cellular adhesion molecule (EpCAM): most widely used



Speicher & Pantel. Nature Biotech 2014

Circulating tumor cells: platforms

EpCAM-affinity based
- CellSearch® system
- AdnaTest BreastCancerDetect
- CTC-Chip
- Dynal®
- MACS®
- MagSweeper
- On-Q-Ity
- CTC-ETI

Physical properties-based
- ISET
- ScreenCell®
- ApoStream™
- Density Gradient Centrifugation

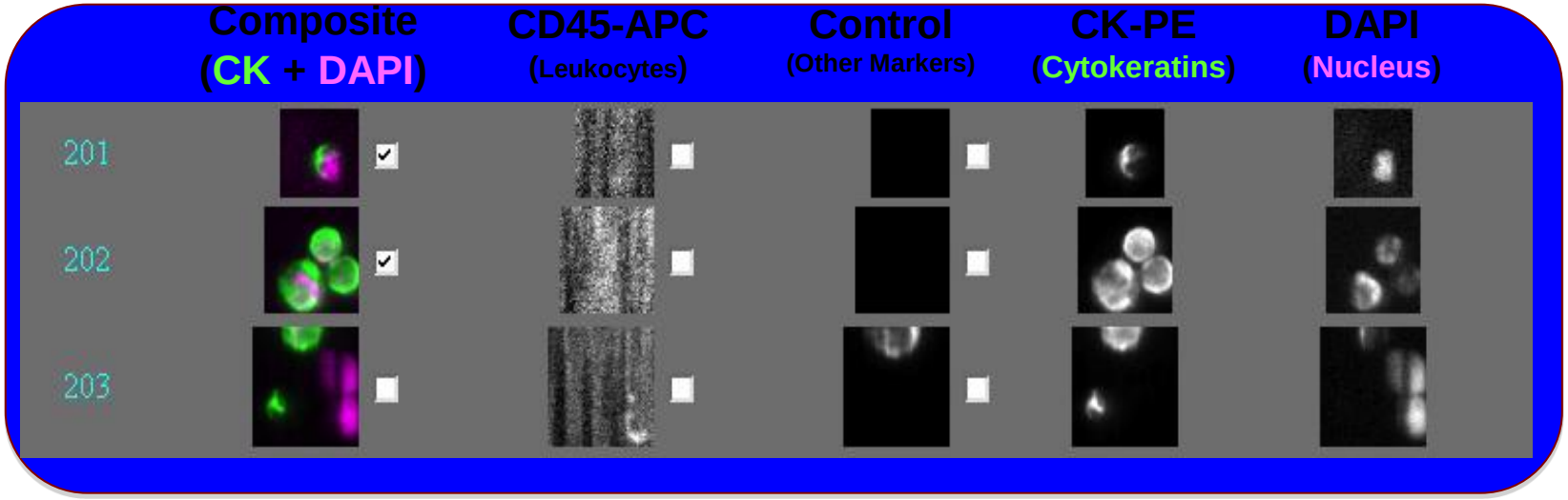
Other methods
- FAST
- EPISPOT
- Flow cytometry (FACS)
- PRO Onc Assay

De Mattos-Arruda, L. et al. Nat. Rev. Clin. Oncol.2013

Circulating tumor cells: platforms

CellSearch System

- Only FDA-approved technique for CTC enumeration
- Isolates CTCs by immunomagnetic capture (EpCAM)
- Confirms CTCs by immunofluorescent microscopy (Cytokeratin, DAPI, and CD45).



