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January 27, 2018

40 YEAR ANNIVERSARY
SAN ANTONIO BREAST CANCER SYMPOSIUM®

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San Francisco, CA
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January 27, 2018

New Directions in Translational Research, Prognostic Markers, and Resistance Mechanisms

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Disclosure(s)

LAURA van ’t VEER, PhD

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Speaker’s Bureau:	**N/A**
Advisory Panel/Consultant:	**N/A**
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Dr. van t’ Veer will not discuss investigational or off-label use

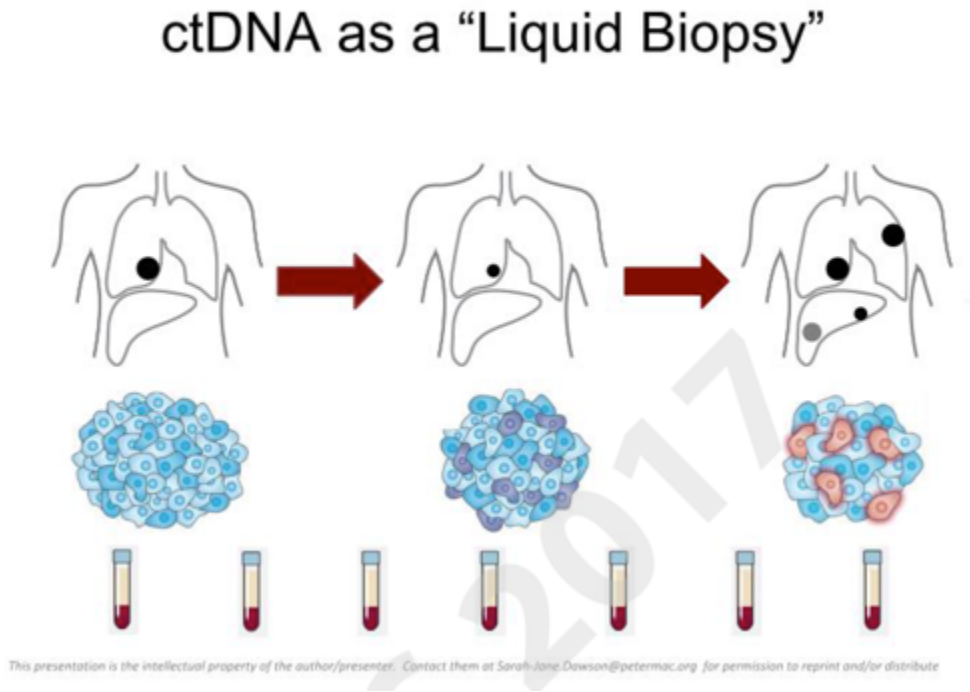
Review of Presentations

- Liquid Biopsies in Breast Cancer
  Sarah Dawson, Melbourne; educational
- Liquid Biopsies in Breast Cancer
  Nick Turner, Royal Marsden; AACR outstanding investigator award
- CTC's and Late Recurrence
  Joe Sparano, ECOG-ACRIN; GS06-3
- Intratumoral Heterogeneity
  Sarat Chandarlapaty, MSKCC; Basic Science Forum BSF03-1
- BRCA2 VUS implications for risk and treatment
  Fergus Couch, Mayo Clinic; GS4-06

Liquid Biopsies in Breast Cancer

Sarah Dawson, Melbourne; educational

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



ctDNA =
circulating DNA
Also called
cfDNA =
cell-free DNA

Different from
CTC =
circulating tumor cells
(same concept)

