Circulating tumour DNA in breast cancer

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Conflicts of Interest

- I have no financial relationships to disclose
- I will not discuss off label use and/ or investigational use in my presentation

Summary

- Circulating tumour DNA (ctDNA)
  - Role as a predictor of outcome in early breast cancer
  - Mutation tracking
- Challenges
  - Intra-tumour genetic heterogeneity
  - *De novo* discovery of mutations

Circulating tumour DNA

Circulating cell-free plasma DNA (cfDNA)

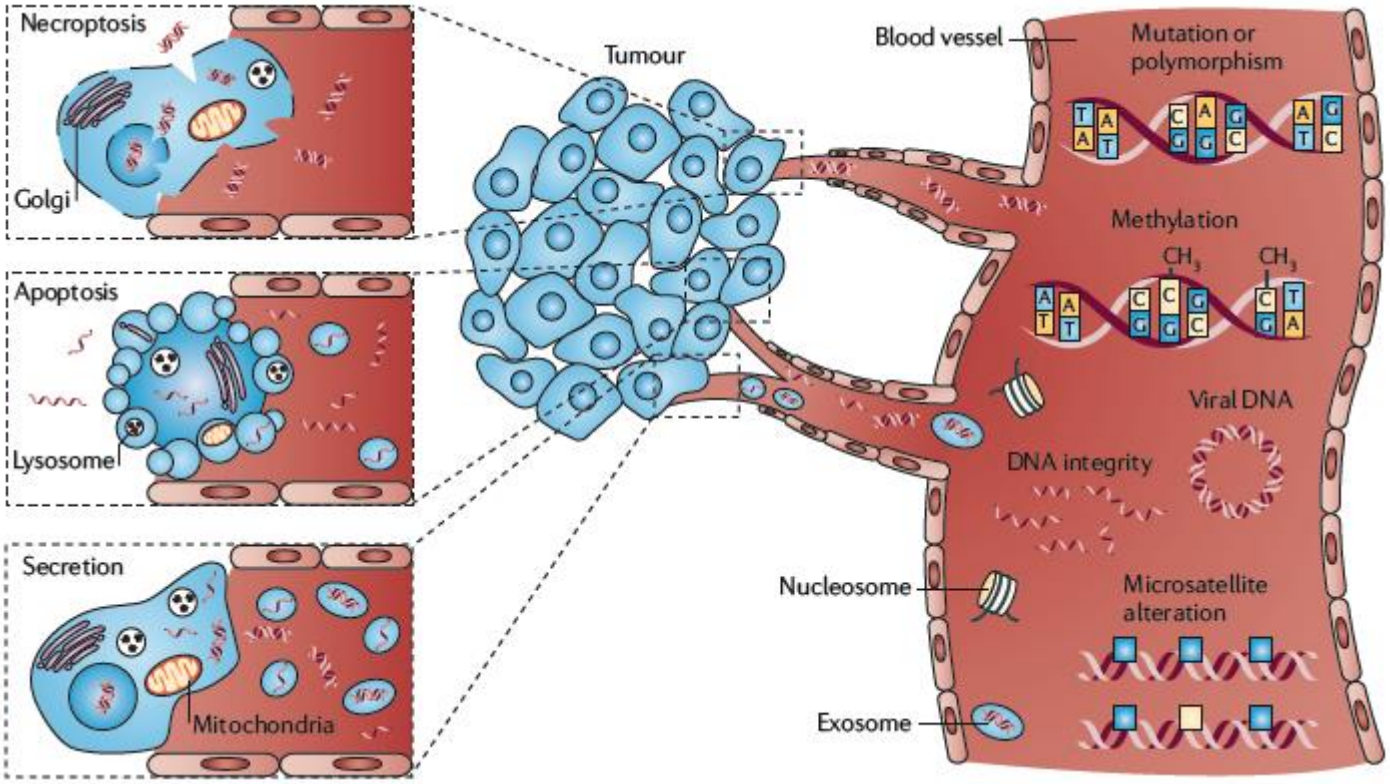
- DNA present in the plasma
- Fragments are small (140 - 170 base pairs)
- Not cancer-specific
  - Healthy individuals: ~1 ng DNA/ml
  - Inflammatory diseases, stroke, trauma, surgery, sepsis, PE
  - Cancer patients: 4-fold greater levels
- Cancer: small % of circulating DNA is tumour-derived (ctDNA)

Lee et al. 2001; Schwarzenbach et al. 2011

Circulating tumour DNA (ctDNA)

