La biopsia liquida nel carcinoma mammario

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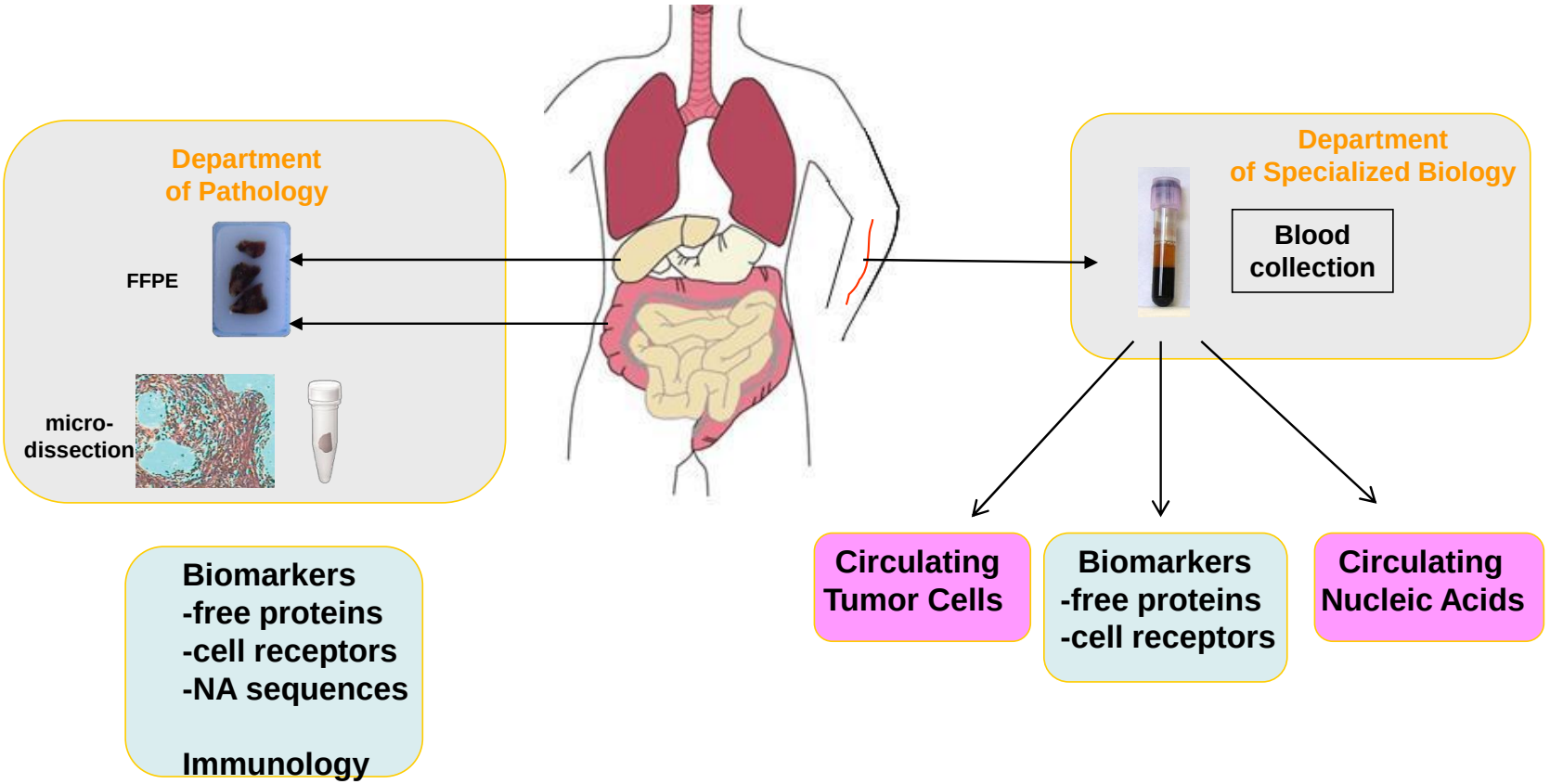
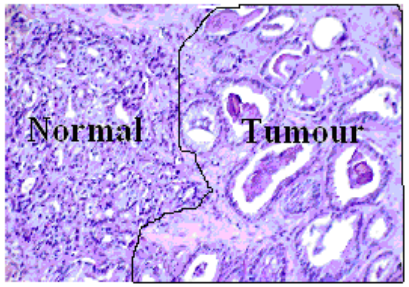
Biomarkers for solid tumor