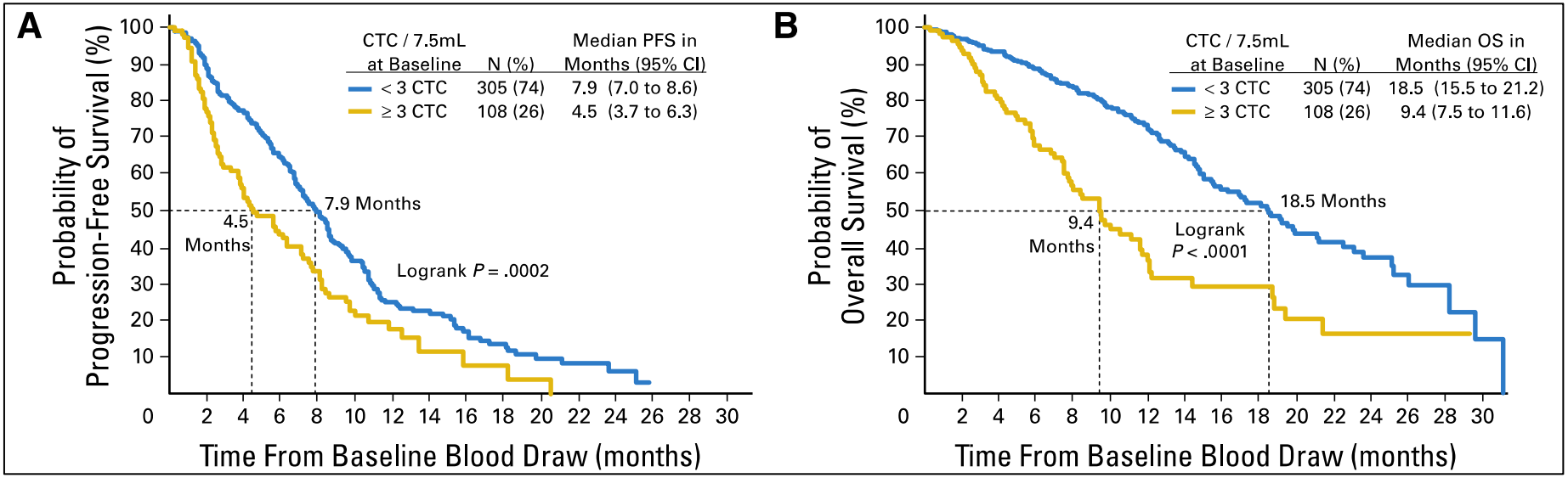
Prognosis

Prognostic role of CTCs in metastatic CRC

- 30-40% metastatic CRC patients have detectable CTCs (CellSearch)
- More CTCs are detected in the mesenteric / portal venous blood vs peripheral blood
- Detection of CTCs is associated with poor prognosis

Rahbari NN et al. Ann Surg Oncol 2012
Cohen et al. J Clin Oncol 2008
Cohen et al. Ann Oncol 2009

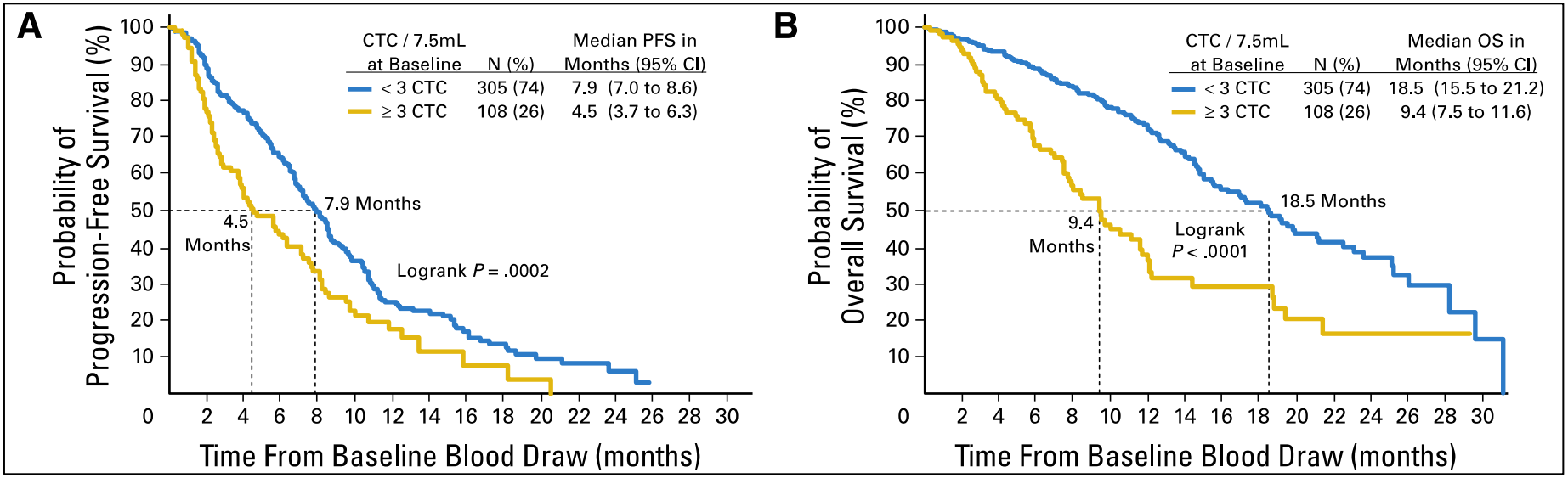
Metastatic CRC patients with higher CTC counts have a worse prognosis