Sources of DNA release

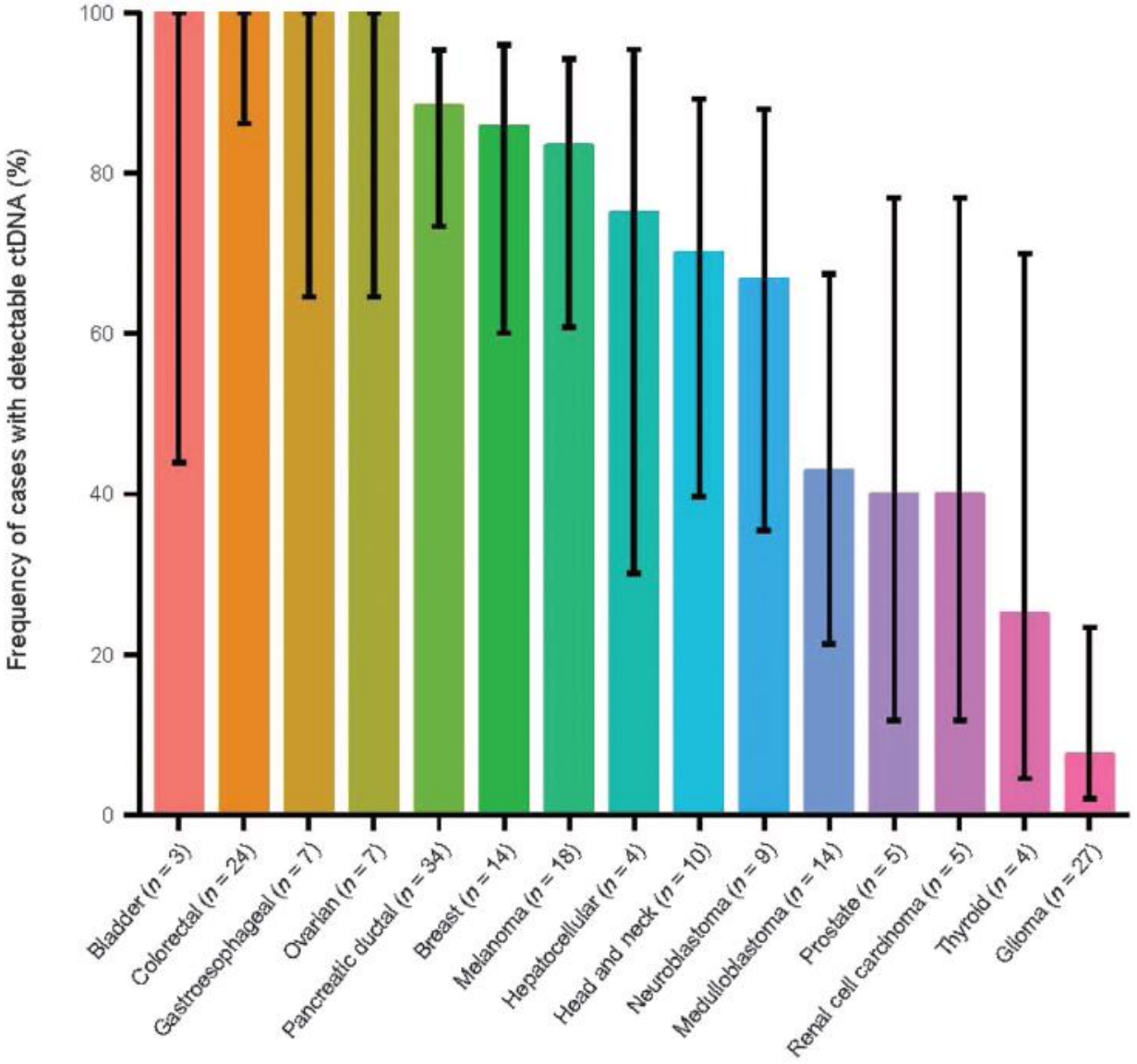
Types of DNA alterations



The tumour burden and rate of tumour cell proliferation/ apoptosis have a substantial role!

Schwarzenbach et al. Nat Reviews Cancer. 2011

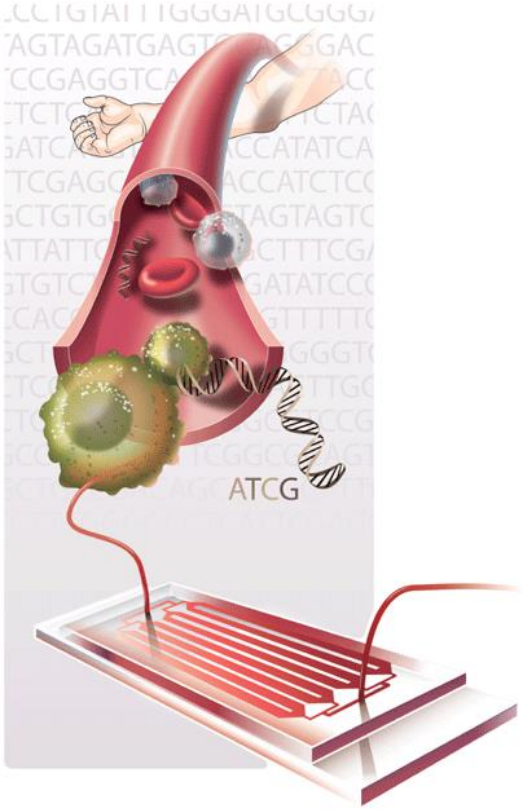
ctDNA and tumour type



Bettegowda et al. Sci Transl Med 2014

Potential uses of ctDNA

- Estimation of risk for metastatic relapse or disease progression
- Real-time monitoring of therapy response
- Identification of therapeutic targets and resistance mechanisms

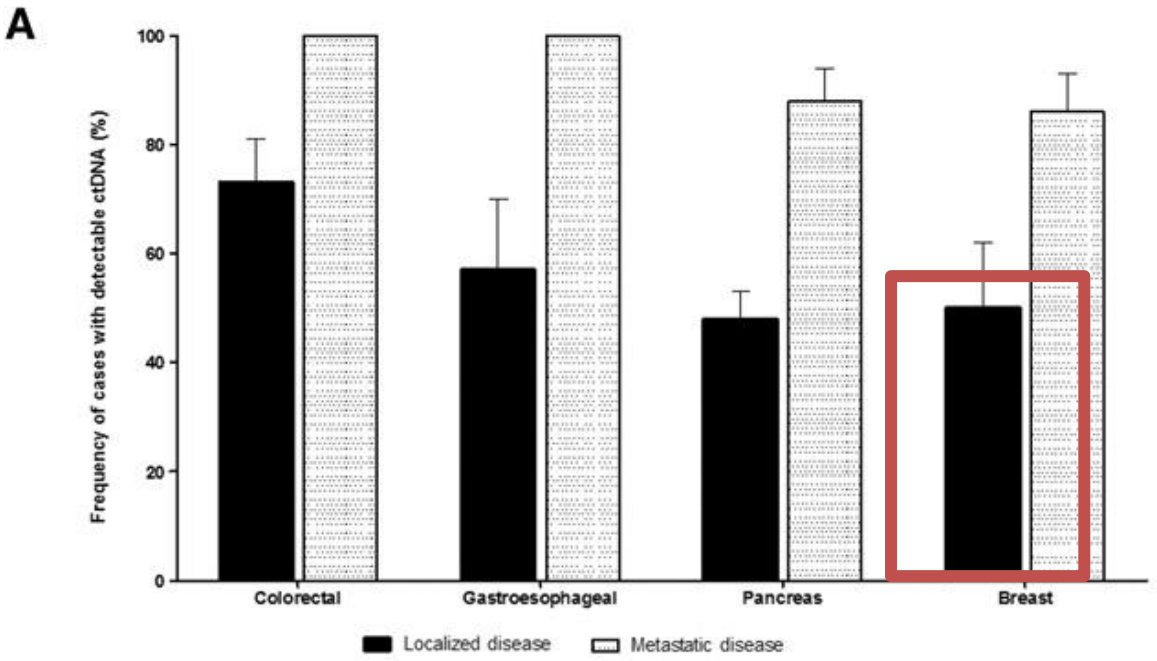


METASTATIC BREAST CANCER

Dawson et al. NEJM 2013; Murtaza et al. Nature 2013; Bidard et al. Sci Trans Med 2013

Early vs. advanced breast cancer

- Fraction of patients with detectable ctDNA in localized breast cancer (stages I to III)



Bettegowda et al. Sci Transl Med 2014

Circulating Tumor DNA (ctDNA) in Early Breast Cancer

- Can ctDNA be detected in patients with early breast cancer?