Liquid Biopsies in Breast Cancer

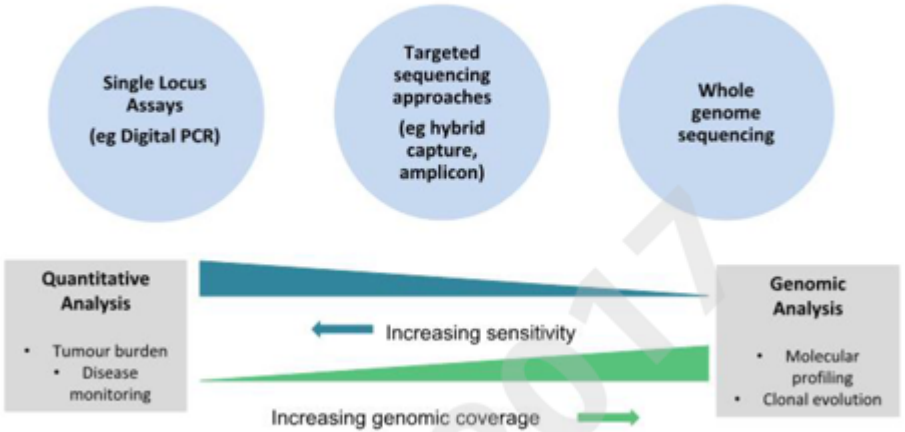
Sarah Dawson, Melbourne; educational

Pre-analytical considerations

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	

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

Methods of ctDNA analysis



Liquid Biopsies in Breast Cancer

Sarah Dawson, Melbourne; educational

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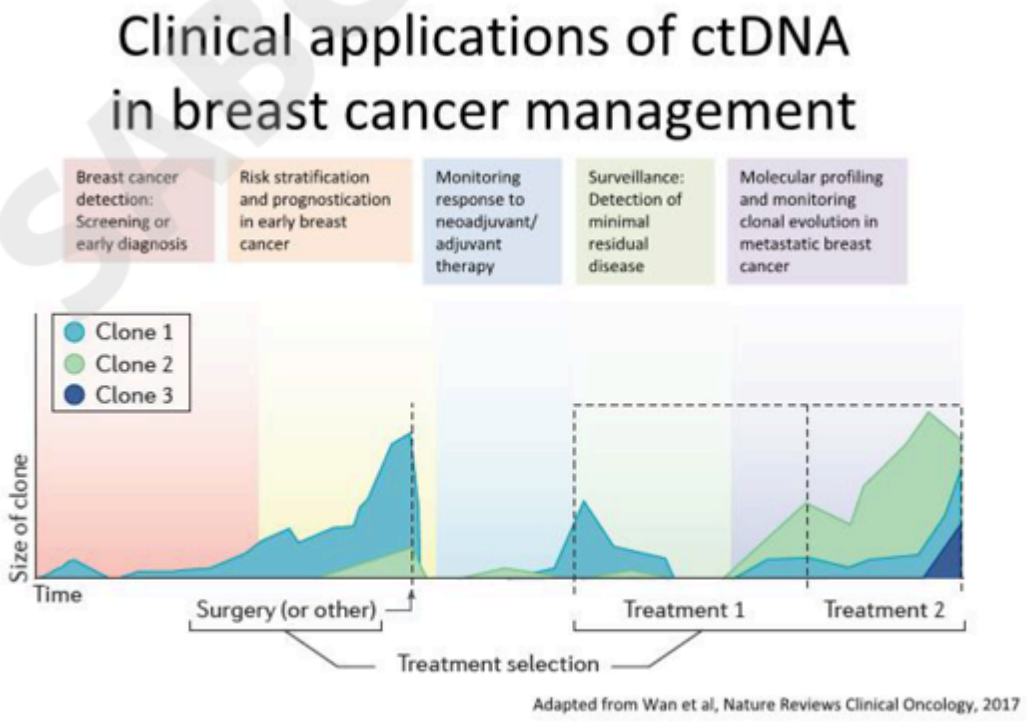
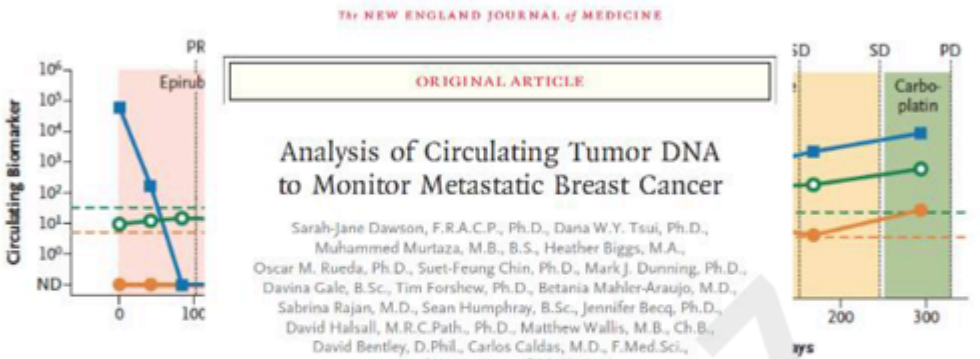


