GBCC2018
Global Breast Cancer Conference 2018
April 5 (Thu) - 7 (Sat), 2018
Songdo ConvensiA, Incheon, Korea

Liquid Biopsy: CTC, ctDNA, Exosome etc.

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5th April, 2018
Incheon, Korea

HKBOG
Hong Kong
Breast
Oncology Group

Disclosures

Consultant or Advisory Role:

AstraZeneca, Aptus, Astellas, DeNovo Service, Eisai, Foundation Medicine, Novartis, Pfizer & Roche

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SAPIENTIA·ET·VIRTUS

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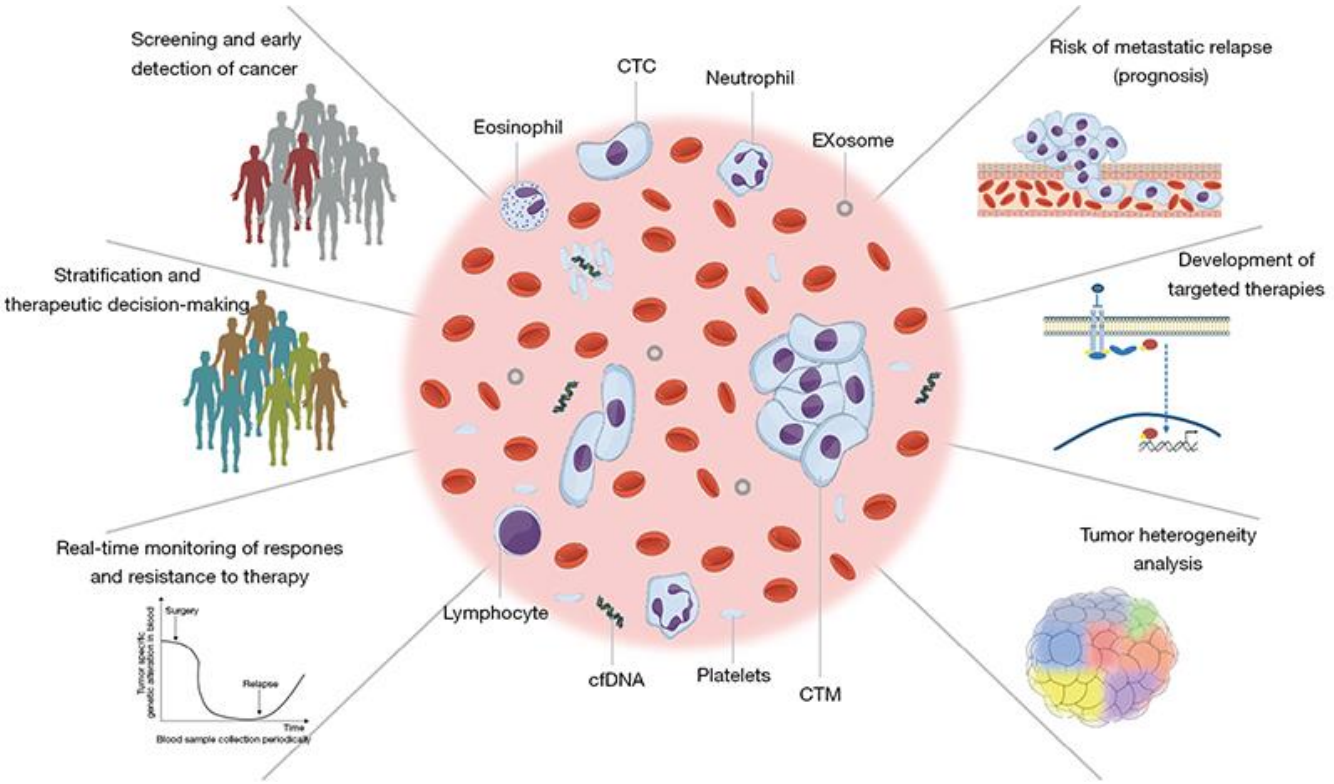
Liquid Biopsy
(Fluid Biopsy, Fluid Phase Biopsy)

Sampling and analysis of non-solid biological tissue, primarily, blood
– as a diagnostic and monitoring tool for diseases s.a. cancer
-largely non-invasive
-can be done more frequently, taking multiple samples



Opportunities and Challenges of

- Circulating Tumour Cells (CTCs)
- Circulating Tumour DNA (ct DNA)
- Exosome and EVs

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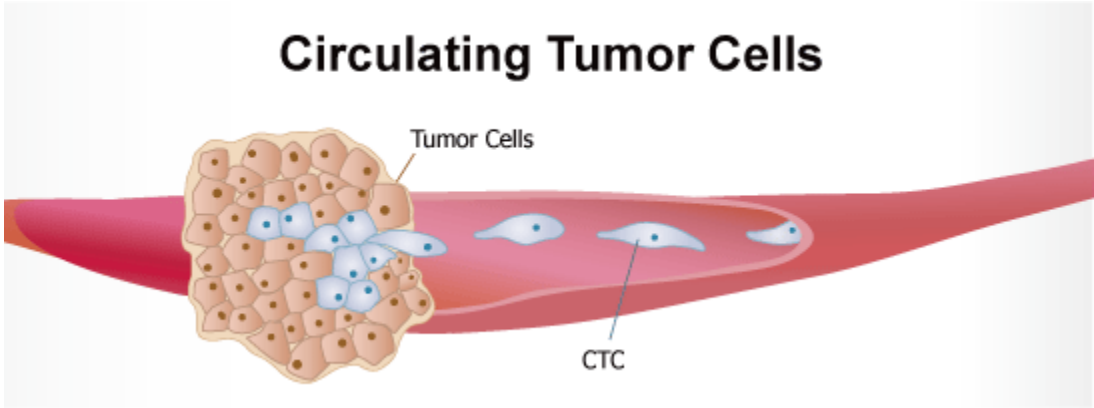
Circulating Tumour Cells (CTCs)

- **1869 Thomas Ashworth**
  - First described “Circulating Tumour Cells (CTCs)” in a metastatic cancer patient
- **1976 Nowell**
  - Amended the definition as tumor cells derived from primary tumors or metastatic tumors with the ability to get out of the basement membrane and invade into the blood vessels through the tissue matrix
- **2004 Cristofallini**
  - Showed the finding of at least five CTCs in 7.5ml blood was associated with reduced PFS and OS in MBC patients.



Paget S. Lancet 1889; Bidard F-C et al. Lancent Oncol, 2015

40th Annual San Antonio Breast Cancer Symposium, December 5-9, 2017

Circulating Tumor Cells and Late Recurrence of Breast Cancer

Joseph A. Sparano, MD[1], Anne O'Neill, MS[2], Katherine Alpaugh, PhD[3], Antonio C. Wolff, MD[4], Donald W. Northfelt, MD[5], Chau T. Dang, MD[6], George W. Sledge, MD[7], Kathy Miller, MD[8]

1. Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2. Dana Farber Cancer Institute, Boston, MA; 3. Fox Chase Cancer Center, Philadelphia, PA; 4. Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD; 5. Mayo Clinic, Scottsdale, AZ; 6.Memorial Sloan Kettering Cancer Center, New York, NY; 7. Stanford Cancer Center, Palo Alto, CA; 8. Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN

ECOG-ACRIN cancer research group

Reshaping the future of patient care

Background: Rationale for Evaluation of Circulating Tumor Cells (CTCs) as a Biomarker for Late Recurrence

- **Analytical validity**
  - FDA-cleared blood test for enumerating CTCs in metastatic breast cancer [1,2]
- **Clinical validity**
  - CTC burden and prognosis in metastatic breast cancer[1,2]
  - CTC presence/burden & recurrence in early breast cancer [3,4]

	Lucci et al [3]	Rack et al [4]	Reithdorf [5]
No.	302	2026	213
CTC-Positive	24%	22%	22%
Median followup	2.9 years	2.9 years	5.6 years
Recurrence risk (CTC + vs. -)	4.6-fold ↑	2.1-fold ↑	2.9-fold ↑

(1) Cristofanilli et al. N Eng J Med 2004; 351:781-91 (2) Smerage et al. J Clin Oncol 2014; 32:3483-89 (3) Lucci et al. Lancet Oncol 2012; 13: 688-95
(4) Rack et al. JNCI 2014:106(5): dju066 doi:10.1093/jnci/dju066 (5) Reithdorf et al. Clin Cancer Res 2017; 23: 5384-5392

Joseph Sparano et al, SABCS 2017

Methods: Study Design

- **Population:** Stage II-III HER2-negative enrolled in E5103 (NCT00433511)
- **Treatment:** AC-weekly paclitaxel ± bevacizumab + endocrine therapy if ER+
- **Selection:** Recurrence-free 4.5-7.5 years after diagnosis & informed consent
- **CTC Assay:** Whole blood (7.5 ml) drawn into fixative-containing tube for CTC identification and enumeration using the CellSearch® system at entry
- **Assay results:** not reported to clinicians or patients due to uncertainty regarding prognostic information

Joseph Sparano et al, SABCS 2017

Results: Patient Characteristics, Recurrences, & CTC Results

(Enrollment Period: February 2013 – July 2016)

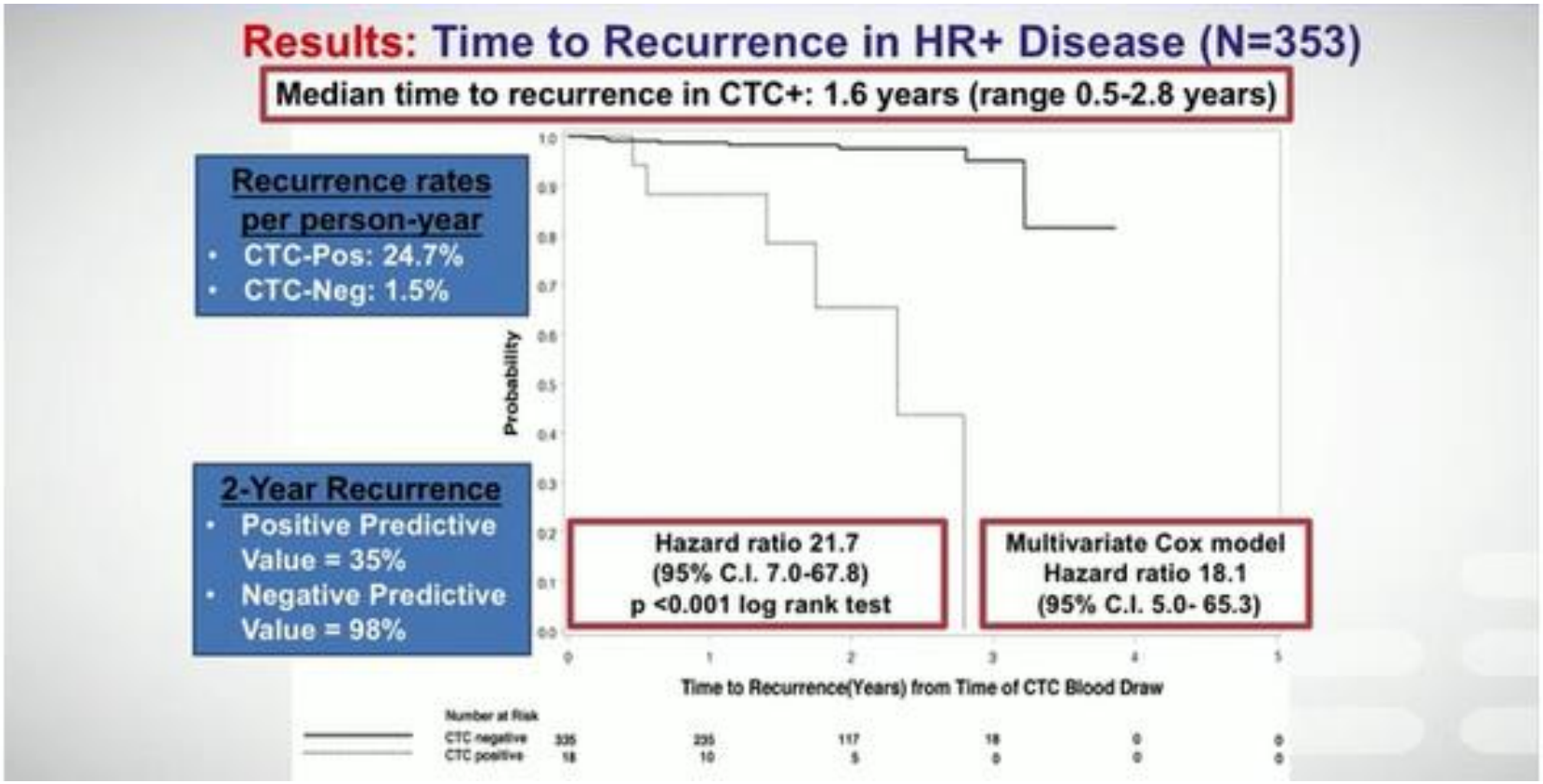
Total	Total (N=547)
Age at diagnosis (n=547)	
< 50 years	44%
>= 50 years	56%
Tumor size (N=547)	
< 2 cm	41%
>/= 2 cm	59%
Nodal Status	
Negative	27%
Positive	73%
HR Expression (N=546)	
Negative	35%
Positive	65%
Histologic grade (N-534)	
Low-intermediate	45%
High	55%
Endocrine Therapy (N=330)	88%