Baseline before treatment	
PFS	OS
✓	✓

Cohen et al. J Clin Oncol 2008

Metastatic CRC patients with higher CTC counts have a worse prognosis



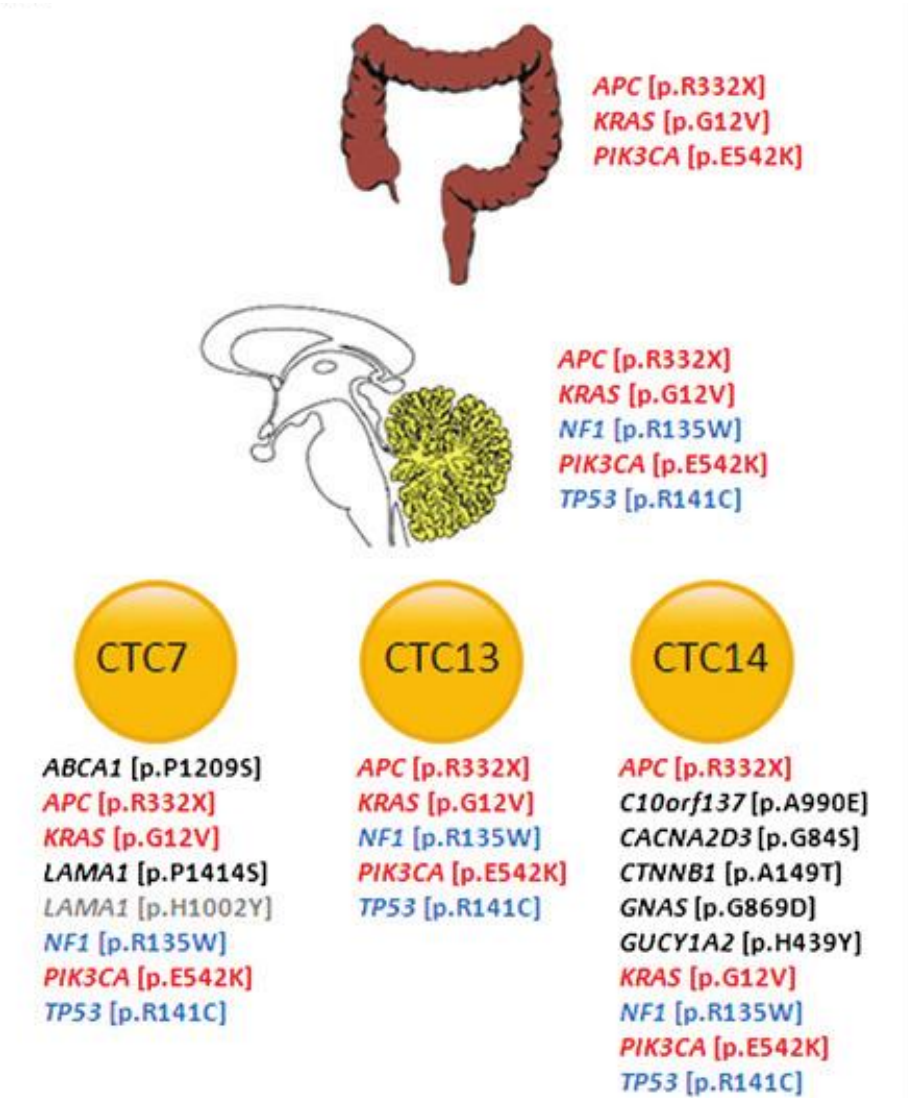
Baseline before treatment	
PFS	OS
✓	✓

Changes during treatment	
PFS	OS
✓	✓

Cohen et al. J Clin Oncol 2008

Characterization of CTCs