- Does ctDNA predict relapse after treatment of primary breast cancer?

ChemoNEAR

- Led by Nick Turner – RMH, London
- 31 patients enrolled in a prospective multi-centre sample collection study (ChemoNEAR, REC Ref No: 11/EE/0063)
- Patients scheduled for standard neoadjuvant chemotherapy

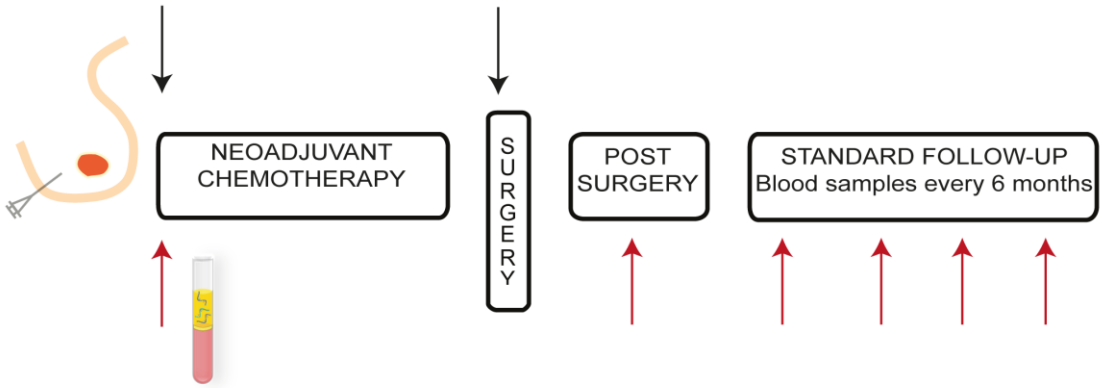
  90% (28/31) ECx4– accPaclitaxel x4 +/- trastuzumab
- Primary breast cancer

  No evidence of clinical distant metastasis

  Staging of all node positive and T3/4 patients according to local guidelines
- Median follow-up 17.4 months from study entry

	All patients (%)	Disease free (%)	Early relapse (%)
N	31	26	5
Median age (range), y	55 (29–68)	55 (29-68)	60 (57-65)
Pathology			
IDC NST	24 (77,5)	21 (81)	3 (60)
ILC	2 (6,5)	2 (7,5)	0 (0)
Mixed IDC/ILC	4 (13)	2 (7,5)	2 (40)
Metaplastic	1 (3)	1 (4)	0 (0)
Histologic grade			
1	0 (0)	0 (0)	0 (0)
2	10 (32)	8 (31)	2 (40)
3	20 (65)	17 (65)	3 (60)
N/A	1 (3)	1 (4)	0 (0)
Receptor status			
ER/PgR+ HER2-	11 (35)	10 (38,5)	1 (20)
ER/PgR- HER2+	7 (23)	7 (27)	0 (0)
ER/PgR+ HER2+	7 (23)	4 (15,5)	3 (60)
Triple negative	6 (19)	5 (19)	1 (20)
Clinical size at presentation (cT)			
1-2	24 (77,5)	21 (80,5)	3 (60)
3-4	6 (19,5)	4 (15,5)	2 (40)
N/A	1 (3)	1 (4)	0 (0)
Nodal status at presentation			
Negative	18 (58)	16 (61,5)	2 (40)
Positive	13 (42)	10 (38,5)	3 (60)
Pathological response to NAC			
No pathCR	20 (65)	17 (65)	3 (60)
pathCR	10 (32)	8 (31)	2 (40)
N/A	1 (3)	1 (4)	0 (0)

Prognosis in early stage breast cancer



Tumour - Diagnostic biopsy

Surgery (for patients with residual disease)

Plasma for extraction of cell free DNA (EDTA)