- **Median followup – 1.8 years**
  - Range 0-3.9 years
- **Recurrences**
  - **HR-Positive (N=14/353): 4.0%**
    (95% CI 3.0 to 7.9%)
  - **HR-Negative (N=1/193): 0.5%**
    (95% CI 0, 2.9%)

- **CTC-Positive (1 cell/7.5 ml blood)**
  - **Overall (N=26): 4.8%**
    95% CI 3.1%-6.9%
  - **HR-Positive (N=18/353): 5.1%**
    95% CI 3.0%-7.9%
  - **HR-Negative (N=8/193): 4.1%**
    95% CI 1.8%-9.0%

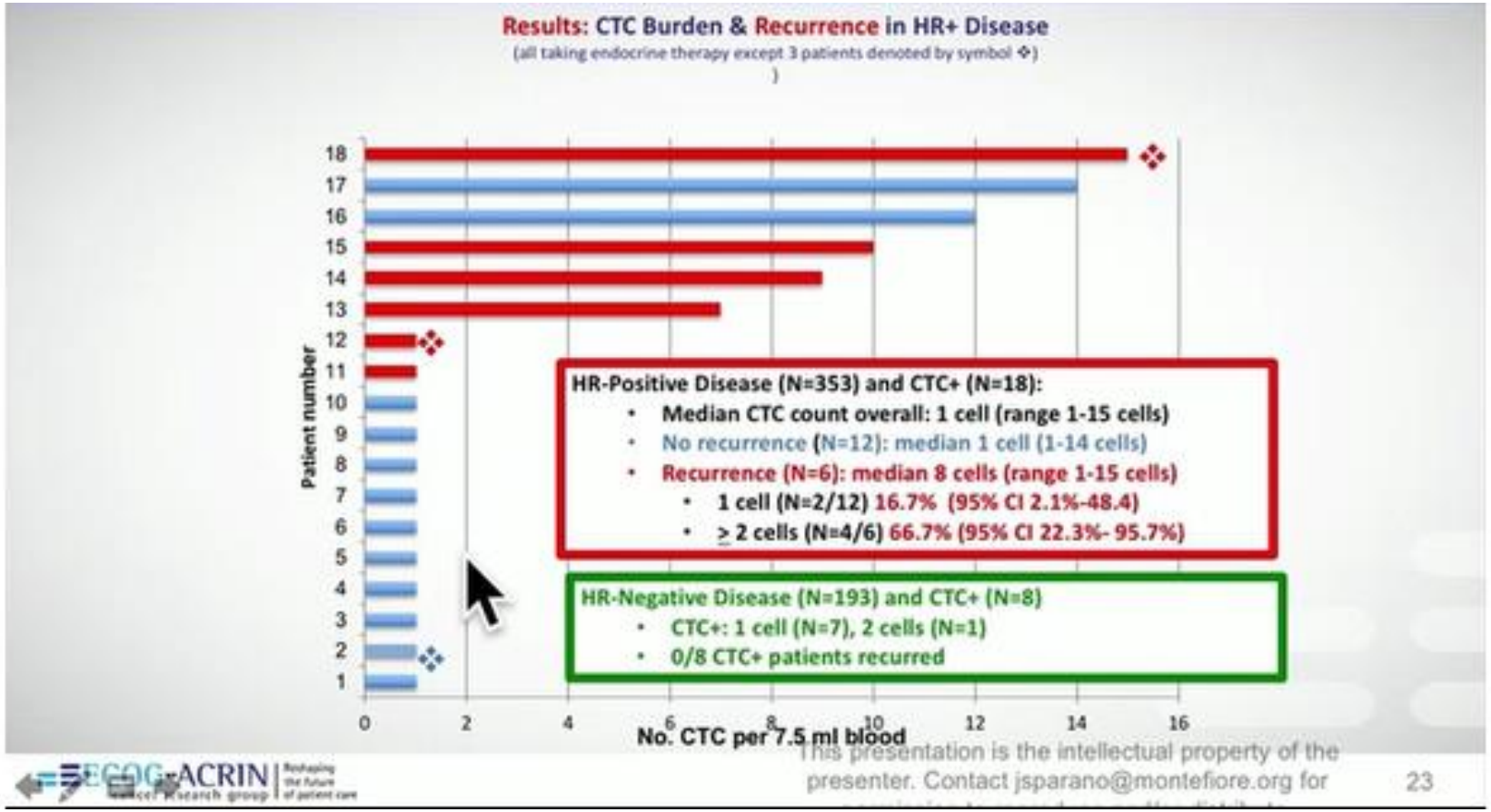
Joseph Sparano et al, SABCS 2017

SABCS 2017 –Predicting late relapse with CTC?



Joseph Sparano et al, SABCS 2017

High risk of recurrence- having high levels of CTCs (not so predictive for low number of CRC)



Joseph Sparano et al, SABCS 2017

Conclusions

- **Study objective 1: prevalence of detectable CTCs**
  - Detectable in 5% with localized HR+, HER2- breast cancer 5 years or more after diagnosis
  - After adjuvant chemotherapy and concurrent endocrine therapy
  - Also detected in 4% of HR-, HER- ("triple-negative") disease
- **Study objective 2: CTCs and clinical recurrence**
  - Prospective study - level 1 evidence supporting clinical validity of a positive CTC assay with clinical recurrence in HR+ breast cancer
    - Robust risk stratification (hazard ratio ~20x↑)
    - High negative predictive value (98%)
  - No association with recurrence in ER- disease, although few events in this population

Joseph Sparano et al, SABCS 2017


AGO-B
BREAST STUDY GROUP
San Antonio Breast Cancer Symposium, December 5-9, 2017
SUCCESS
BIG-Member
Extended adjuvant bisphosphonate treatment over five years in early breast cancer does not improve disease-free and overall survival compared to two years of treatment: Phase III data from the SUCCESS A study
Wolfgang Janni, Thomas WP Friedl, Tanja Fehm, Volkmar Mueller, Werner Lichtenegger, Jens Blohmer, Ralf Lorenz, Helmut Forstbauer, Emanuel Bauer, Visnja Fink, Inga Bekes, Jens Huober, Julia Jückstock, Andreas Schneeweiss, Hans Tesch, Sven Mahner, Sara Y Brucker, Georg Heinrich, Lothar Häberle, Peter A. Fasching, Matthias W Beckmann, Robert Coleman, Brigitte Rack

San Antonio Breast Cancer Symposium, December 5-9, 2017

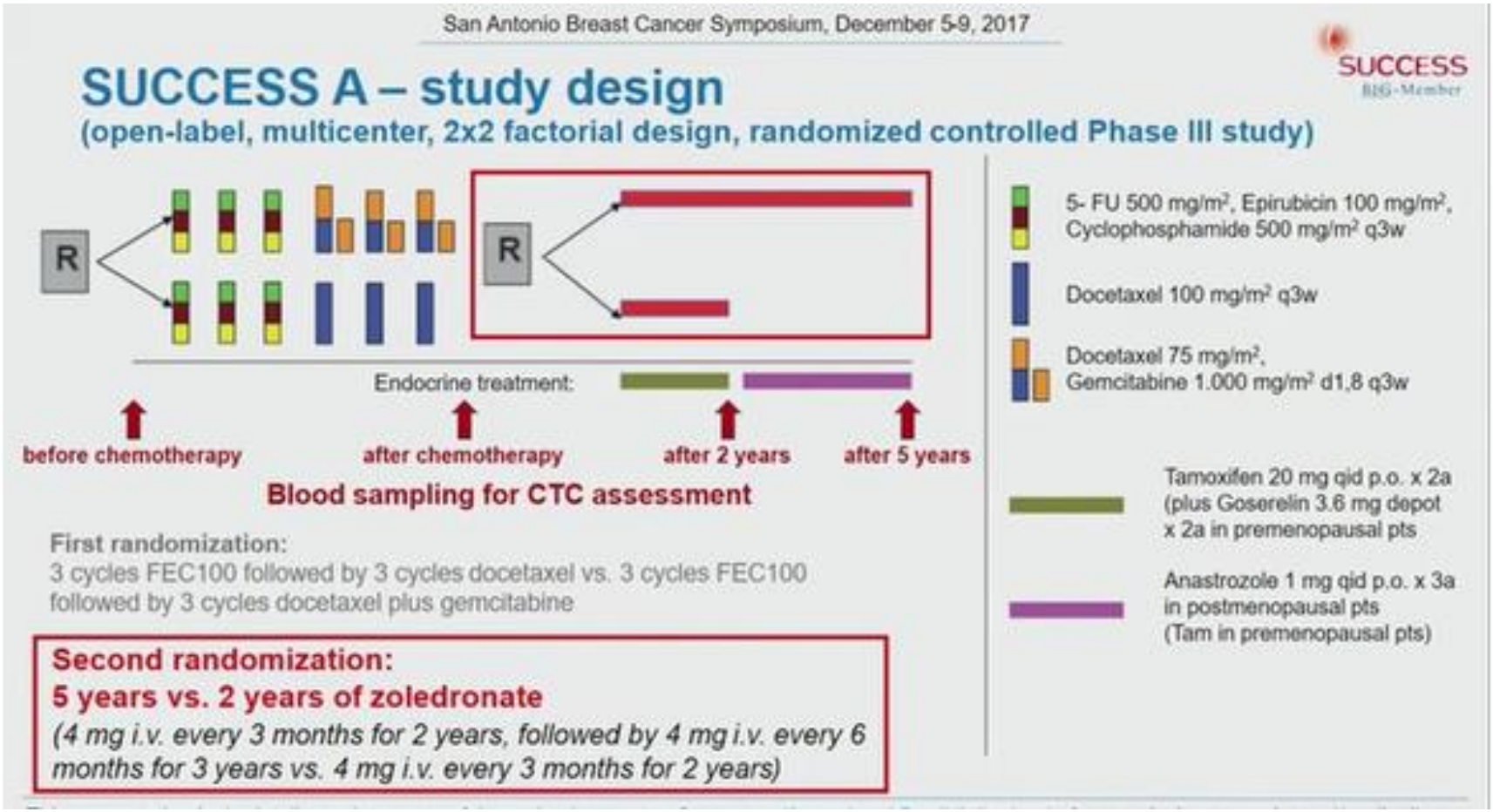
SUCCESS
BIG-Member

Background: Circulating tumor cells

- Circulating tumor cells (CTCs) detectable in early and metastatic breast cancer
- CellSearch System® (Menarini) FDA-cleared for enumeration of CTCs
- Prognostic relevance in the primary[1] as well as metastatic[2] setting established
- CTC dynamics as early treatment monitoring tool in metastatic breast cancer[3]
- Recent data indicate prognostic role of CTCs assessed during long-term follow-up in early breast cancer[4, 5]
- Bisphosphonates may play a role for the elimination of CTCs and DTCs