Tissue biopsy versus liquid biopsy

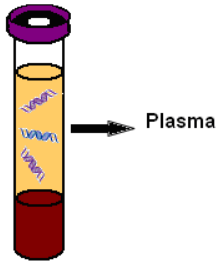
Tissue biopsy

- Cancer is a heterogeneous disease
  - Molecular properties differ within a tumor
  - Primary tumor biopsy may not reflect current disease
  - Therapy causes changes in tumor cells
- Biopsy is invasive
  - May not be feasible based on patient condition or tumor accessibility
  - Impractical for periodic monitoring for recurrence/PD
- Biopsy tissue is limited
  - Greater demand due to molecular profiling
  - Surgery is costly
- Technical considerations
  - Mixture of normal and tumour DNA
  - Long time to process by histopathologists
  - Macrodissected to enrich tumour content
- Some patients have no tumour sample available
- The sample represents the tumour at one fixed time point



Liquid biopsy

- CTC/ctDNA shed directly from tumour
- Allows early disease detection
- Allows evaluation of metastasis in real-time and monitoring the actual treatment response
- Non-invasive blood test
- Enables assessment of tumor heterogeneity and monitoring of tumor dynamics
- Is much faster than classical biopsy testing
- Can be cheaper than classical biopsy testing
- Serial samples can be taken at various time points during the patient's treatment