Liquid Biopsies in Breast Cancer

Sarah Dawson, Melbourne; educational

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

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

Liquid Biopsies in Breast Cancer

Sarah Dawson, Melbourne; educational

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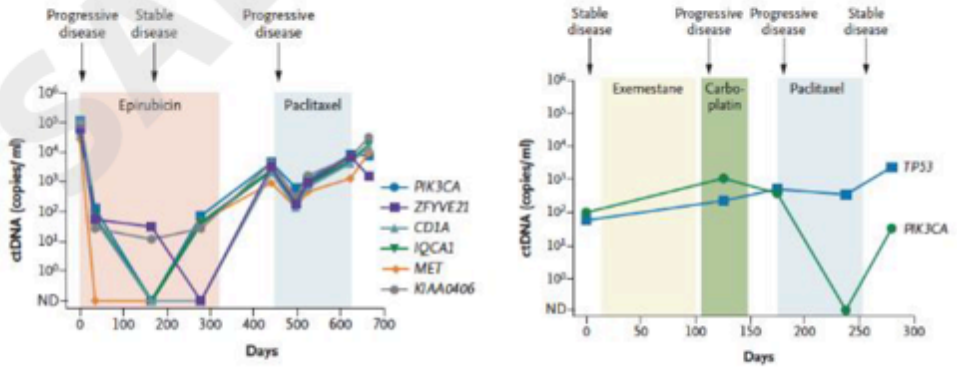
ctDNA to monitor treatment responses



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

Liquid Biopsies in Breast Cancer

Nick Turner, Royal Marsden; AACR outstanding investigator award

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

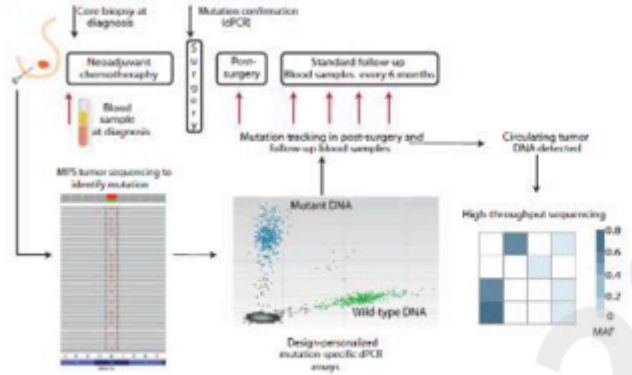
ctDNA during surveillance to detect early relapse

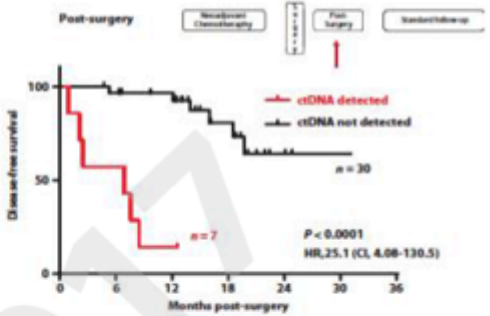
RESEARCH ARTICLE

CANCER

Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer

Isaac Garcia-Murillas,[1]* Gaia Schiavon,[1,2]† Britta Weigelt,[3] Charlotte Ng,[3] Sarah Hrebien,[1] Rosalind J. Cutts,[1] Maggie Cheang,[4] Peter Osin,[2] Ashutosh Nerurkar,[2] Iwanka Kozarewa,[1] Javier Armisen Garrido,[1] Mitch Dowsett,[1,2] Jorge S. Reis-Filho,[3] Ian E. Smith,[2] Nicholas C. Turner[1,2]†