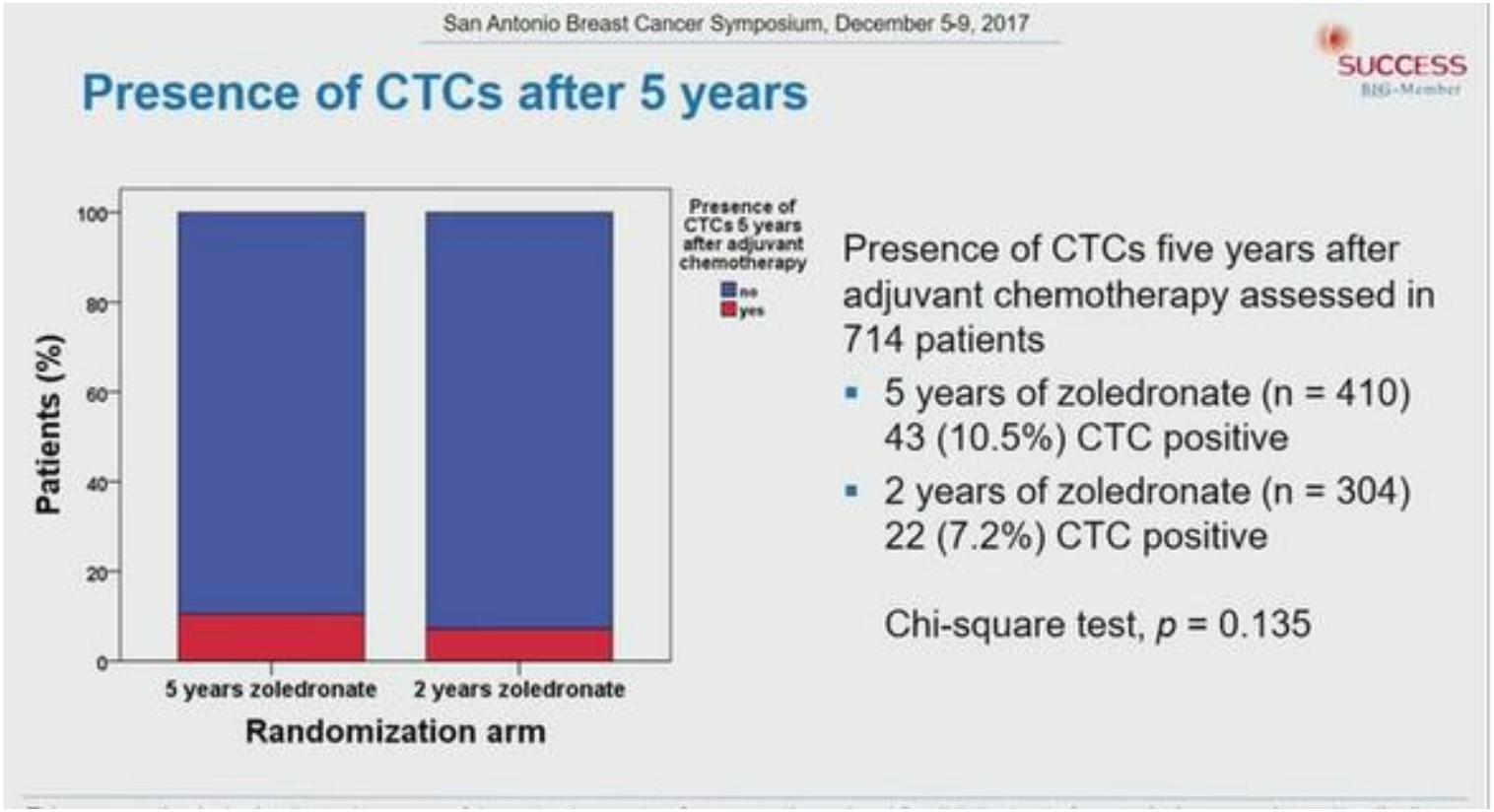
[1] *Rack B, et al., J Natl Cancer Inst. 2014; 106(5); Janni W, et al, Clin Cancer Res. 2016; 22(10):2583-93.*
[2] *Bidard FC et al. Lancet Oncol. 2014; 15(4):406-14.*
[3] *Smerage JB, et al. J Clin Oncol. 2014; 32(31):3483-9.*
[4] *Janni W, et al. SABCS 2015,* [5] ***Sparano J, et al. SABCS 2017 (GS6-03)***

Wolfgang Janni et al, SABCS 2017



Wolfgang Janni et al, SABCS 2017

Lack of significant difference with regard to prevalence of CTCs 5 years after adjuvant chemotherapy in accordance to survival analysis



Wolfgang Janni et al, SABCS 2017

Background: Late Recurrence in ER+ Breast Cancer

- **Late recurrence – 5 or more years after diagnosis**
  - Accounts for ~ 50% of recurrences in ER+ breast cancer
  - In EBCTCG metaanalysis[1], the 10-year recurrence risk in patients recurrence-free after a 5-year course of endocrine therapy:
    - 5% if 0 LN+
    - 10% if 1-3 LN+
    - 22% if 4-9 LN+
- **Adjuvant therapy and biomarkers for late recurrence**
  - Adjuvant chemotherapy ↓ *early* recurrence within 5 years [2]
  - Extended adjuvant endocrine therapy ↓ late recurrence by ~ 2-4% [3-8]
  - Some gene expression assays prognostic for late recurrence
    - ~ 2.5-fold ↑ recurrence risk in high vs. low risk groups [9,10]

(1) EBCTCG, NEJM 2017; 377; 1836-46; (2) EBCTCG. Lancet 2012; 379:432-44 (3) Goss PE et al, J Natl Cancer Inst 2005;97:1262-71. (4) Mamounas EP et al., J Clin Oncol 2008;26:1965-71 (5) Jakesz et al., J Natl Cancer Inst. 2007; 99(:1845-53. (6) Davies C et al., Lancet. 2013 ;381:805-16. (7) Gray et al., J Clin Oncol 31, 2013 (suppl; abstr 5). (8) Goss PE et al., N Engl J Med. 2016; 375:209-219 (9) Sgroi et al. J Natl Cancer Inst 2013; 105:1036-42 (10) Wolmark et al. J Clin Oncol 2016; 34:2350-58

Joseph Sparano et al, SABCS 2017

Implications of BC Clinical Practice & Research

- **Proof of Concept – "Clinical Validity"**
  - Biomarker prognostic for late recurrence in hormone positive HER2 negative EBC
  - Supports concept of a "second decision point" to tailor therapy based on biomarkers results
- **Further Study – "Clinical Utility"**
  - **Positive CTC assay** – may benefit from novel therapeutic approaches (s.a. oral SERDS, CDK4/6 inhibitors)
  - **Negative CTC assay** – spared from extended adjuvant hormonal therapy?
  - To explore the comparison and/or in combination with ct DNA or other biormarkers

Opportunities and Challenges of

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- **Circulating Tumour DNA (ct DNA)**
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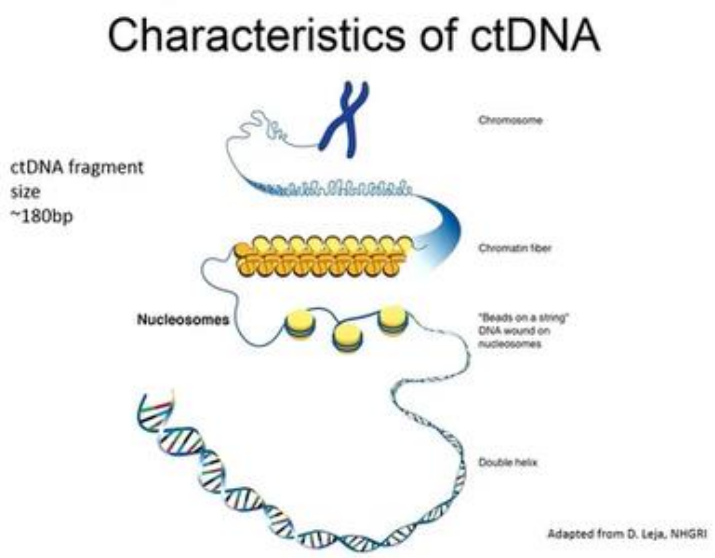
Emerging potential added value of ctDNA

Circulating tumour DNA are released into the blood of patients with a wide range of malignancies

Detectable in ~90% of patients with advanced cancer

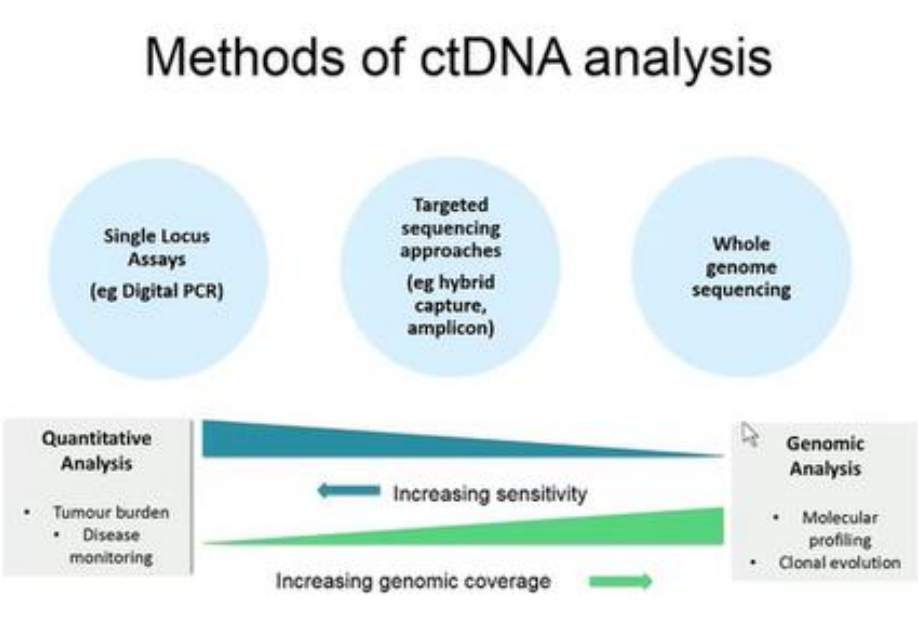
Frequently present at low levels



Diehl et al Nat Med 2008
Perkins G et al PLoS ONE 2011
Forshew etal STM 2012
Dawson et al NEJM 2013
Crowley et al Nat Rev Clin Oncol 2013
Bettegowda et al STM 2014

Pre-Analysis Consideration & Methodology

Blood Collection	EDTA	cfDNA Specific Tubes
Plasma Centrifugation	RT, within 1 h	RT, within 96 h
	Plasma can be kept frozen at -80°C	
cfDNA Preparation	cfDNA extraction: standard protocol	
QC	Quality and quantity check of cfDNA	
Analysis	Various analysis approaches depending on clinical application and/or research question	

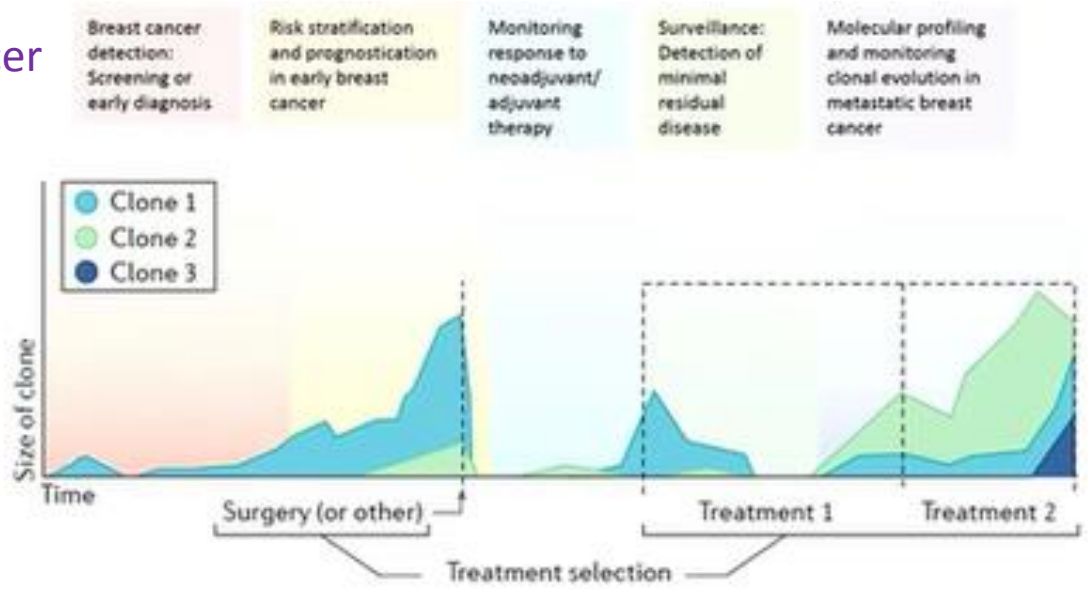


With Courtesy of Sarah-Jane Dawson

Clinical Applications of ctDNA in BC Management

Potential Use of ctDNA Analysis

1) Selecting therapy in metastatic cancer
2) Monitoring of therapy
3) Detecting minimal residual disease
4) Screening for cancer