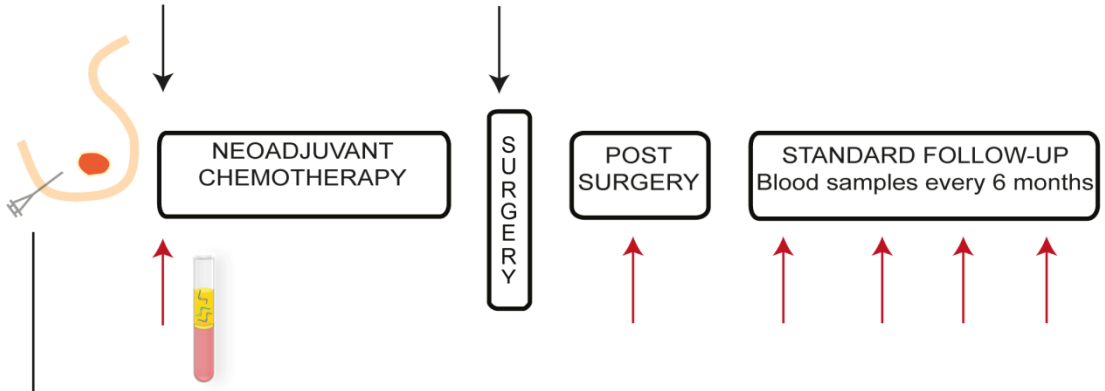
Baseline

Post-surgery

Every 6 months in follow-up

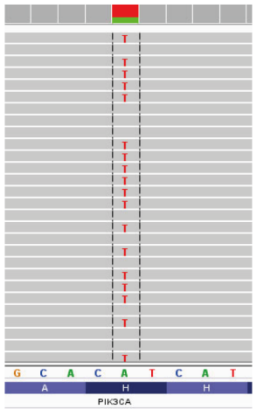
Turner et al., STM 2015

Prognosis in early stage breast cancer

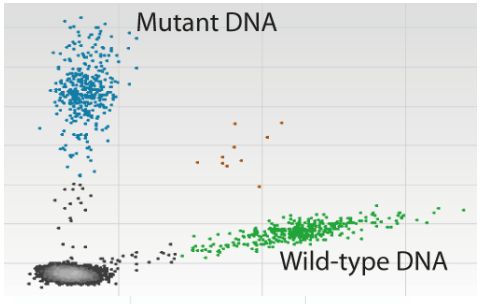


Ion Torrent

AKT1	MAP3K1
BRAF	PIK3CA
CDH1	PIK3R1
GATA3	PTEN
KIT	RUNX1
KRAS	SF3B1
MAP2K4	TP53



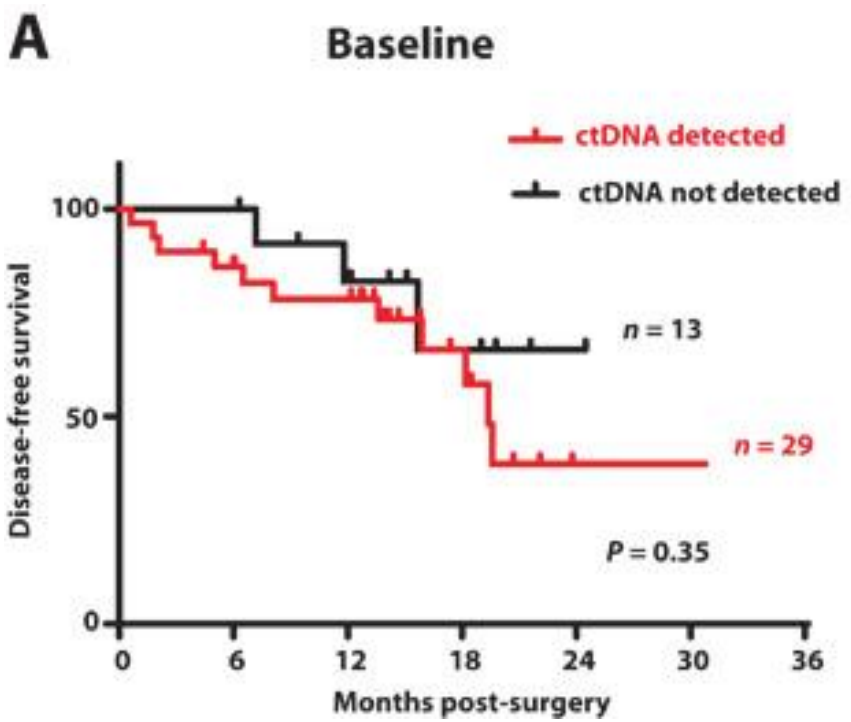
Develop personalized tumor specific digital PCR assays

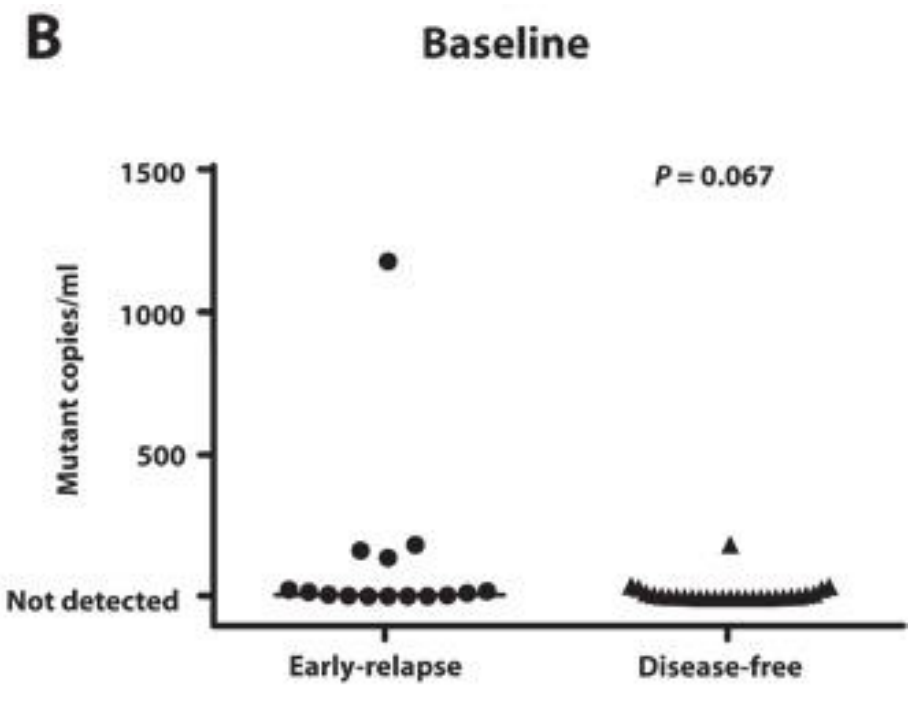


Track mutations in circulating free plasma DNA

Turner et al., STM 2015

Early relapse detection



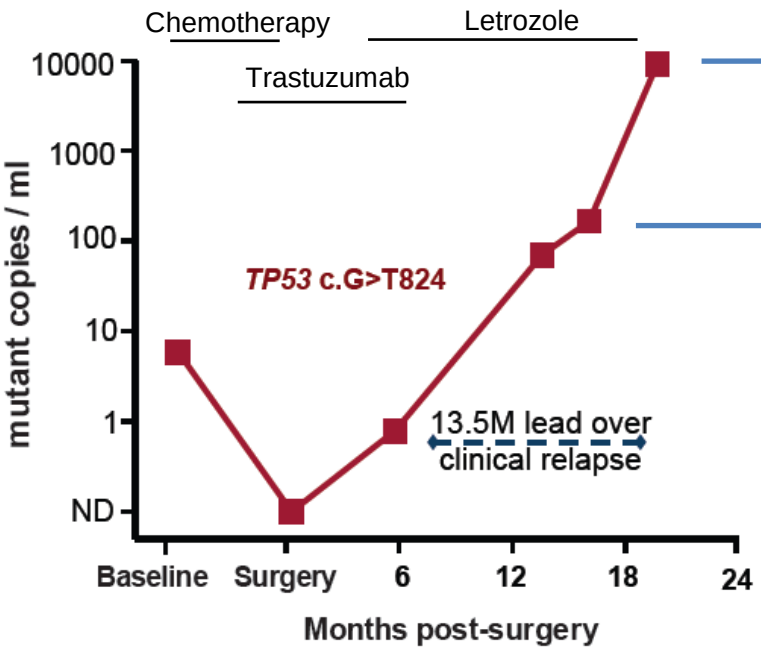


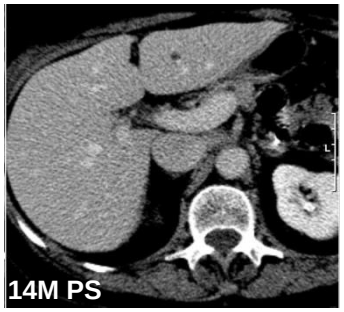
Baseline ctDNA burden is not predictive of disease free survival