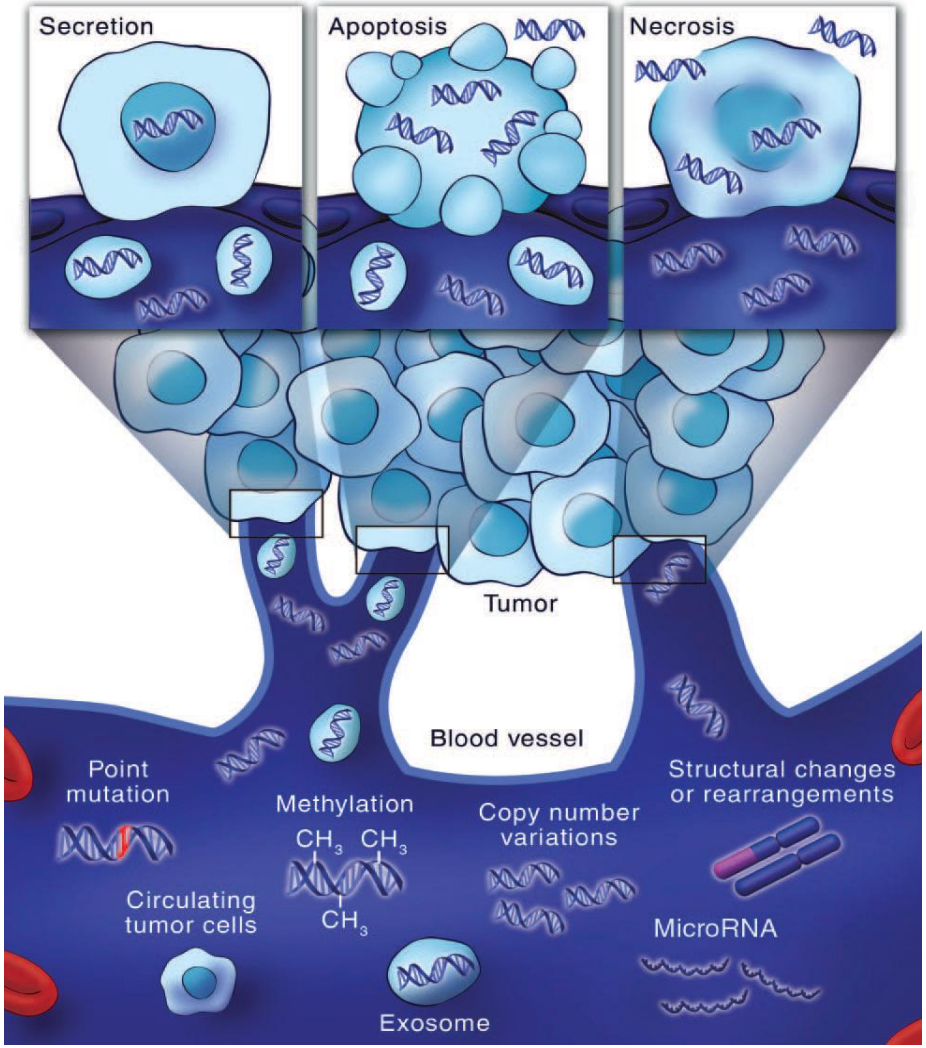


CTC analyses versus ctDNA analyses

	CTC	ctDNA
Equipement	Dedicated instrumentaction needed	None
Isolation	Complex CTC isolation and single cell transfer for further processing	Not required; standard preparation of plasma DNA
WGA required for DNA analysis	Yes	No
Information on heterogeneity or clonality	Yes, if enough CTCs are captured and successfully analyzed	No, results represent an average from cells shedding ctDNA
Dependance on EpCAM markers	Yes for most CTC capture systems. EpCAM free systems exist but await validation in clinical studies	Independent of any marker