Liquid Biopsies in Breast Cancer

Sarah Dawson, Melbourne; educational

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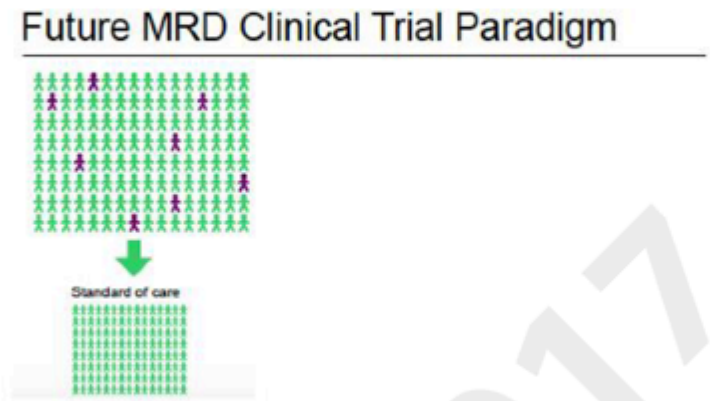
ctDNA, risk stratification and future clinical trial design

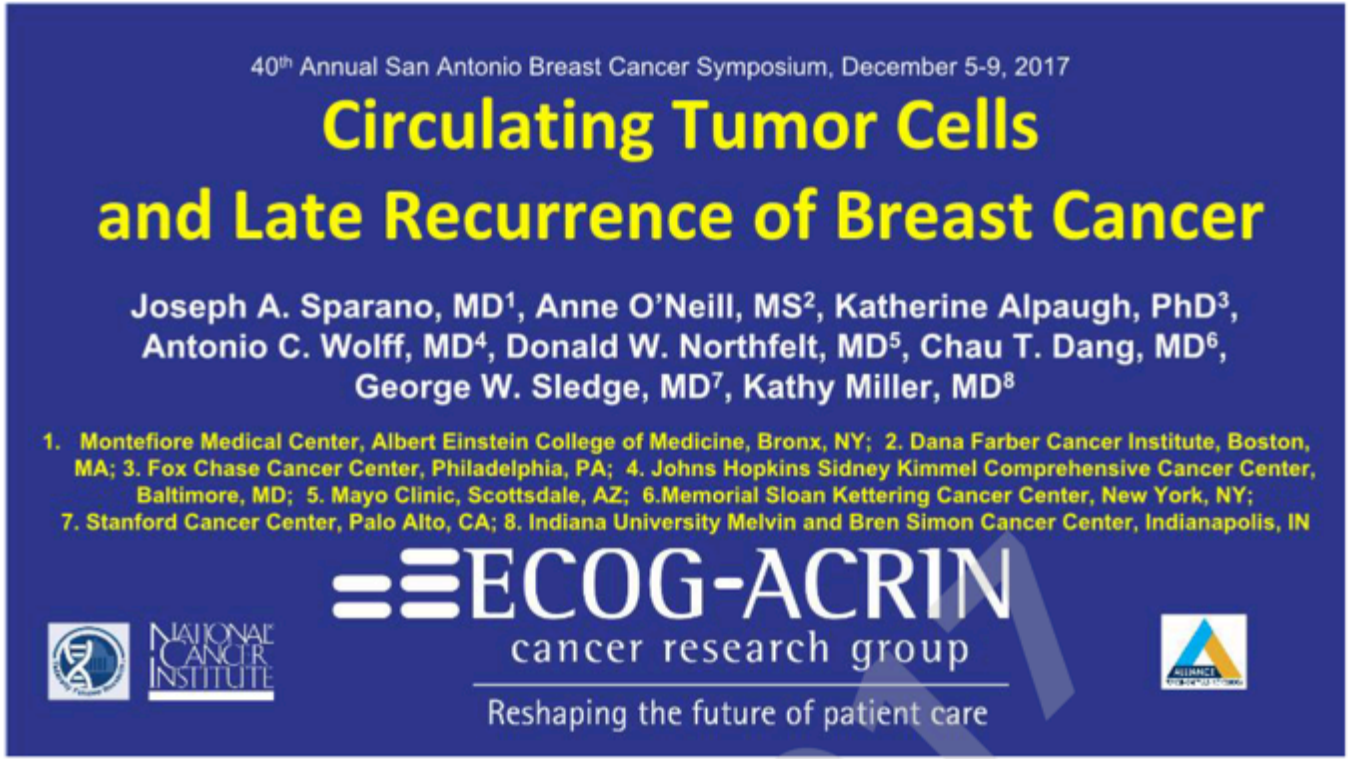


MRD
Minimal residual disease

ctDNA negative → • De-escalate adjuvant therapy to reduce toxicity and costs
ctDNA positive → • Prioritize intensive and novel approaches for those most likely to benefit

CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3



CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3

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

CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3

San Antonio Breast Cancer Symposium December 5-9, 2017

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

ECOG-ACRIN cancer research group

CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3

Methods: Hypothesis & Study Objectives

Hypothesis:

CTCs are prognostic for late recurrence

Study Objectives:

1. Prevalence of CTCs ~ 5 years after diagnosis
2. Association between CTCs and recurrence

Methods: Analysis Plan

- **Prospective study:** planned in accordance with REMARK[1]
- **Sample size:** distinguish CTC+ rate <1% vs. 5-10%
  - Interim after 233 patients, halt if the CTC+ rate <1% (β=0.10, α=0.05)
  - Accrual continued after interim analysis, but eventually halted after 3.4 years due to slowing accrual and close to target
- **Primary analysis population:** evaluable CTC sample and no invasive recurrence before or within 30 days of entry
- **Primary endpoint: time to recurrence (TTR):** date of sample to first invasive distant and/or local/regional recurrence

(1) McShane et al. J Natl Cancer Inst 2005;97:1180-4

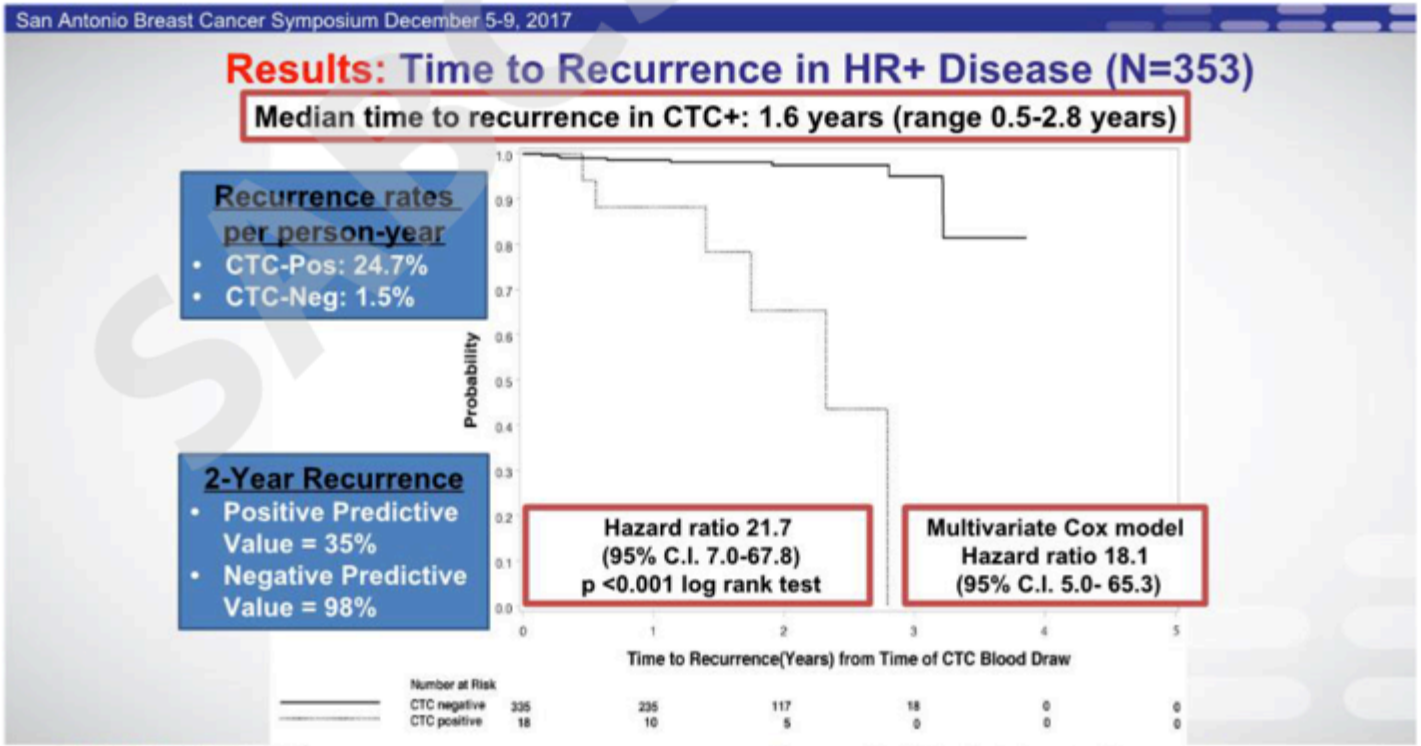
Methods: Study Design

- **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

ECOG-ACRIN

CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3



CTC's and Late Recurrence

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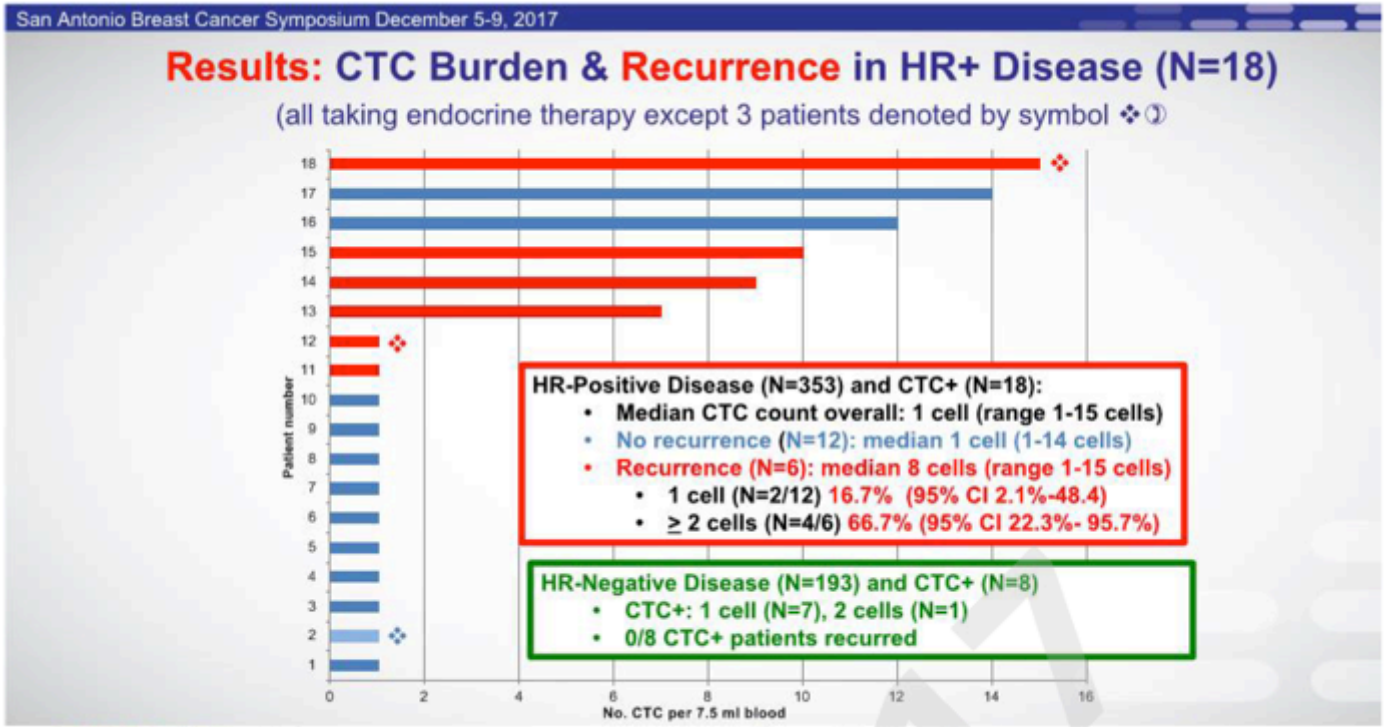