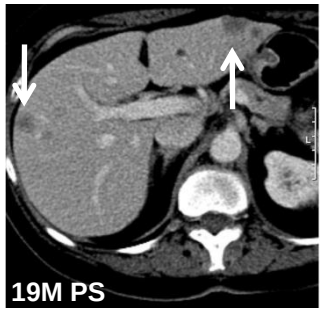
Baseline ctDNA burden not different between early relapse and disease free

Turner et al., STM 2015

Lead time over clinical relapse



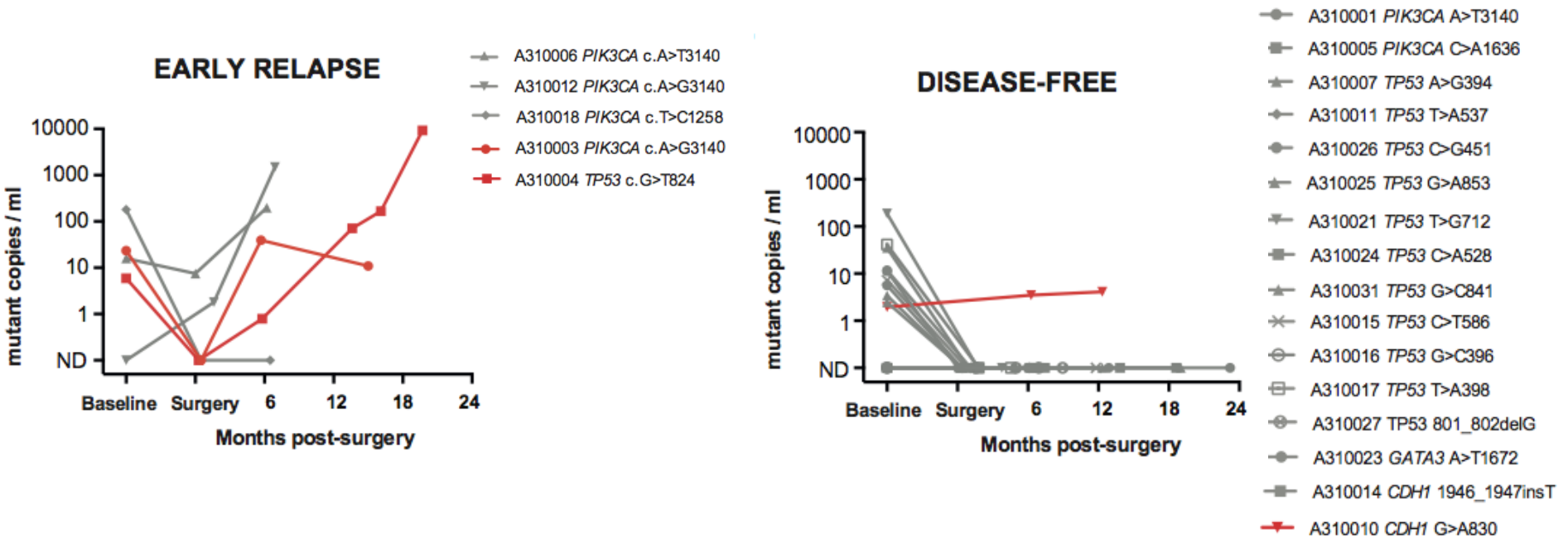




Median lead time over clinical relapse for all relapses 8 months

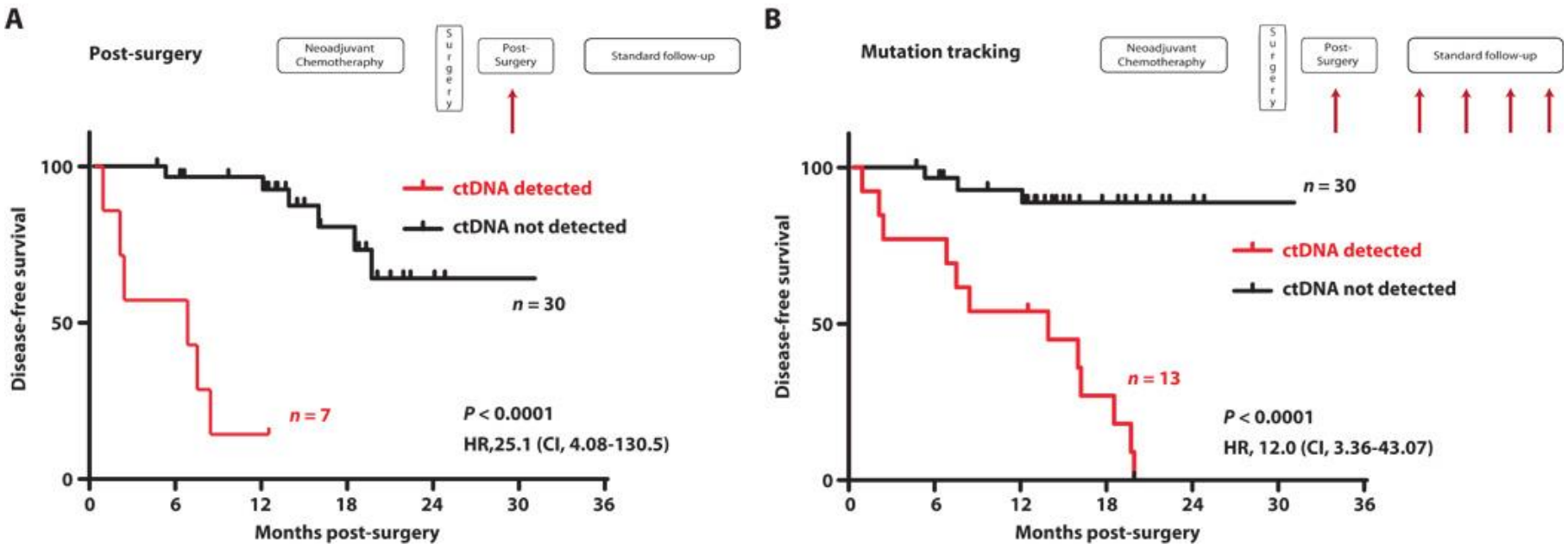