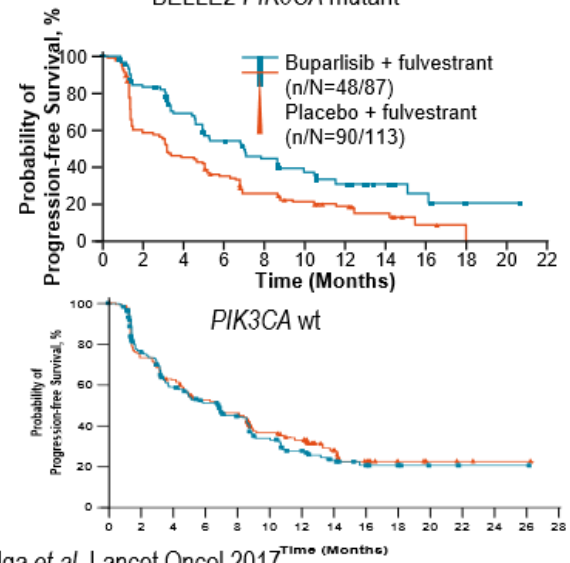
Wan et al, Nature Reviews Clinical Oncology, 2017

Selecting therapy for MBC patients

PI3K INHIBITORS ARE EFFECTIVE IN *PIK3CA* MUTANT BC

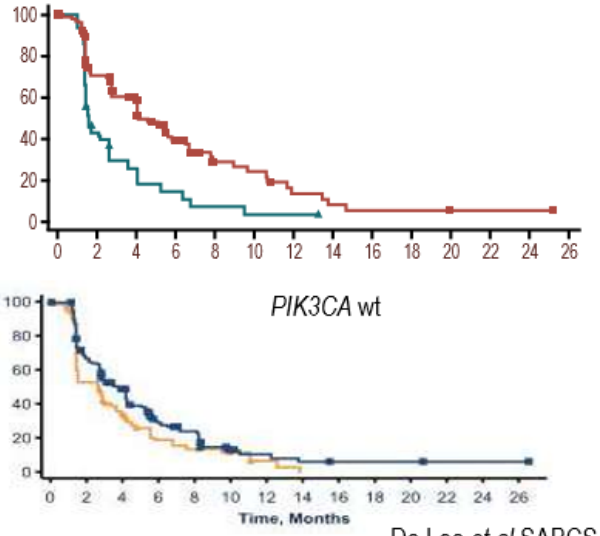
Buparlisib - Exploratory analysis from BELLE2 and pre-planned BELLE3

BELLE2 *PIK3CA* mutant



Baselga *et al* Lancet Oncol 2017

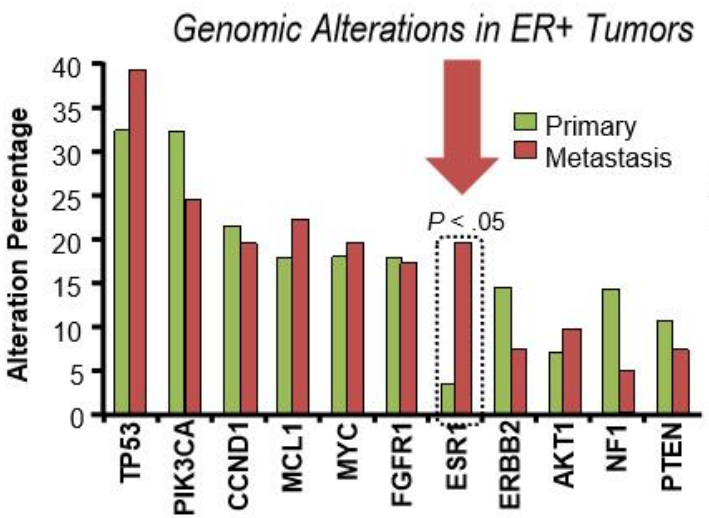
BELLE3 *PIK3CA* mutant



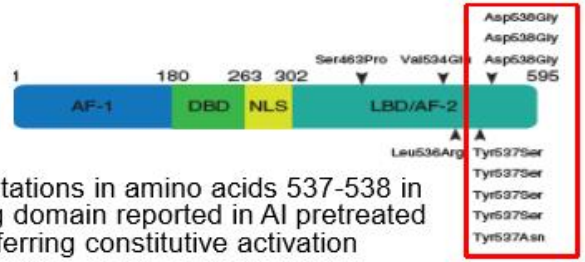
De Leo *et al* SABCS 2016

Selecting therapy for MBC patients

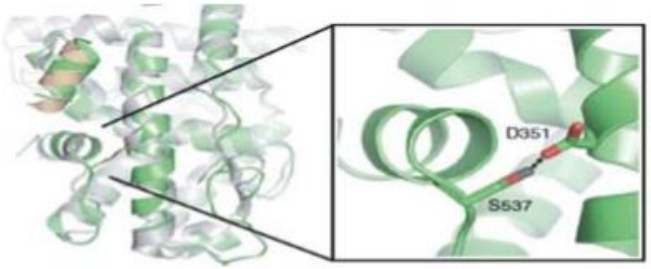
BACKGROUND - ESR1 MUTATIONS IN ADVANCED BREAST CANCER



ESR1 mutations occur in ~20% of endocrine resistant ER positive breast cancer

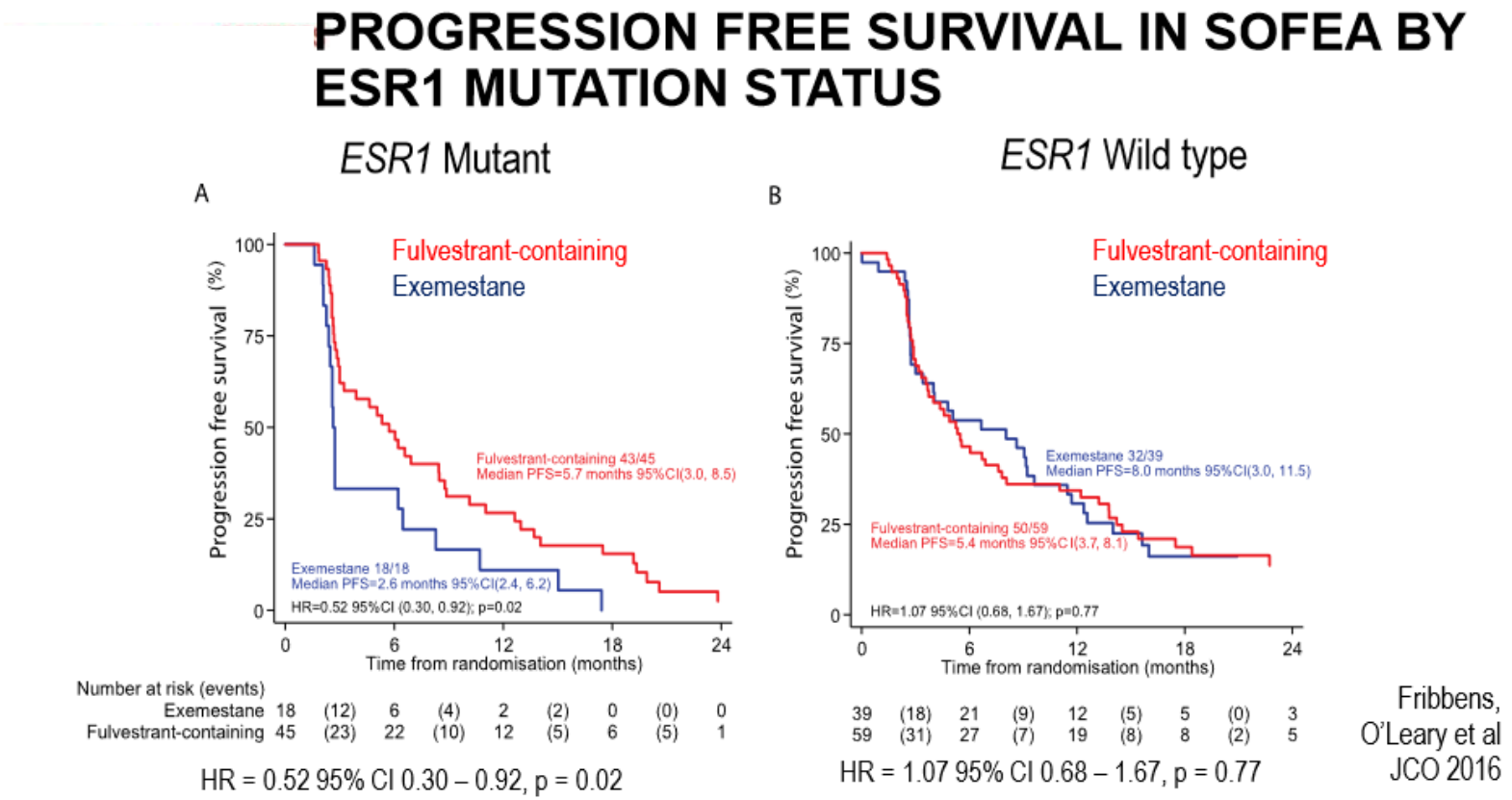


Cluster of mutations in amino acids 537-538 in ligand-binding domain reported in AI pretreated pts, conferring constitutive activation



Zhang, Q.X. et al. *Cancer Res* 1997, Li, S. et al. *Cell Reports* 2013, Toy, W. et al. *Nat Gen* 2013,

Selecting therapy for MBC patients

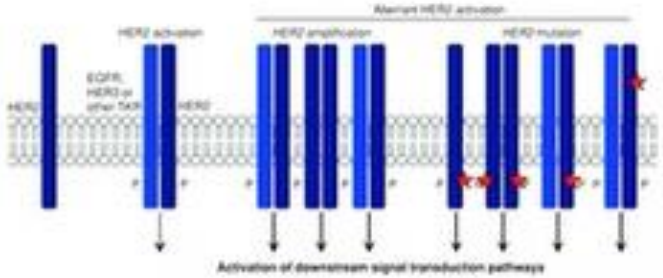


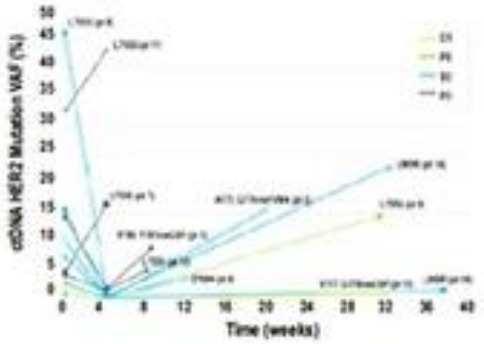
Selecting therapy for MBC patients

Plasma HER2 mutations and targeted therapy

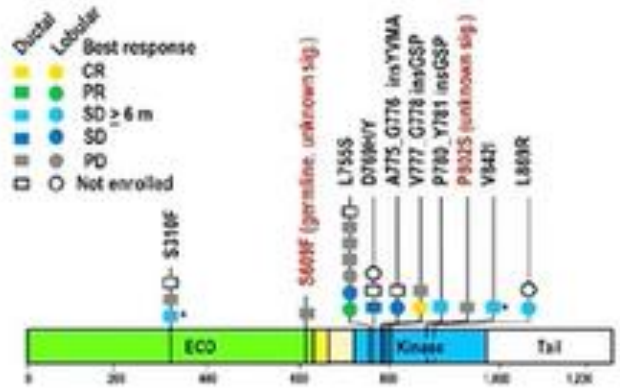
Mutant HER2 trial:

- Plasma HER2 mutations identified in 79% of tumour positive cases
- Neratinib showed a CBR of 31% in patients with HER2 mutations