Characterization of CTCs



CTCs are heterogeneous

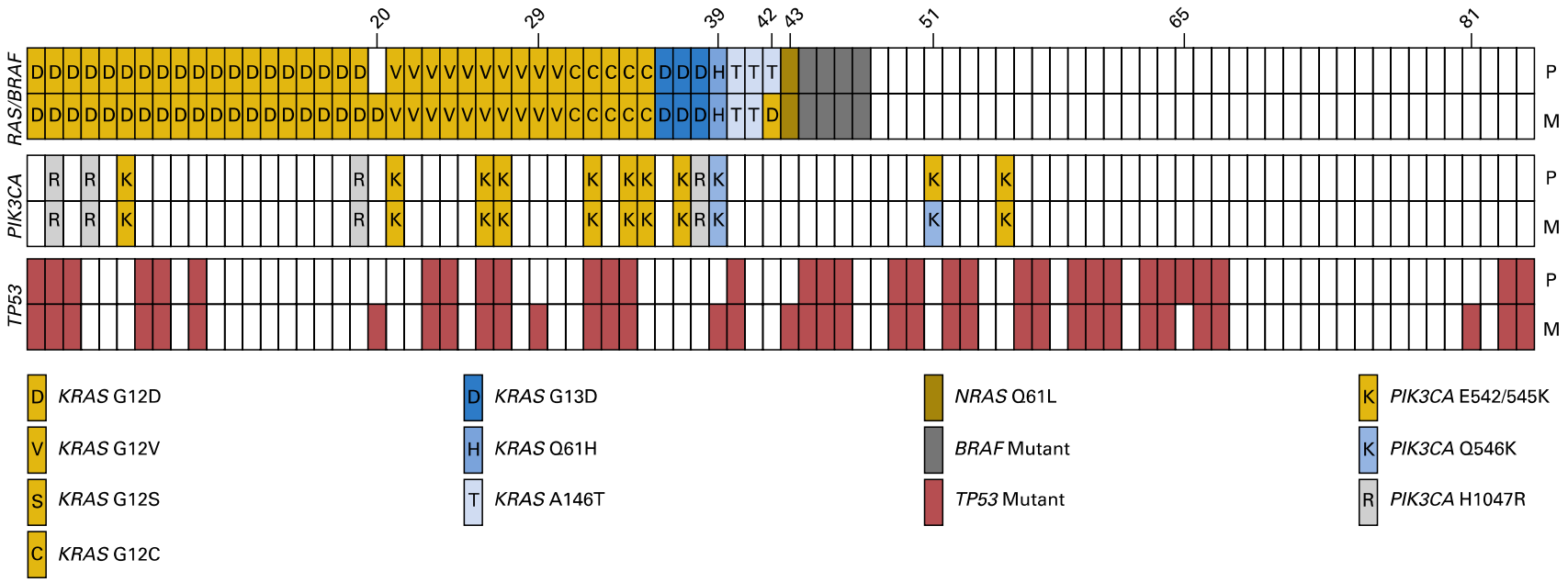
Heitzer E et al. Cancer Res 2013

Take home message: CTCs

- Detection approaches usually based on epithelial markers
- CTCs enumeration: Prognostic in metastatic CRC (level I evidence)
- CTC characterization: single-cell promising