Turner et al., STM 2015

Predicting early relapse – mutation tracking



Turner et al., STM 2015

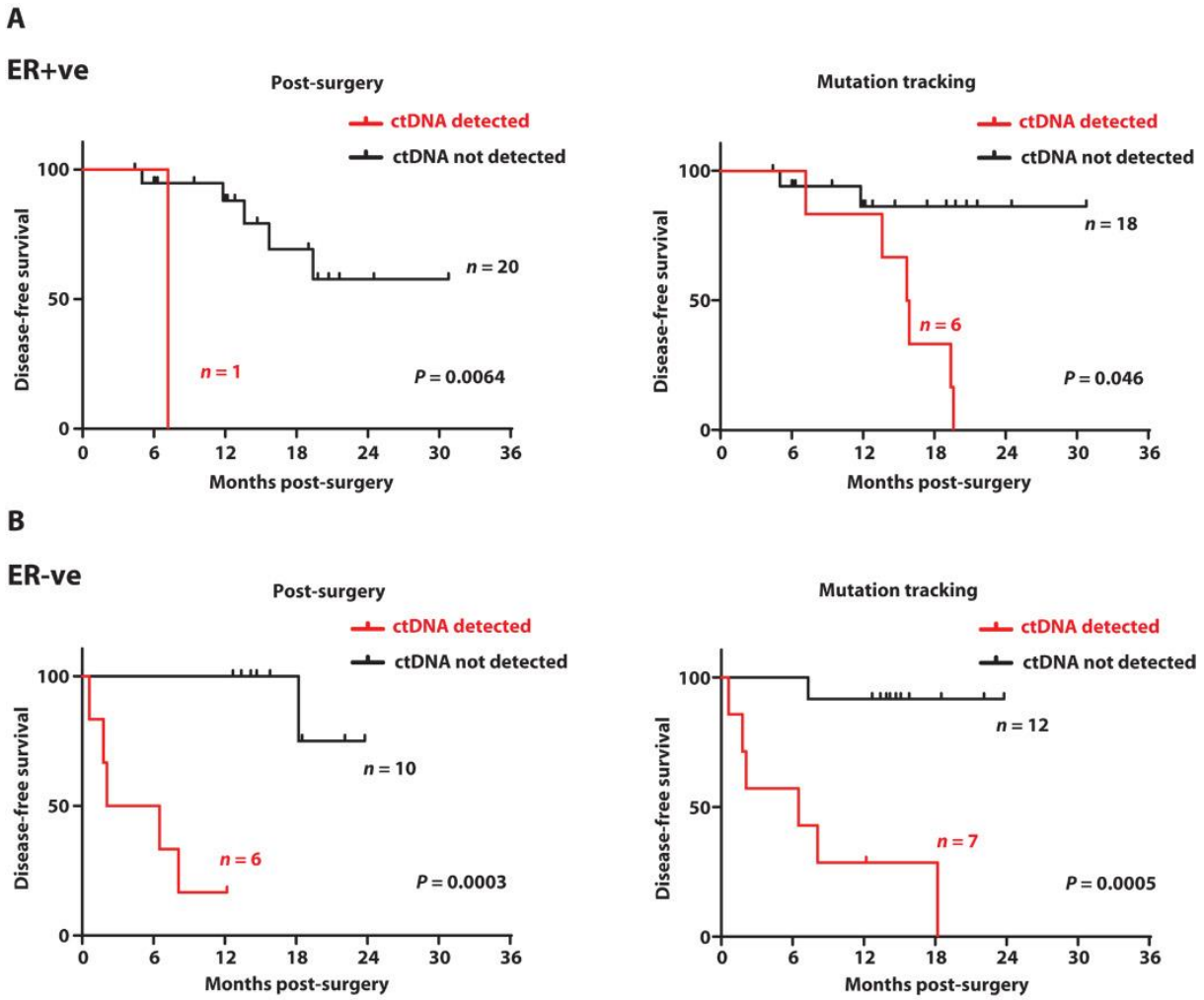
Predicting early relapse



Mutation detection in the immediate post-surgical sample and dynamic mutation tracking are both highly accurate in predicting relapse