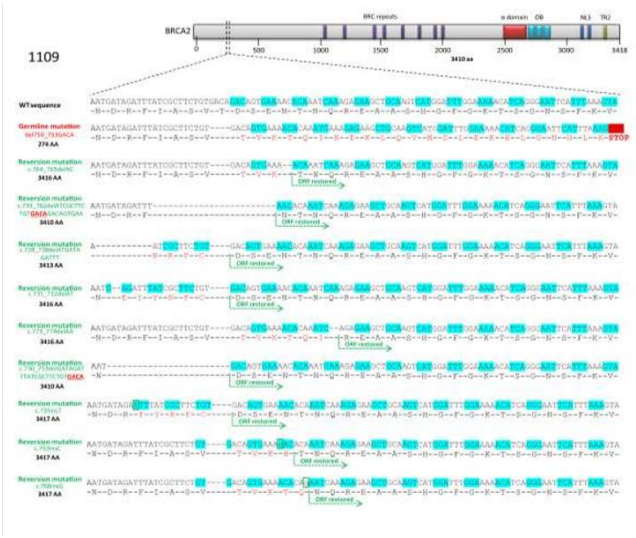


Ma et. al., CCR, 2017



Courtesy of Sarah-Jane Dawson

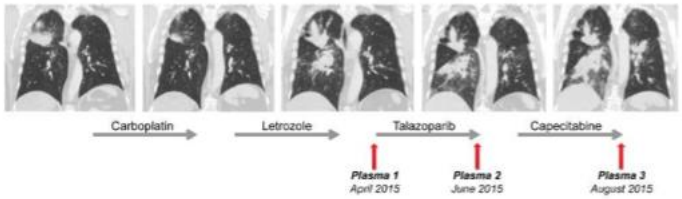
Selecting therapy for MBC patients

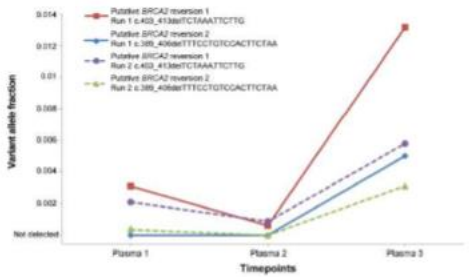


DETECTION OF BRCA REVERSION MUTATIONS IN BRCA MUTANT CANCERS

Weigelt *et al* CCR 2017

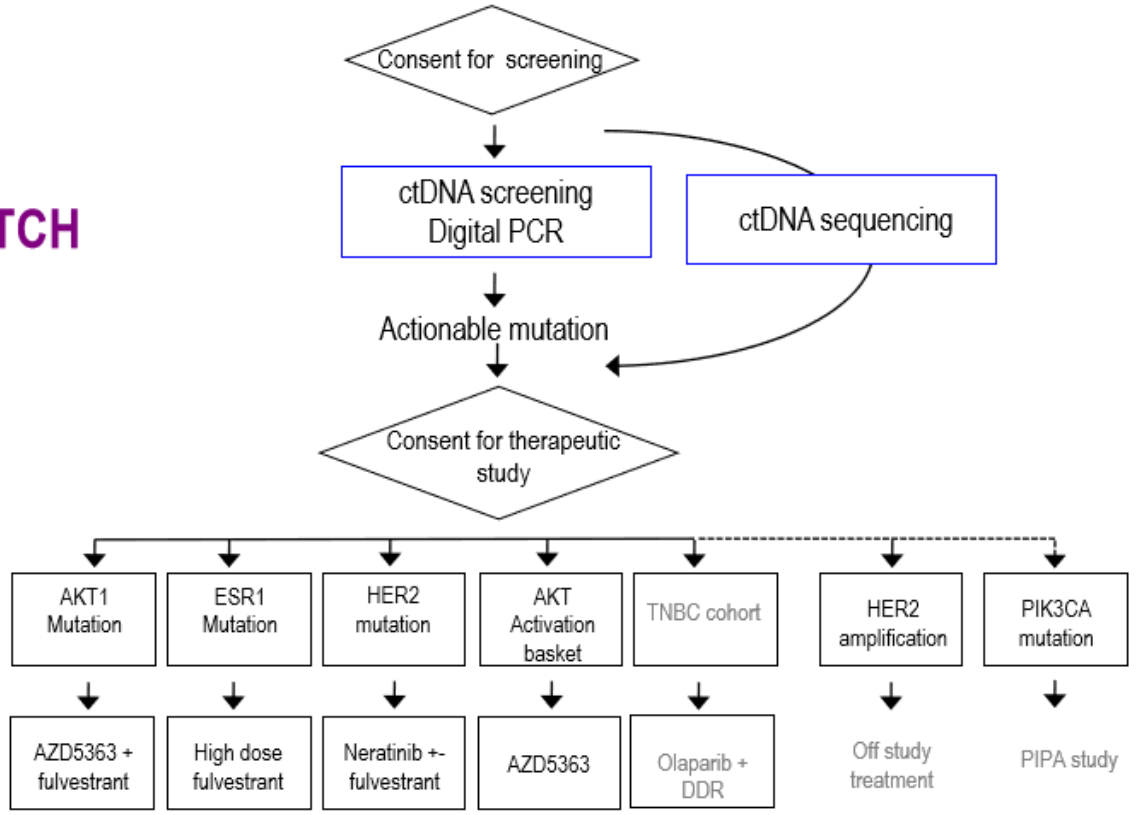
Detection of BRCA reversion mutations





Selecting therapy for MBC patients

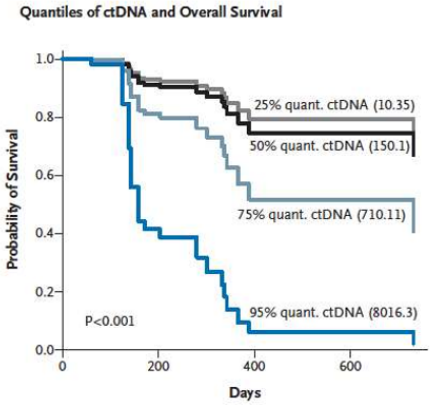
UK plasma MATCH



CANCER RESEARCH UK

Monitoring of Therapy

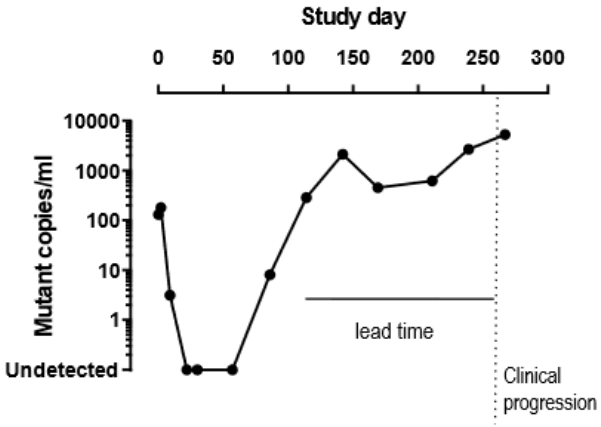
TUMOUR BULK SURROGATES

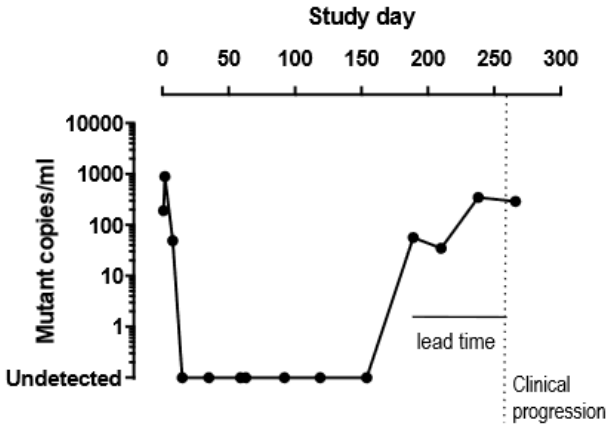


Level of circulating tumour DNA is prognostic

Can ctDNA be used to predict response to therapy?

Changes in ctDNA abundance through therapy

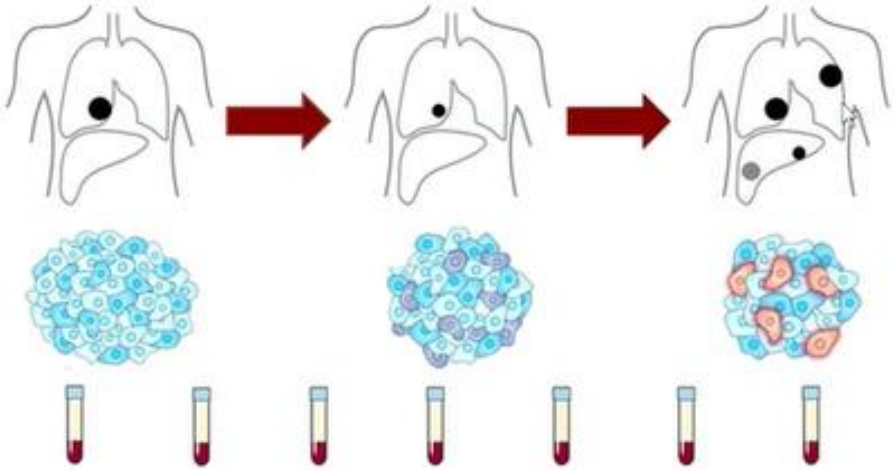




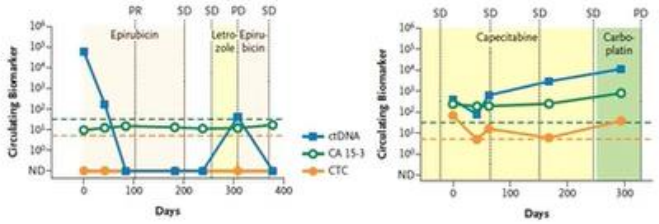
Turner *et al* AACR 2015

Monitoring of Therapy

ctDNA as a "Liquid Biopsy"

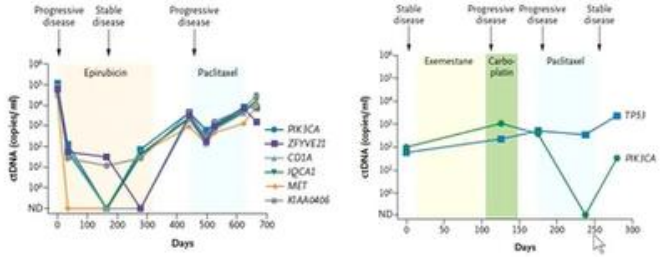


ctDNA to monitor treatment responses



- Changes in levels of circulating tumour DNA closely follow treatment responses
- Tracking levels of circulating tumour DNA may provide an early indicator of treatment resistance

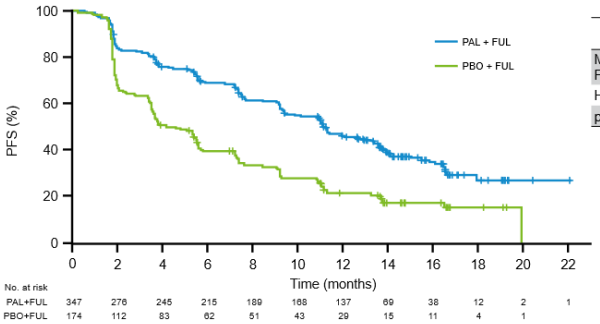
ctDNA to monitor treatment responses



- Multiple mutations often show similar dynamic changes when tracked simultaneously
- Evidence of clonal heterogeneity: different clones show diverging patterns over the course of treatment

Monitoring of Therapy

PALOMA3 – palbociclib and fulvestrant



	PAL+FUL (n=374)	PBO+FUL (n=174)
Median (95% CI) PFS, mo	11.2 (9.5, 12.9)	4.6 (3.5, 5.6)
HR (95% CI)	0.497 (0.398, 0.620)	
p value	<0.00001	

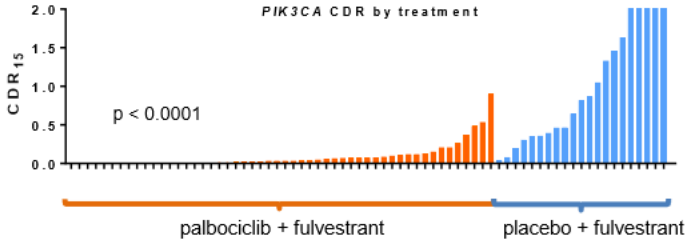
Turner et al NEJM 2015, updated SABCS 2016