Applicability for diagnostic or monitoring purpose	Established for CTC enumeration; advancements will depend on improvements in CTC capturing, analysis tolls and associated costs	Needs to be determined in clinical studies.

Modified from Heitzer et al . Genome Med 2013

Circulating tumor DNA (ctDNA)

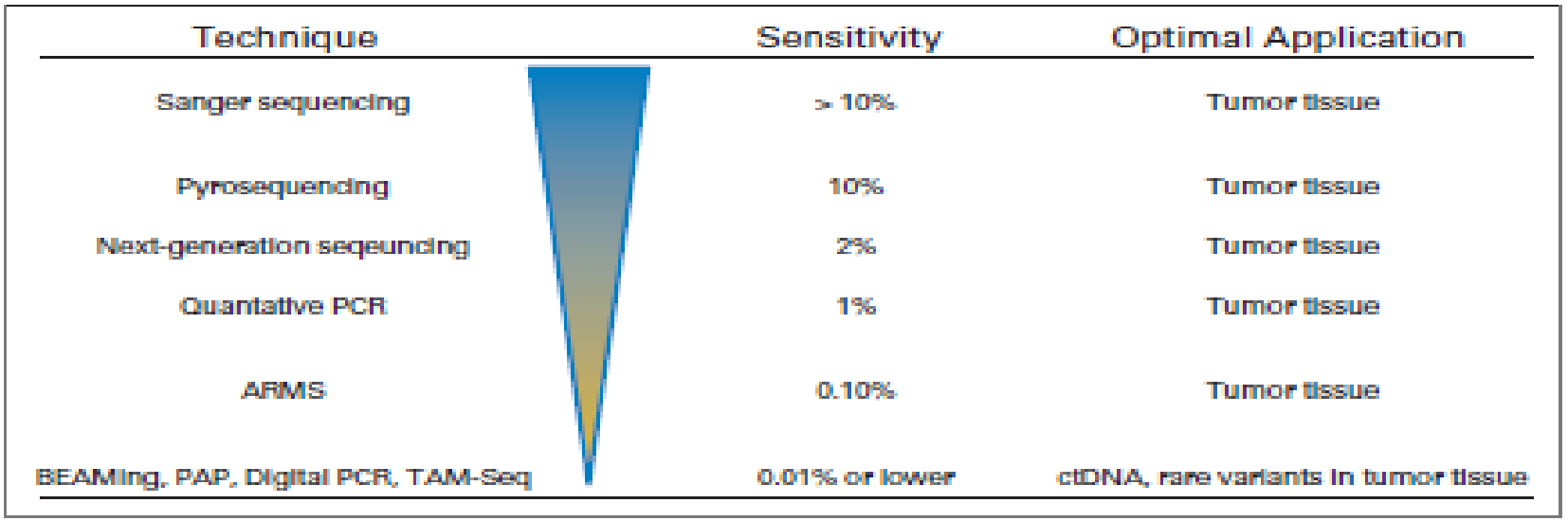


- ctDNA is tumour DNA that has been shed into the bloodstream
- ctDNA can be present in 0.01% - >90% of the total Cell Free DNA (cfDNA)
- The amount of ctDNA is related to the tumour burden and varies between patients with different clinical presentations
- ctDNA has a very short half life ranging from 15 minutes to several hours
- It is stable in plasma at – 80 ° C
- Preservative tubes can be used to stabilise the cfDNA in blood for up to 14 days at room temperature.