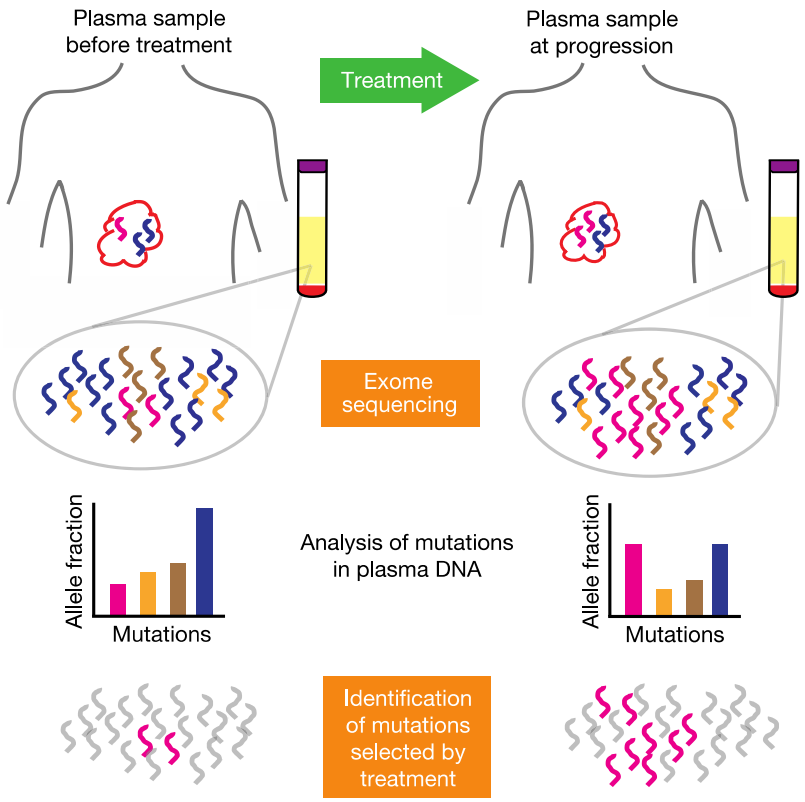
Turner et al., STM 2015

Predicting early relapse



Turner et al., STM 2015

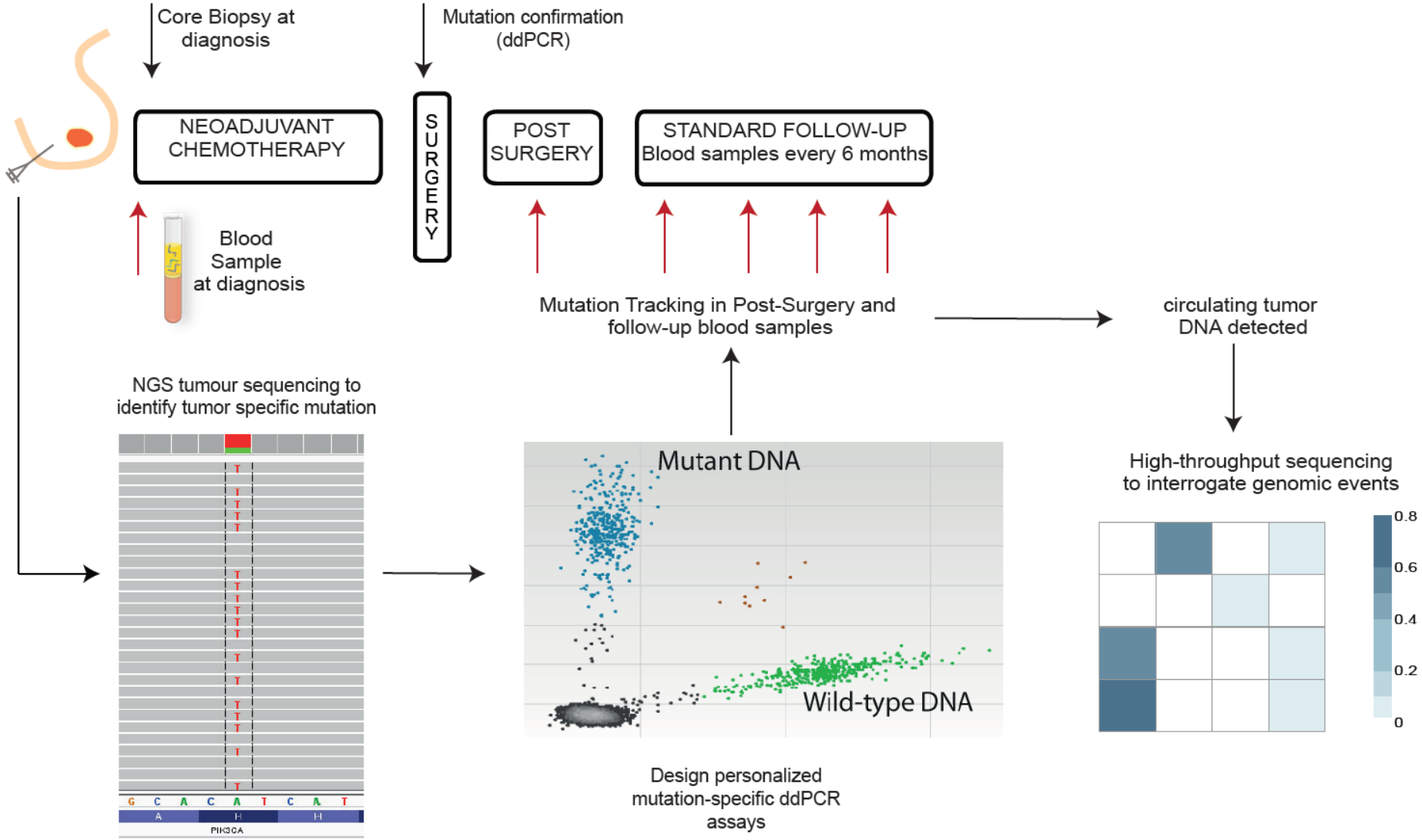
Tracking secondary (‘acquired’) resistance



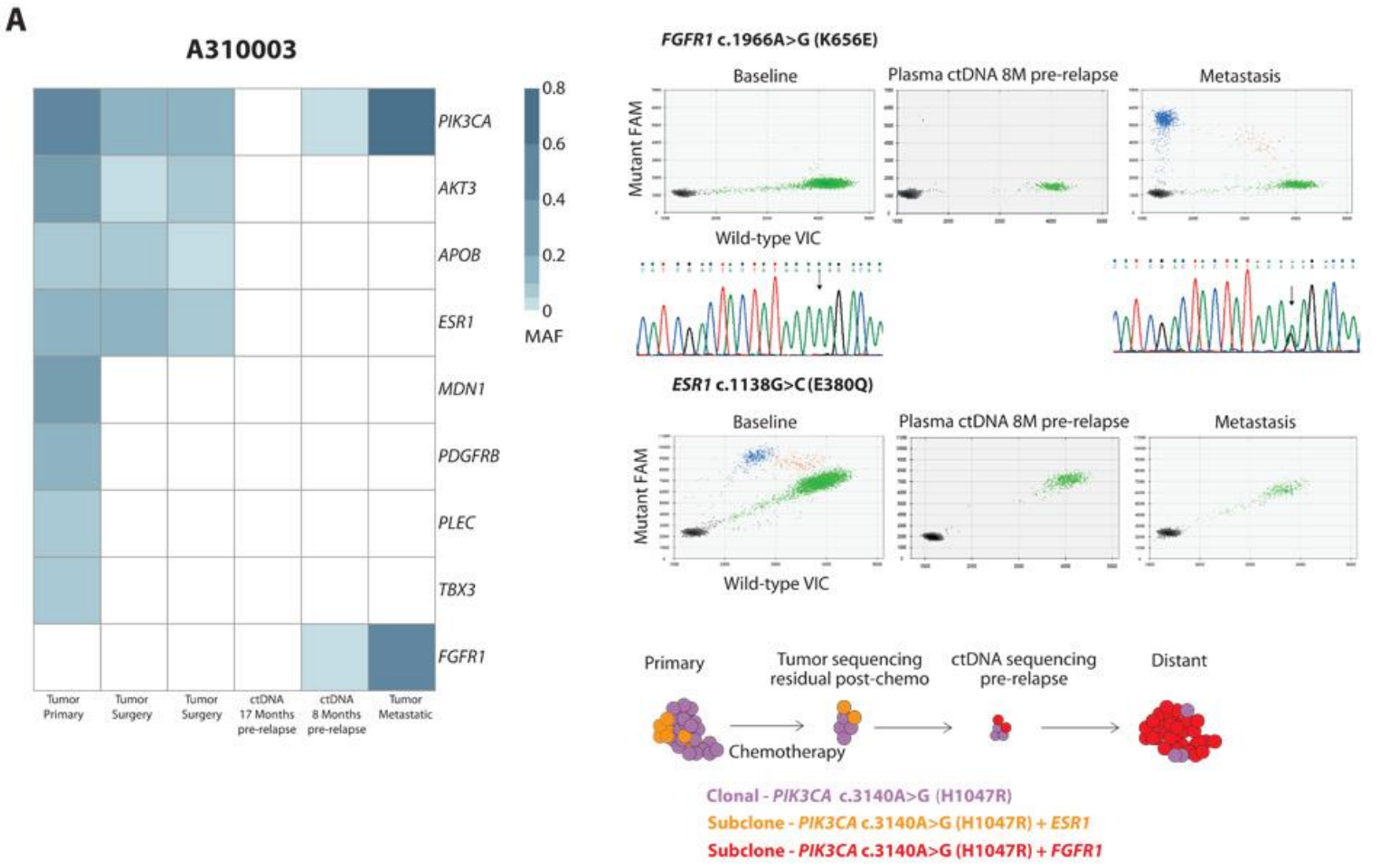
Identification of treatment-associated mutational changes from exome sequencing of serial plasma ctDNA.