ctDNA DYNAMICS AND TREATMENT MONITORING

Fulvestrant +- palbociclib

ctDNA samples
C1 day1 and day 15

Circulating dynamic ratio
day15 relative to day1 level

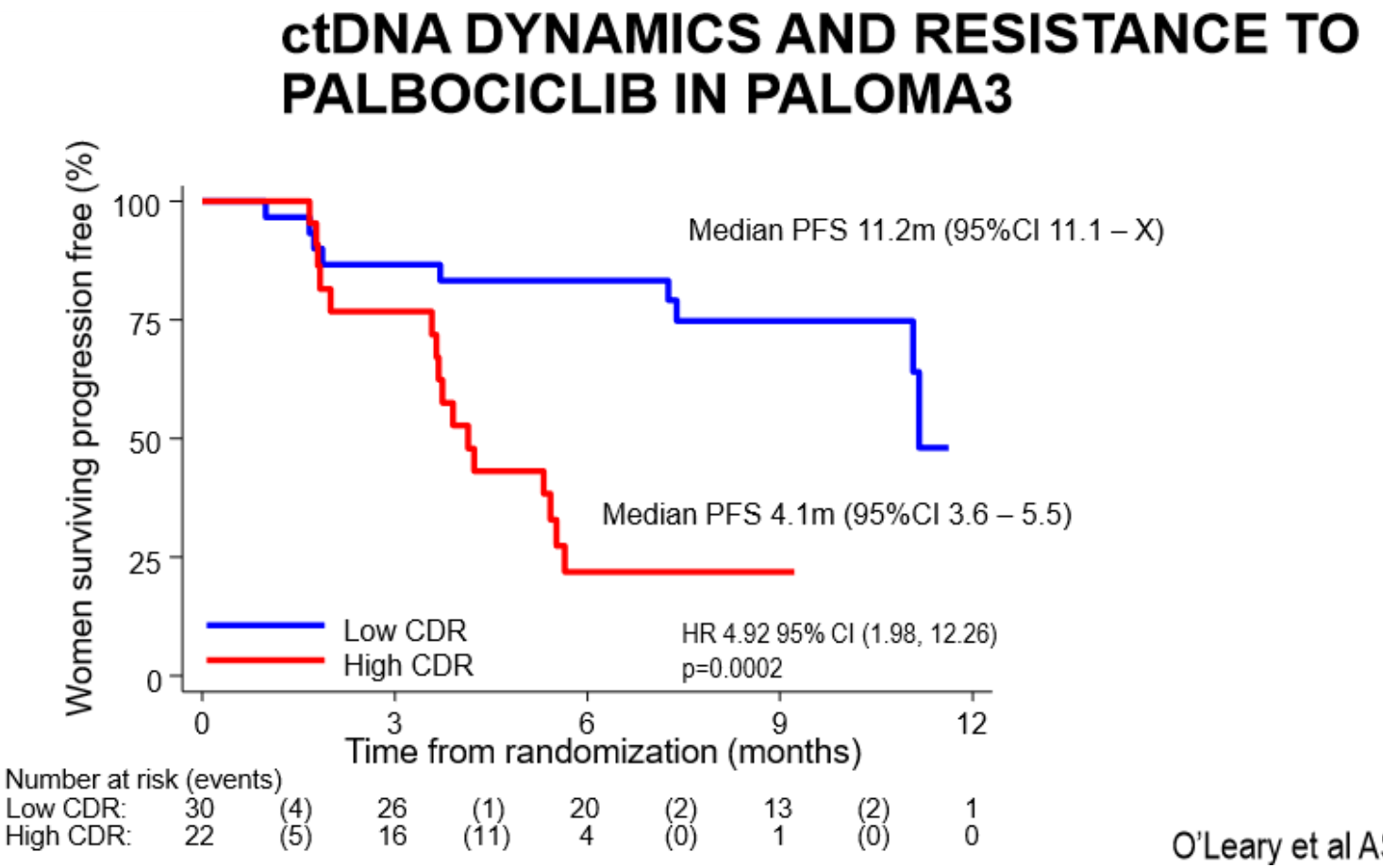


- Palbociclib + fulvestrant suppressed day 15 *PIK3CA* ctDNA levels to a greater extent than placebo + fulvestrant (p<0.0001).

O'Leary et al ASCO 2017

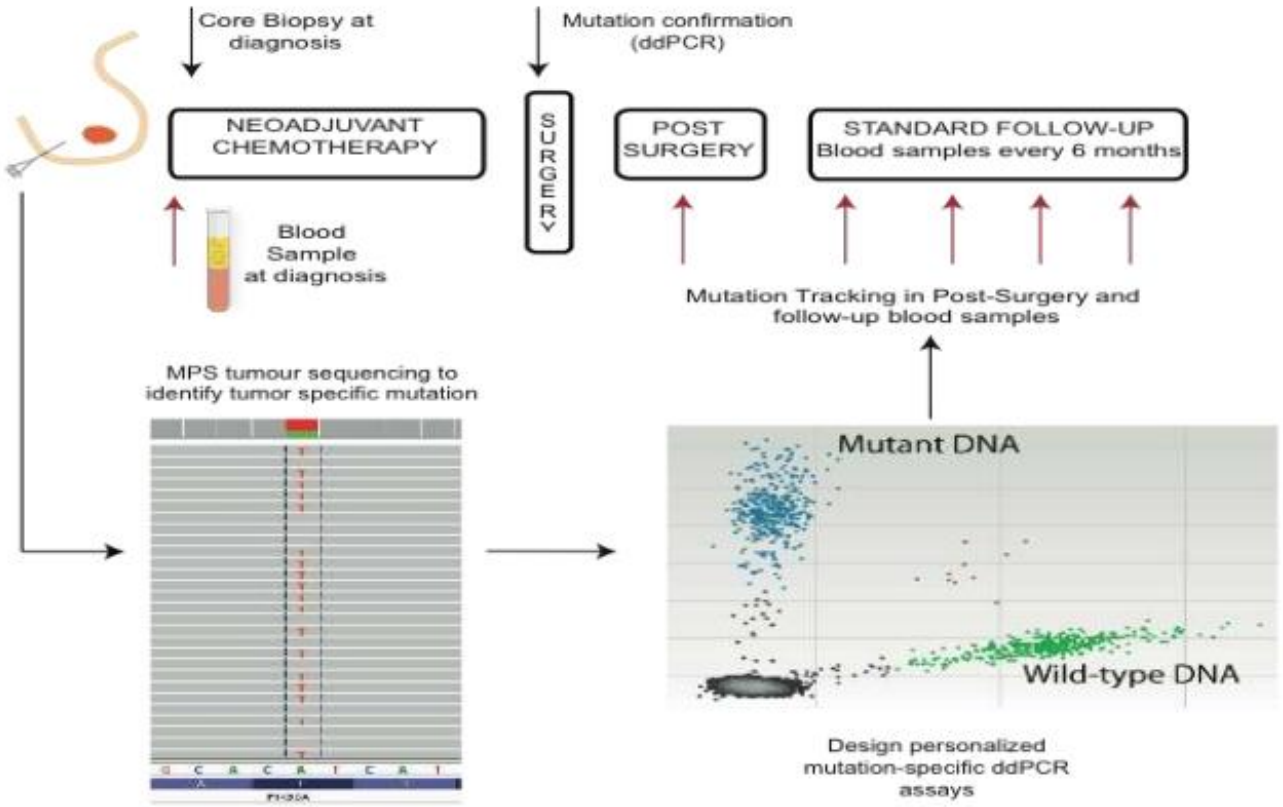
CDR – circular dorsal ruffles

Monitoring of Therapy



O'Leary et al ASCO 2017

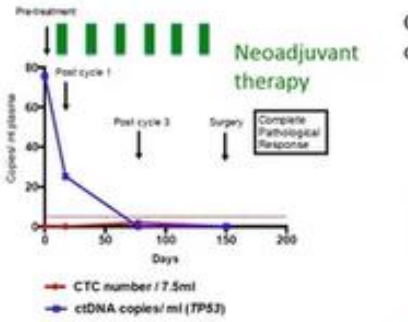
Detecting Minimal Residual Disease Predicting Early Relapse in EBC…



Garcia-Murillas et al Science Trans Med 2015

Detecting Minimal Residual Disease

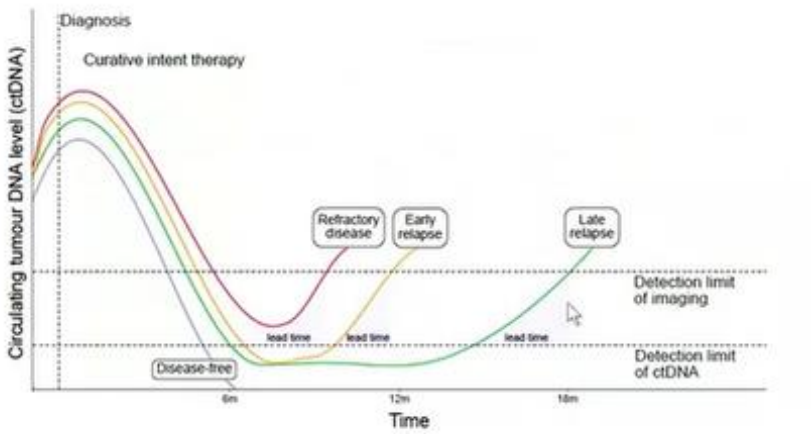
ctDNA and monitoring response to neoadjuvant therapy



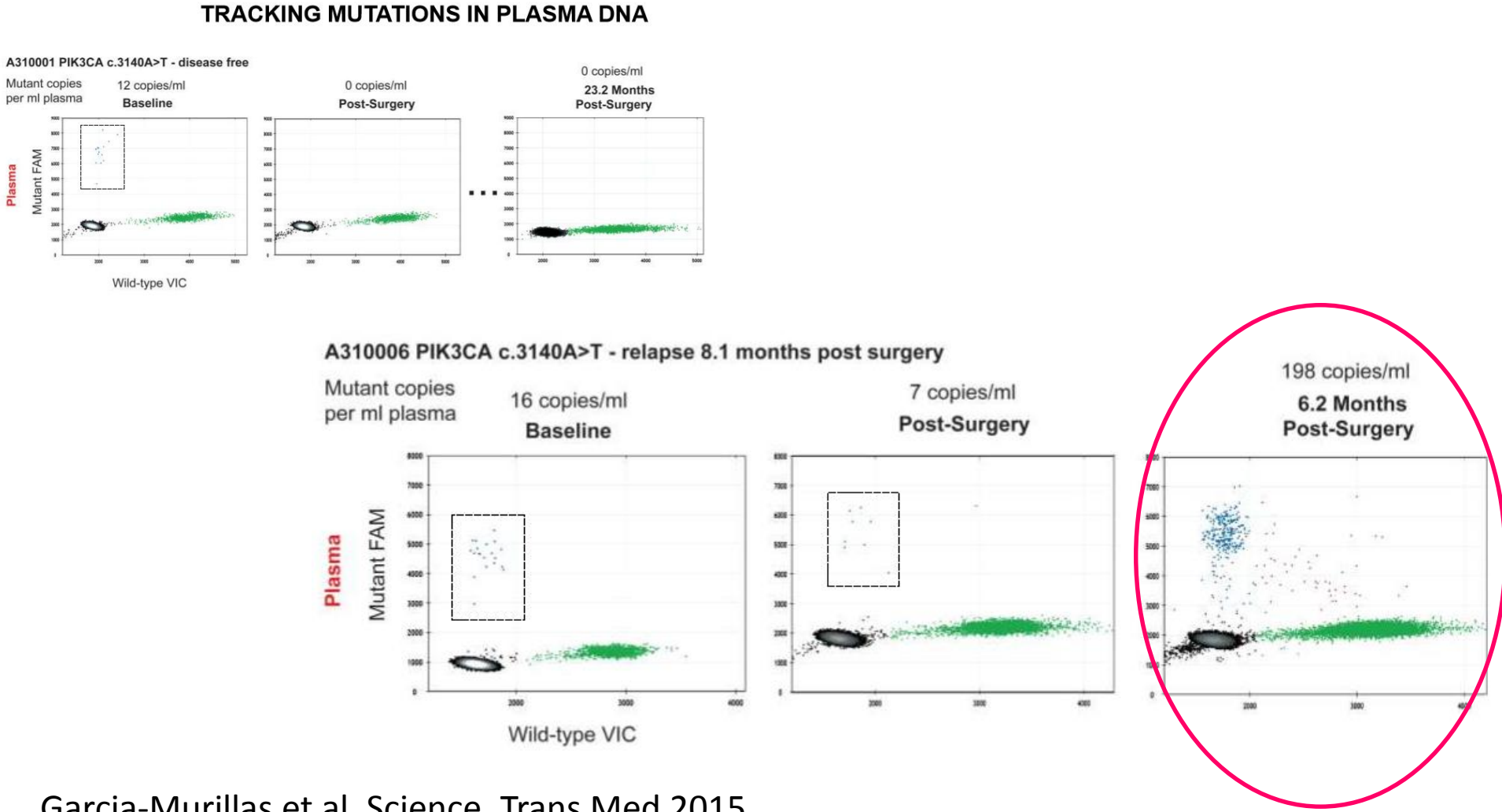
Circulating tumor DNA in HER2 amplified breast cancer NeoALTTO phase 3 trial : Abstract 371, PD3-03