Diaz and Bardelli, JCO 2014

Caveats with ctDNA

- Due to the unstable nature of ctDNA the sample is has to be collected and processed correctly
- Only get 30 ng of cfDNA per 5ml plasma extraction
- Difficult to discriminate ctDNA from normal cfDNA
- The technique used must be sensitive enough to pick up the low level variants



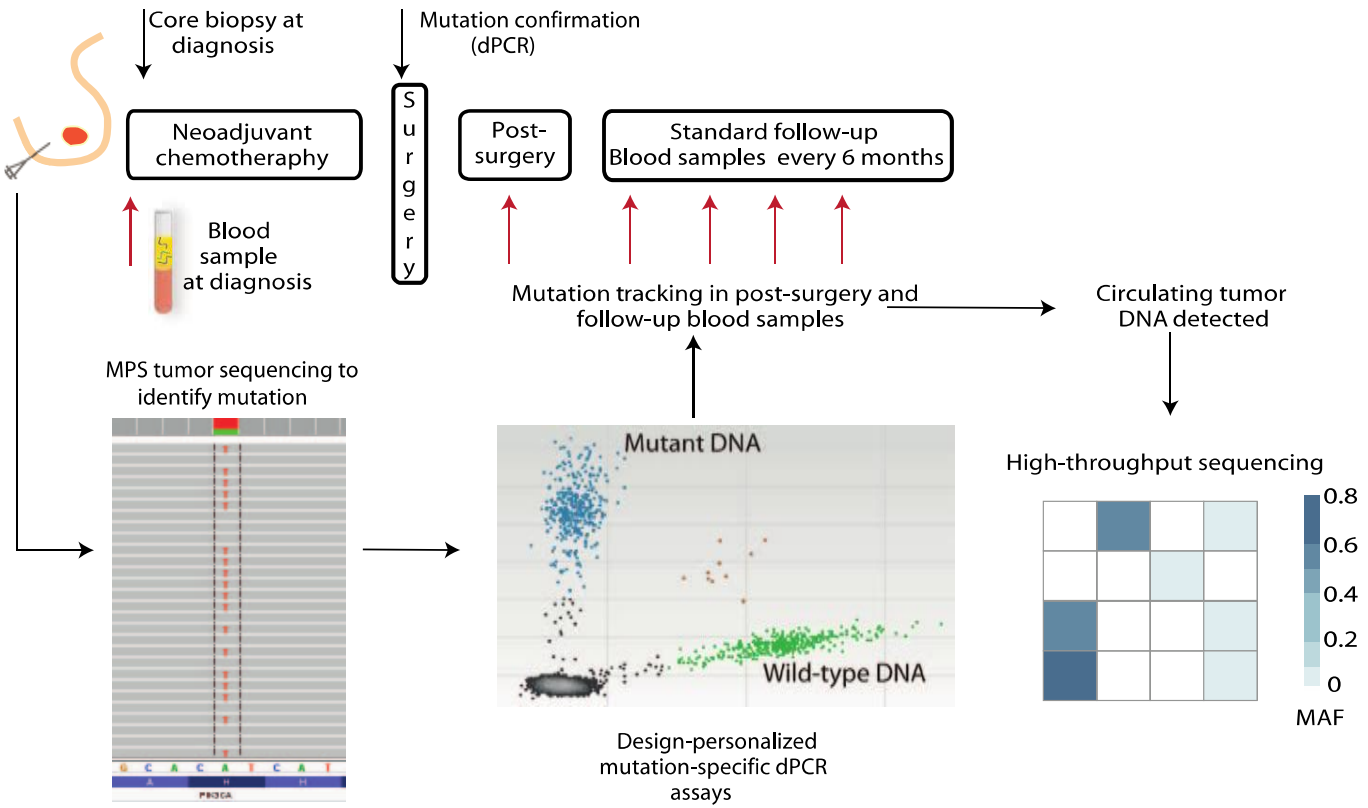
Diaz and Bardelli, JCO 2014

Clinical application of liquid biopsy

1. Prediction of relapse in early BC disease

1. Biology of metastasis (ESR1 mutation/HER2 amplification)

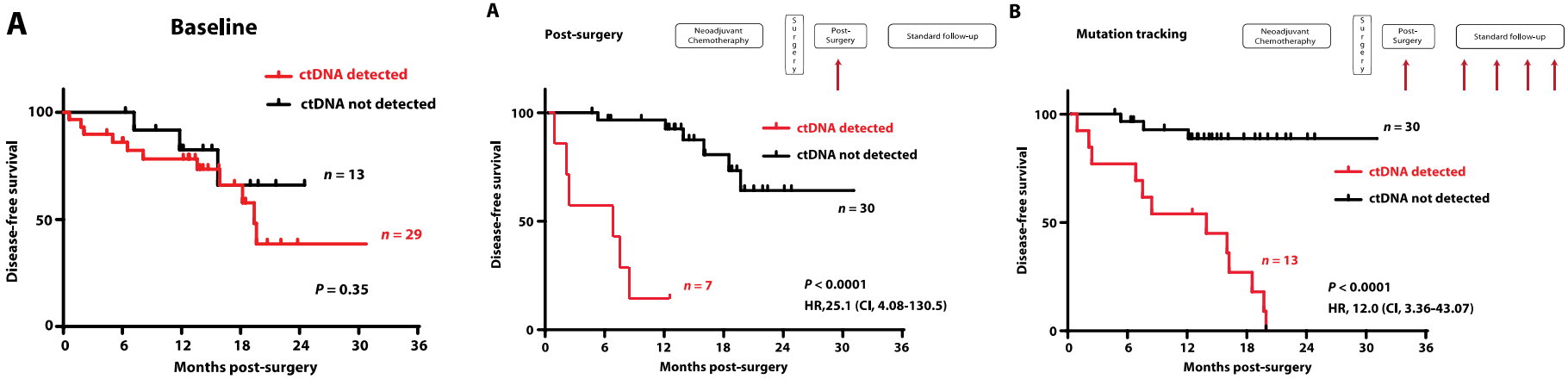
Prediction of relapse in early disease



- Cohort of 55 pts treated with neoadjuvant chemotherapy before surgery
- 78% of primary tumor had one or more somatic mutations
- MPS and dPCR analysis had high level of agreement