Intra-tumor heterogeneity

Intra-tumor heterogeneity and the potential role of circulating biomarkers



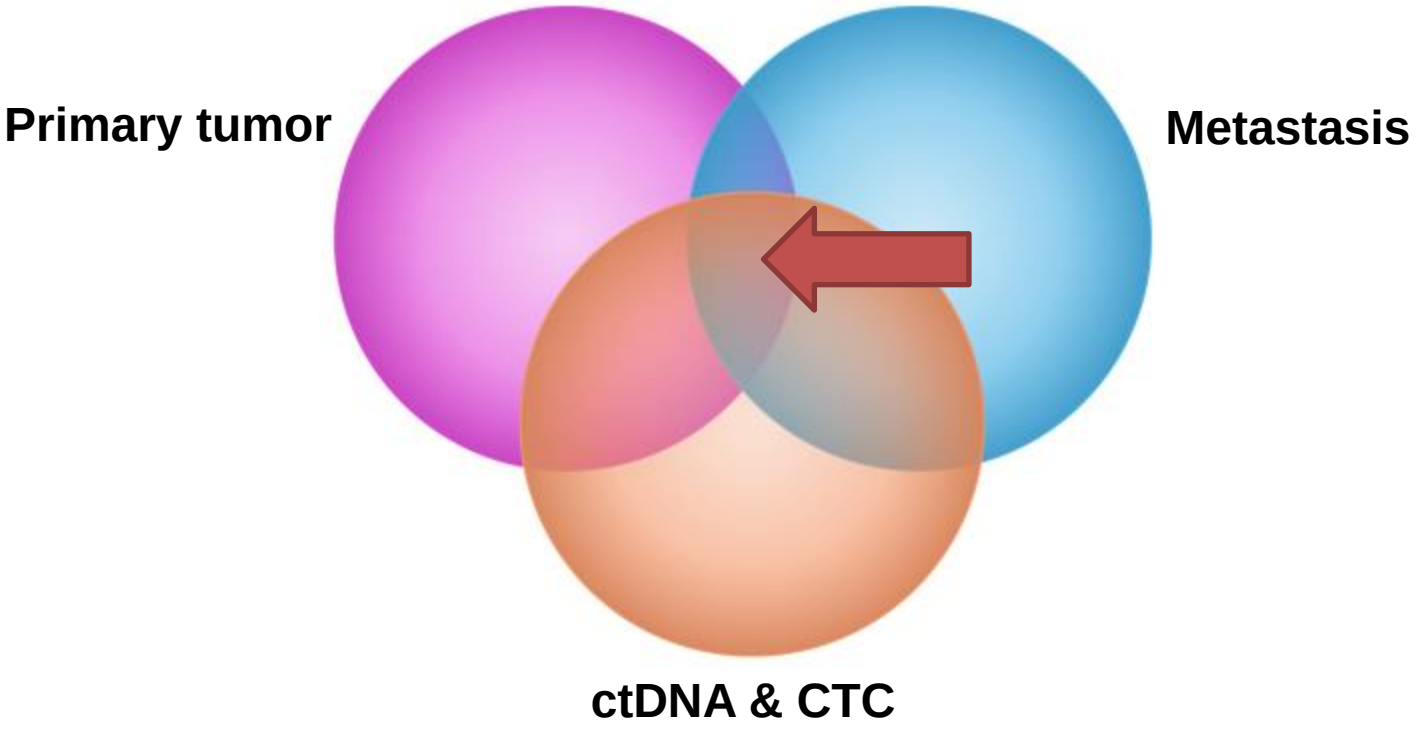
Primary tumor vs. Metastasis:

- High concordance *KRAS* and *BRAF*

-Discordances vary by gene, site of metastasis and prior treatment.

Vakiani et al. J Clin Oncol 2012
Kopetz et al. 2014 ASCO Meeting

Intra-tumor heterogeneity and the potential role of circulating biomarkers



Circulating biomarkers may capture tumor heterogeneity

De Mattos-Arruda et al. Annals Oncol *in press*

Conclusion

ctDNA and CTCs are potential valuable resources for personalized cancer therapy in CRC

- Prognosis
- Prediction of targeted therapy response
- Monitoring disease
- Tracking secondary (‘acquired’) resistance
- Assessing intra-tumor heterogeneity

Acknowledgements

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