Murtaza et al. Nature. 2013

Identifying new mutations in micrometastatic residual disease



MPS of plasma DNA



Pilot

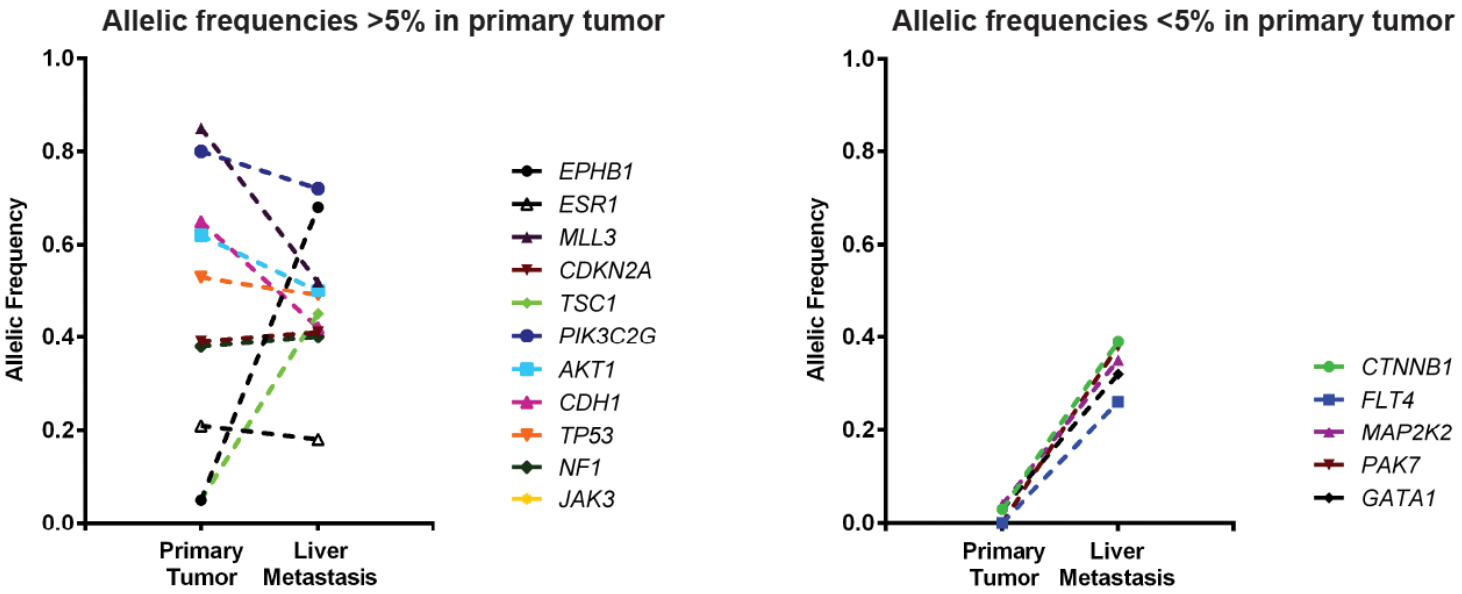
- Metastatic breast cancer patient (ER+ / HER2-)
  - Phase I study PAM4743g (Clinicaltrials.gov, NCT01090960).
  - GDC0068 – pan-AKT inhibitor
  - VHIO – Joan Seoane and Leticia De Mattos-Arruda
  - DNA extracted from
    - diagnostic biopsy of the primary tumour (FFPE)
    - diagnostic biopsy of the liver metastasis (FFPE)
    - peripheral blood leukocytes
    - four plasma samples during AKT inhibitor-based therapy (4th line)
- Targeted massively parallel sequencing
  - IMPACT – collaboration with Mike Berger
- Response was assessed using the RECIST criteria (1.1)

ctDNA analysis captures the heterogeneity of primary tumour and metastasis

Gene	Mutation	Class	Primary tumor (85x)	Liver metastasis (139x)
AKT1	p.E17K	Missense	80%	72%
CDH1	p.159_171PPISC PENEKGPF>L	In Frame Deletion	62%	50%
CDKN2A	p.S12*	Nonsense	85%	52%
TP53	p.K132N	Missense	65%	42%
NF1	p.V2420fs	FrameShift Deletion	53%	49%
TSC1	p.S1046C	Missense	39%	41%
JAK3	p.T21M	Missense	38%	40%
MLL3	p.G292E	Missense	21%	18%
EPHB1	p.I332M	Missense	6%	26%
PIK3C2G	p.K978N	Missense	5%	45%
ESR1	p.E380Q	Missense	5%	68%
MAP2K2	p.E207Q	Missense	4%	35%
GATA1	p.K315N	Missense	3%	32%
CTNNB1	p.A522G	Missense	3%	39%
FLT4	p.R282Q	Missense	0%	26%
PAK7	p.E494*	Nonsense	0%	38%

De Mattos Arruda et al. Ann Oncol, 2014

Longitudinal monitoring of the mutant alleles in plasma-derived ctDNA



De Mattos Arruda et al. Ann Oncol, 2014

Take home messages

- Source of genetic material from patients with early breast cancer
- Methods for sequencing should be carefully chosen
- Potential use
  - Prediction of early relapse
  - Longitudinal monitoring somatic genomic alterations
  - Tracking secondary (‘acquired’) resistance
- ctDNA analysis remains a research tool

ctDNA sequencing in the metastatic setting
Oligometastatic patients

- Potentially useful for longitudinal monitoring
- High depth required for mutation calls
- 250x-400x – insufficient in most cases
- *De novo* discovery - challenging

Take Home Messages

- ctDNA – potential surrogate of tumour tissue
- ctDNA analysis
  - Prognostication
  - Prediction
  - Monitoring therapeutic resistance
  - Tacking intra-tumour heterogeneity
- *De novo* mutation calling
  - Challenging
  - Dependent on disease burden
  - More sensitive techniques required

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Brigitte Sigal
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Together, let's beat cancer.