Garcia-Murillas I et al. Science Translational Med 2015

Prediction of relapse in early disease

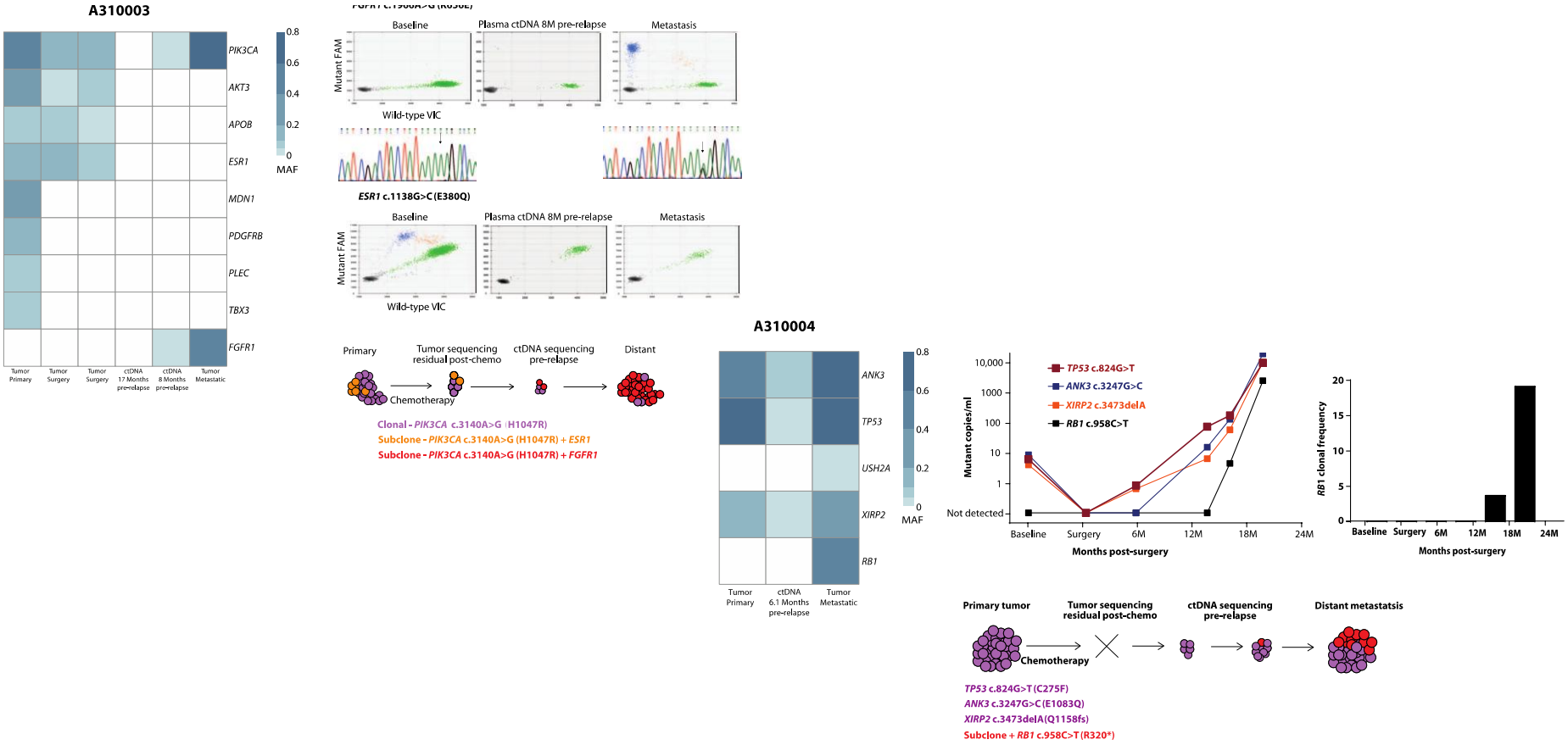


- ctDNA was detected in 69% of baseline plasma samples and was not predictive of DFS
- ctDNA was detected in 19% of single post-operative blood test and was predictive of early relapse (HR: 25.1 –CI: 4.08-130.5)
- “Mutation tracking” was predictive of early relapse (HR: 12.0 –CI: 3.36-43.07)
- Detection of ctDNA had a median of 7.9 months lead time over clinical relapse

Garcia-Murillas I et al. Science Translational Med 2015

Genomic characterization of MRD by high-depth plasma DNA seq

- Custom panel targeting the exons of 273 genes recurrently mutated in BC or involved in DNA repair pathways
- DNA was analyzed from primary tumor, residual primary tumor, plasma and metastasis (n=5)



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ESR1 mutations (ESR1m) in ct DNA from MBC patients

Mutations in ESR1 gene have recently been described as a major mechanism of R to HT

- Data from a cohort of 171 MBC pts
- Tumor material: metastatic tissue and ctDNA
- ESR1m were detected in the plasma of 14% pts with ER+ MBC and 0% pts with ER- MBC
- The ESR1 analysis on plasma had 97% agreement with concomitant recurrent tissue biopsy
- The prevalence of ESR1m differed between cancer exposed to previous AI (5.8% vs 36.4%)

Schiavon G. et al abt 926 AACR 2015

- Data from a retrospective cohort of 11 ER + MBC pts with known ESR1 mutation status and a prospective cohort of 12 MBC (8 ER +/ 4 ER-)
- Tumor material: metastatic tissue and ctDNA
- ESR1m were detected in the plasma of 75% pts with ER+ MBC (6/8) and 0% pts with ER- MBC (0/4)
- The ESR1 analysis had 80% (15/20) agreement with metastatic tissue biopsy

Chu D, Paoletti C et al. CCR 2015

Identification of HER2 amplification in cf DNA from BC patients

HER2 is a well known predictive marker for response to anti-HER2 treatments

- Data from a cohort of 58 MBC pts
- Method: digital PCR; the HER2/EFTUD2 plasma DNA copy number ratio was calculated
- 64% (7/11) of pts with HER2 + MBC were classified as HER2+ by dPCR and 94% (44/47) of HER2 – MBC were classified as HER2-

Gevensleben H et al. CCR 2013

- Data from a cohort of 130 BC pts
- Method: quantitive PCR; the HER2/CNTNAP I plasma DNA copy number ratio was calculated
- 19% (11/57) of pts with HER2 + MBC were classified as HER2+ by dPCR but 24 pts received trastuzumab as last treatment before venepuncture

Page K et al. BJC 2011

Conclusions

- Liquid biopsy is a rapidly advancing field
- Less invasive blood test has the potential to improve the management of many cancer patients
- The clinical utility of using dPCR for ctDNA analyses to guide therapy for cancer patients requires careful prospective studies before adoption into clinical practice

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