Detection of ctDNA before commencement of neoadjuvant targeted therapies was associated with decreased rate of pathological complete response

ctDNA and minimal residual disease



Detecting Minimal Residual Disease Predicting Early Relapse in EBC…

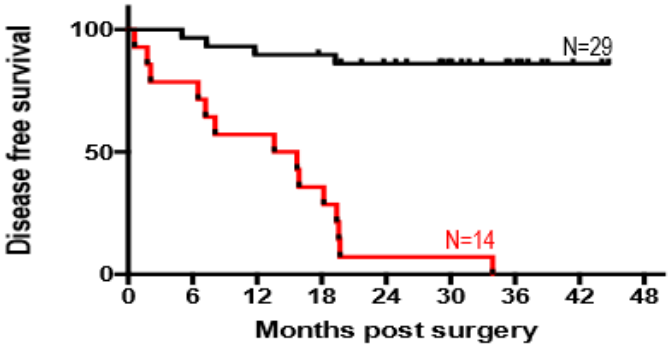


Garcia-Murillas et al, Science, Trans Med 2015

Detecting Minimal Residual Disease Predicting Early Relapse in EBC…

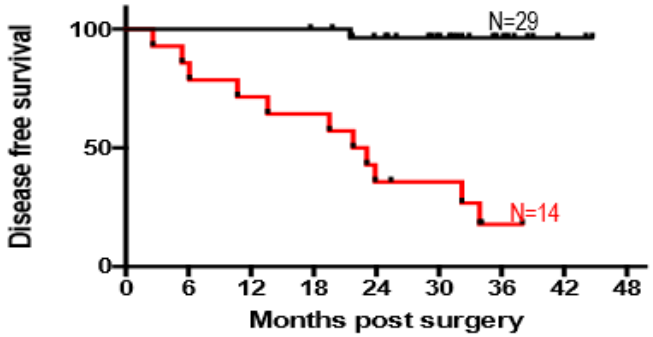
PREDICTING RELAPSE WITH SERIAL SAMPLE TRACKING

Disease free survival



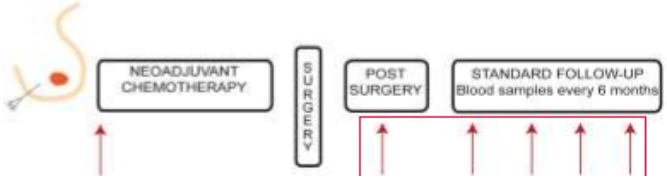
HR=25.7 05% CI(8.3, 79.8) p<0.001

Overall survival



HR=47.1 05% CI(6.1, 366.1) p<0.001

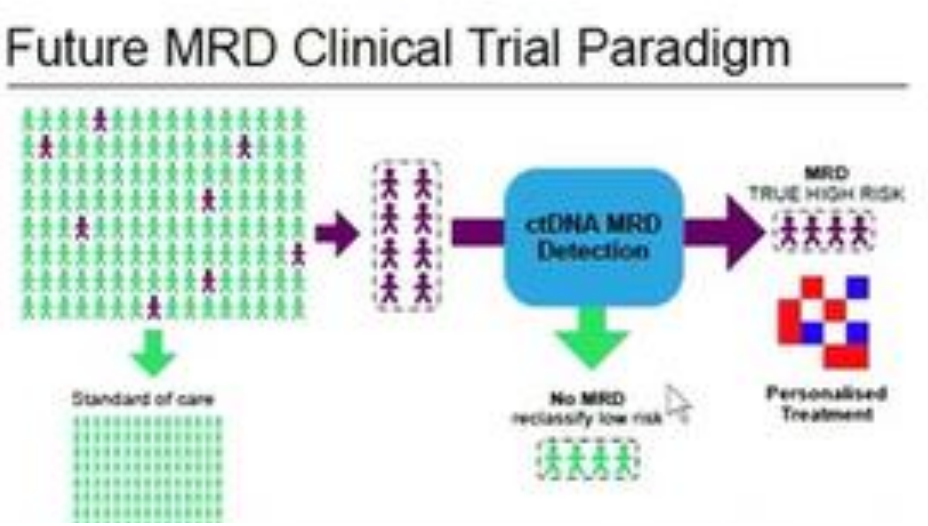
100% positive predictive value for relapse
Median lead time 7.9 months



Better Risk Stratification

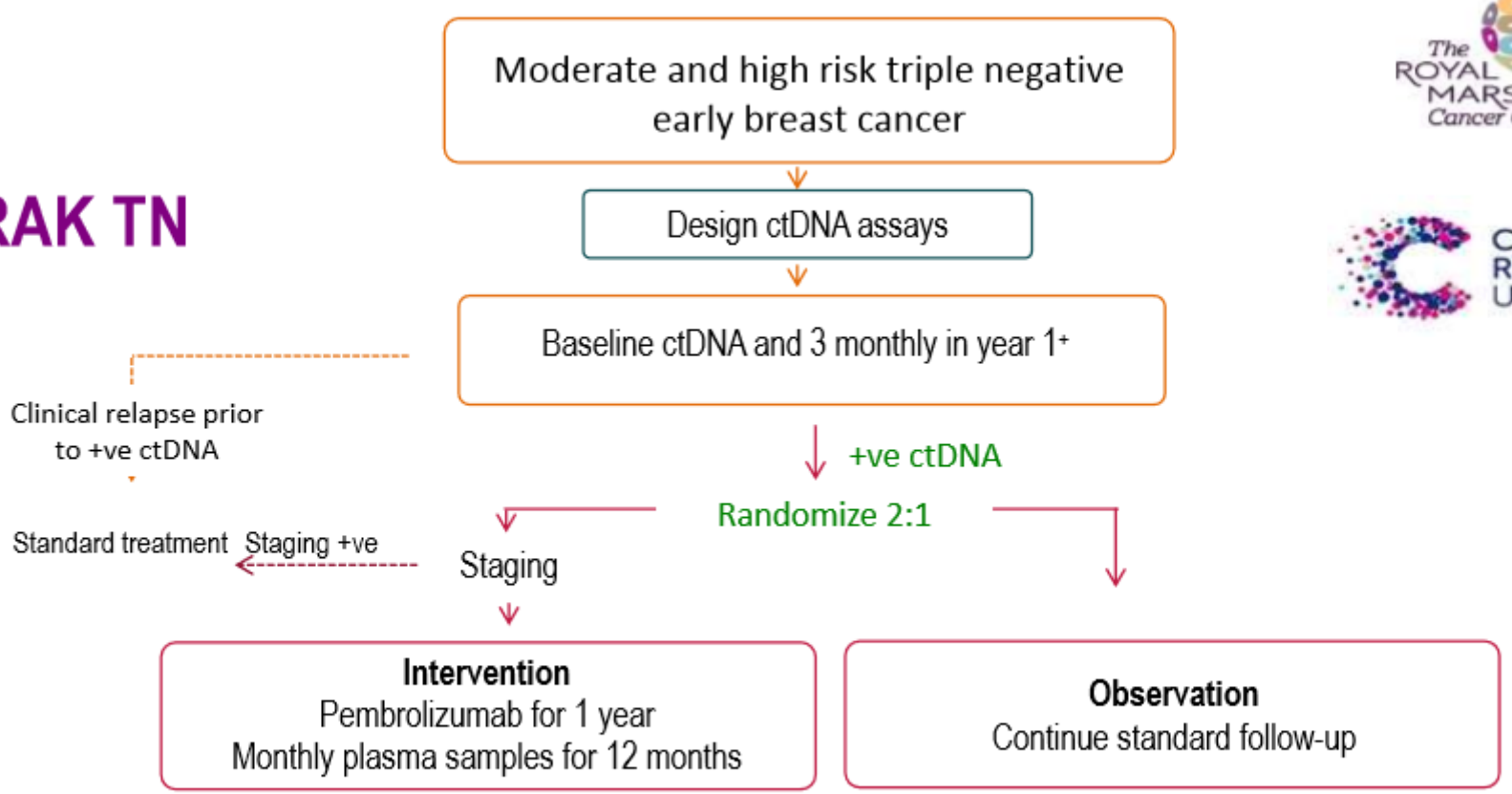
De-escalate adjuvant therapy to reduce toxicity and costs
Prioritize intensive and novel approaches for those likely to benefit

ctDNA, risk stratification and future clinical trial design



Detecting Minimal Residual Disease

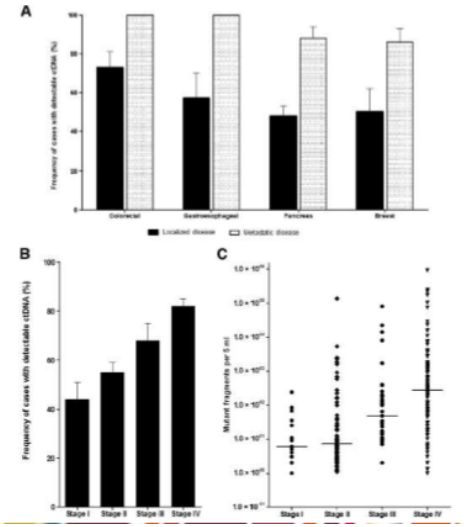
cTRAK TN



+All patients ctDNA negative in the first year have continued blinded ctDNA 3 monthly in year 2 but will not be randomised on the resul

Screening for Cancer

Detection of ctDNA in early stage cancer



Looking for known somatic mutations in blood

ctDNA, early detection and screening

Table 1. Comparison of ctDNA Liquid Biopsy Test to Potential Cancer Screening Test

Indication	Tumor Liquid Biopsy (Genotyping, Monitoring)	Early Cancer Detection
Target population	Patients with known diagnosis of cancer	Asymptomatic individuals
Tissue reference	Can be informed by tissue analyses	No prior knowledge of tissue
Key performance characteristics	Sensitivity and specificity for specific actionable genotypes	• Sensitivity and specificity for clinically detectable cancer • Premium on specificity in individuals without detectable cancer • Tissue of origin needed to guide workup
Clinical Endpoint for Utility	Therapeutic benefit with specific therapies	Net outcome improvement with early detection and local treatment of cancer
Genes Covered	10-50	100-1000s
ctDNA Limit of Detection	0.1%	<0.01%
Importance of Novel Variant Detection	Low	High
Amount of Sequencing	1x	100X
Study Size for Clinical Validity and Utility	100's	10,000 - 100,000 s

A. Arvanis et. al., Cell, 2017

Opportunities and Challenges of

- Circulating Tumour Cells (CTCs)
- Circulating Tumour DNA (ct DNA)
- **Exosome and EVs**

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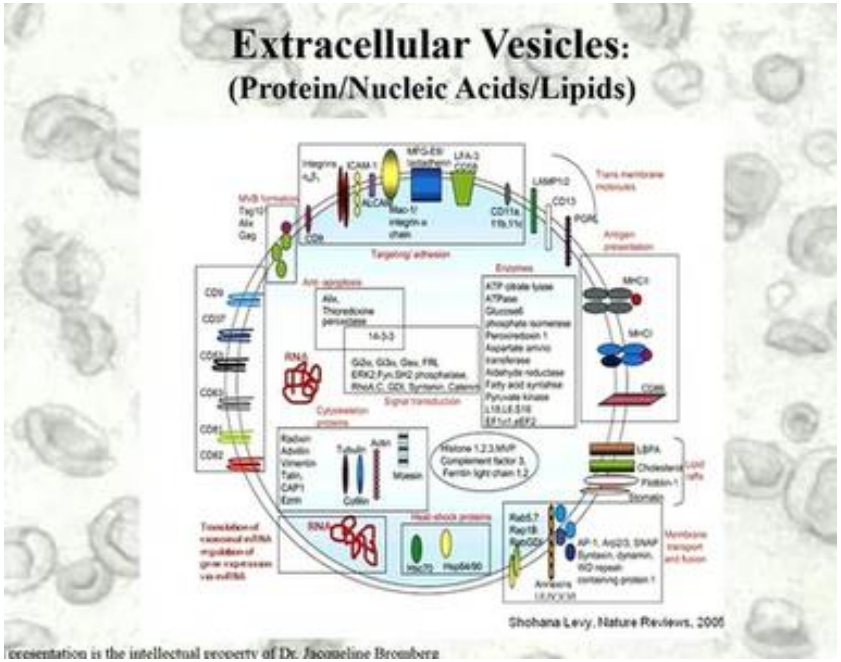
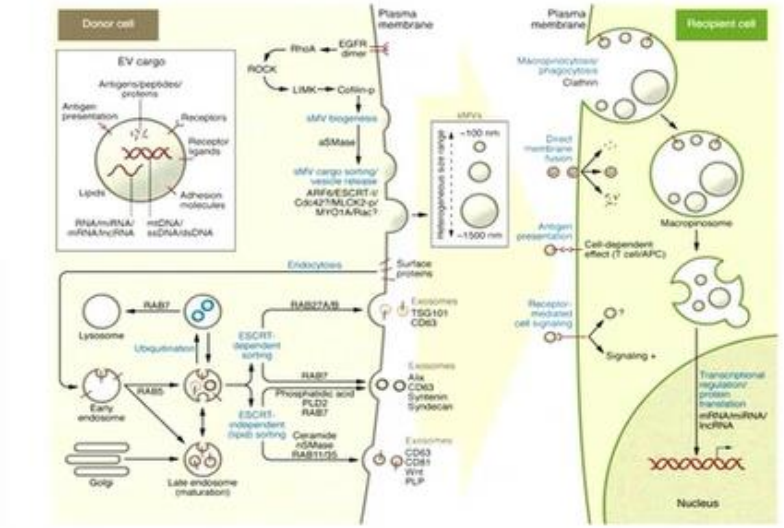
Extracellular Vesicles

- Exosomes & microvesicles
- All cells produce extracellular vesicles
  - Reticulocytes, megakaryocytes
- Tumours
  - Cancer cells, stromal cells, immune cells
- Oxidative Stress
  - Chemotherapy, radiotherapy
- Hypoxia
  - Aging, tumour microenvironment



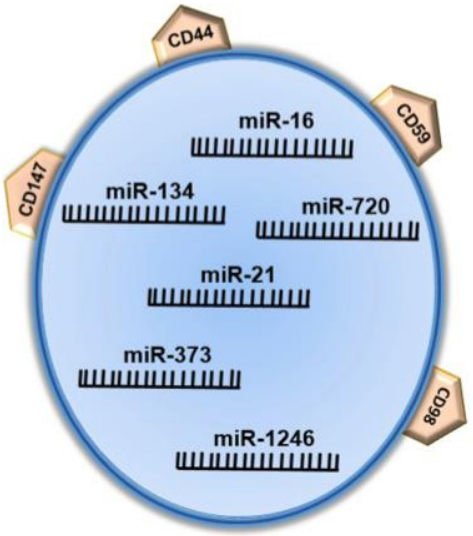
Exosomes

- First reported by Pan and Johnstone in 1983 at McGill University when culturing sheep reticulocytes.
- Lipid bilayered vesicles with endocytic origins, released into extracellular region by a variety of mammalian cells including CANCER cells.
- Exosomes from different types of cells enclose different proteins in their biogenesis.
- Exciting in a vast range of biofluids s.a. serum, urine, plasma, breast milk saliva, malignant pleural fluid, BALs etc.



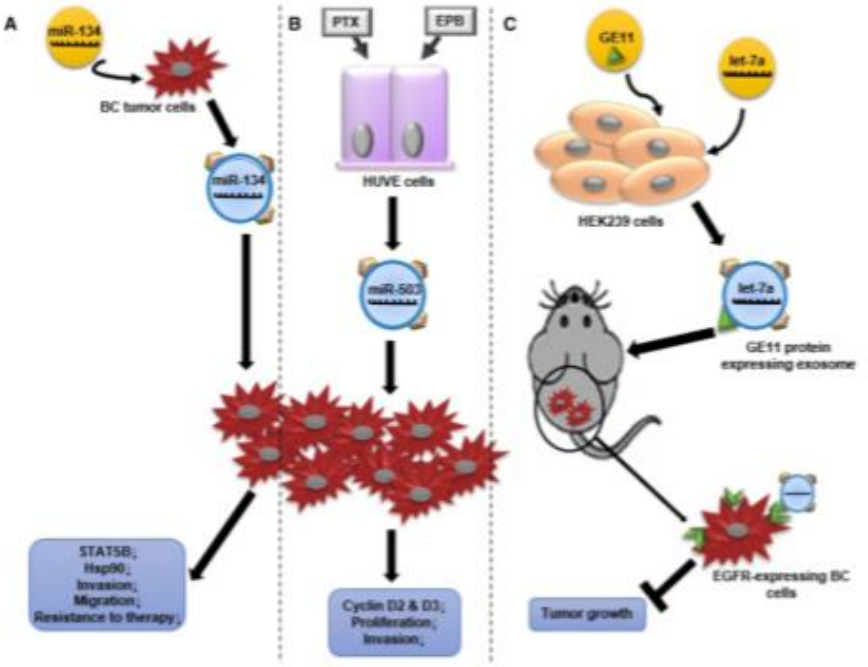
Sina Halvaei et al, 2018

Exosomes



Schematic Representation of TNBC Exosomes

TNBC exosomes represent some surface proteins, including CD98, CD147, and CD59, and some overexpressed miRNAs (miR-134, miR-21, miR-373, and miR-1246).



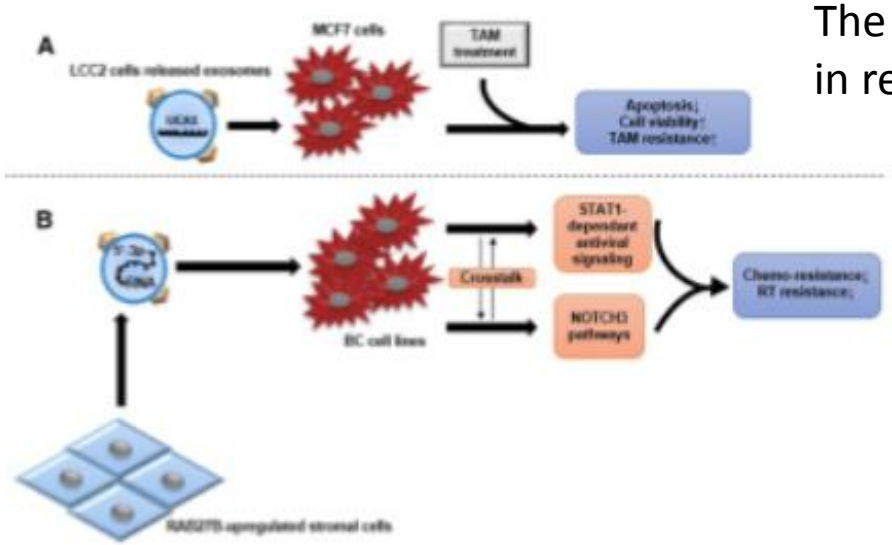
Proposed Exosomes' Role in BC Treatment

(A) miR-134 transfected Hs578Ts cells released miR-134-carrying exosomes that can downregulate STAT5B and HSP90 expression. Also, these exosomes reduce migration and invasion, and increase anti-HSP90 drug sensitivity in secondary Hs578Ts cells. (B) Human umbilical vein endothelial (HUVE) cells released miR-503-over-expressing exosomes after PTX and EPB treatment. These exosomes had the potential to reduce BC invasion and cyclin D2 and D3 expression that led to decline in BC cells proliferation. (C) Human embryonic kidney cells (HEK293) were transfected with GE11 protein (specifically binds to EGFR-expressing cells) and let-7a miRNA. HEK293 cells released GE11-expressing and let-7a-overexpressing exosomes, which bind specifically to EGFR-expressing xenograft BC tissues, and inhibited tumor development in animal model.

Blood-based exosomes,
Breast Milk, breast fluid derived Exosomes

Sina Halvaei et al, 2018

Predicting endocrine resistance



A. Tamoxifen-resistent BC cells secrete UCA-1 over-expressed exosomes which can cause Resistence to Tamoxifen, decreasing apoptosis

B.RAB27B-upregulated stromal cells release Exosomes that contain 5' triphosphate RNAs and activate STAT1 dependent signalling and NOTCH3 pathways in adjuvant BC cells
The cross talk between these 2 pathways result in reduction of chemo-resistance and RT-resistance

Sina Halvaei et al, 2018

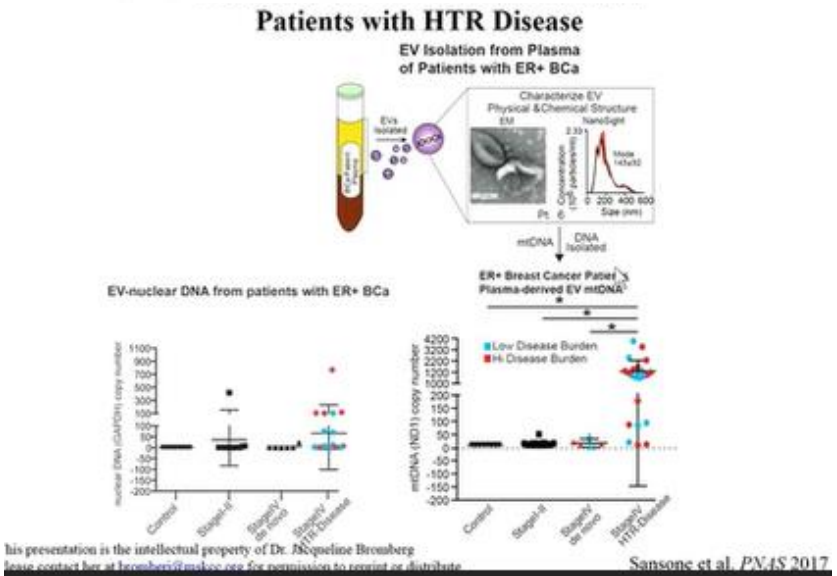
mtDNA in Extracellular Vesicles…

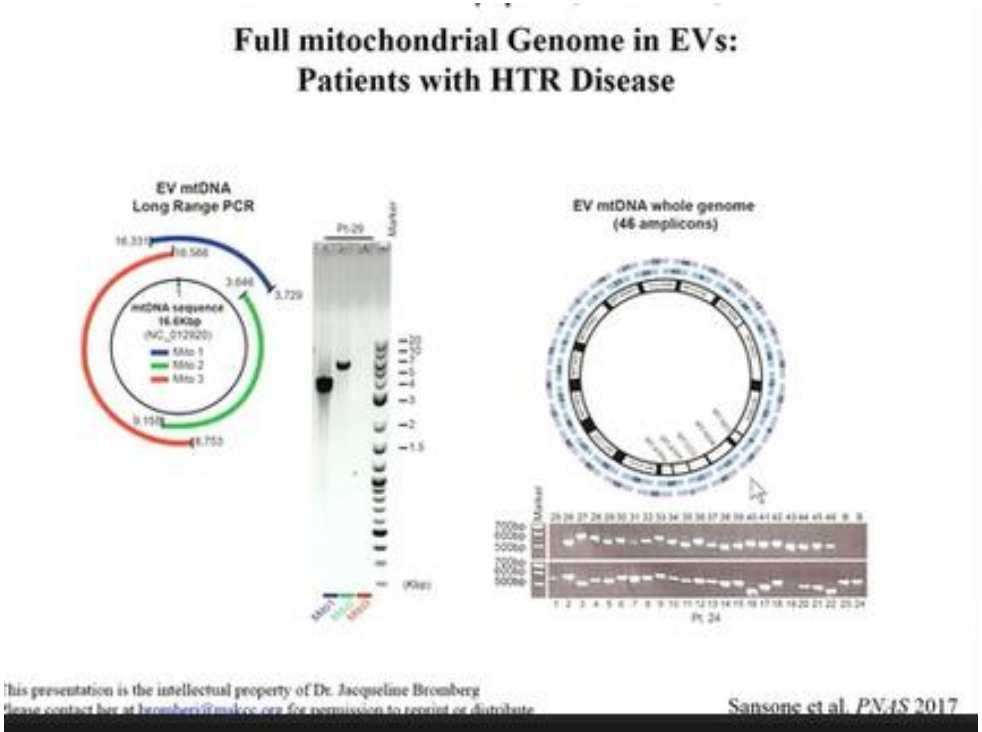
mtDNA Copy Number/Heteroplasmy
Cancer Evolution
Metastasis & Resistance

mtDNA in EVs

Regulation of Metabolism
Resistance to Cancer Therapy

Courtesy of Dr. Jacqueline Bromberg





Conclusion

- There is an emerging added value of liquid biopsy
- The contribution of CTCs, ctDNA and exosomes and others – in the monitoring of treatment response, detecting minimal residual disease, selecting patients for specific therapy and screening for cancer
- From personalized medicine to precision medicine, sparing the low risk group from intesne therapy and putting the high risk group for more intense and innovative therapy with predicted clinical benefits.
- This may reduce the unnecessary exposure to repeated scans or imaging and invasive procedures in the days to come.

Acknowledgement

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Dr. Nicolas Turner
Professor Maria Lung

GBCC 2018 Organizing Committee
Co-Chair and Speakers for this session.

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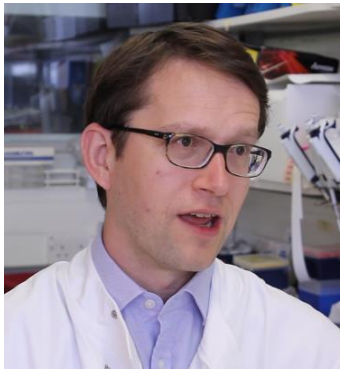
All Council Members and Secretariat
Hong Kong Breast Cancer Foundation (HKBCF)
Hong Kong Society of Breast Surgeons
All Department Heads and Chief of Service
of all Cancer Centres



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Liquid Biopsy: CTC, ctDNA, Exosome etc.

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5th April, 2018
Incheon, Korea

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