CTC's and Late Recurrence

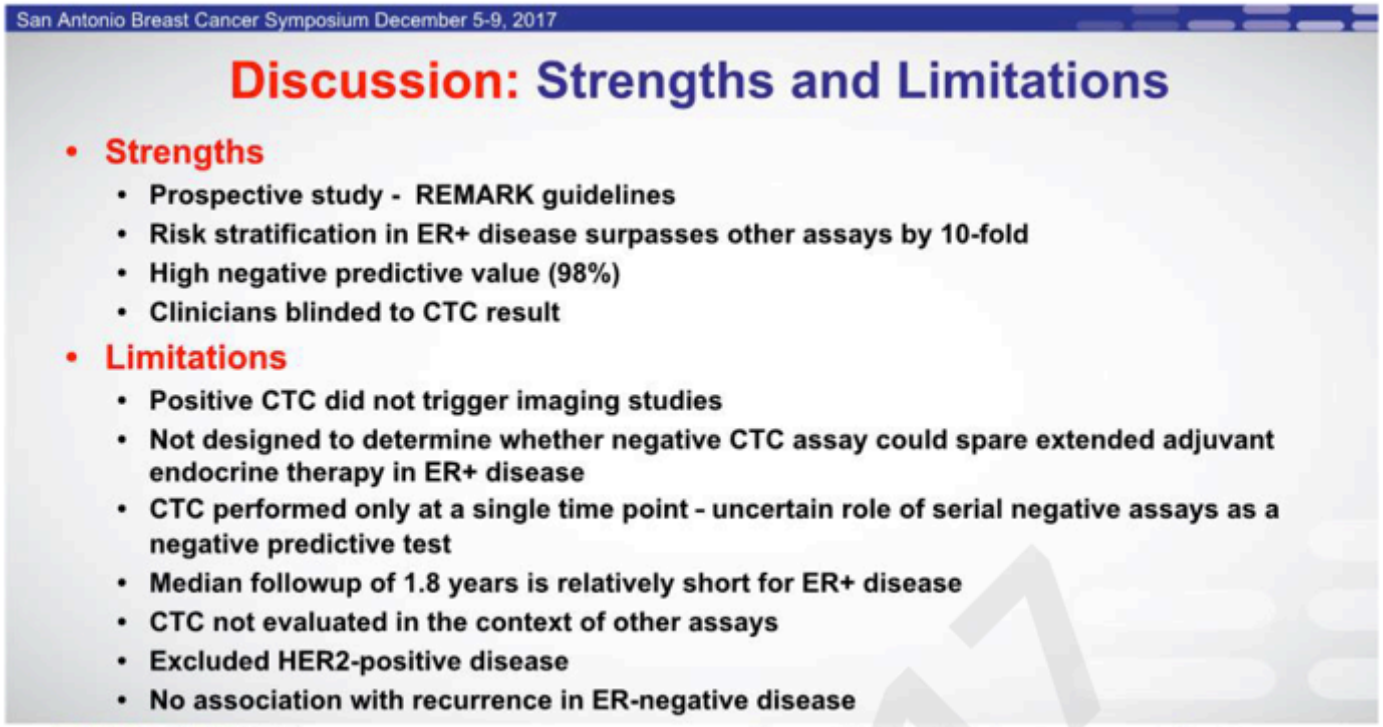
Joe Sparano, ECOG-ACRIN; GS06-3

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

CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3



CTC's and Late Recurrence

Joe Sparano, ECOG-ACRIN; GS06-3

Implications for Clinical Practice and Research

- **Proof of concept - "clinical validity"**
  - **Biomarker prognostic for late recurrence in HR+, HER2- early breast cancer**
  - **Supports concept of a second "decision point" to tailor therapy based on biomarker results**
- **Further study - "clinical utility"**
  - **Negative CTC assay - spare extended adjuvant endocrine therapy**
  - **Positive CTC assay - benefit from novel therapeutic approaches (eg. oral SERDS, CDK4/6 inhibitors)**
  - **Comparison and/or combination with other biomarkers (ie, ctDNA)**

Intratumoral Heterogeneity

Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1

Manifestations and implications of subclonal alterations in metastatic breast cancer

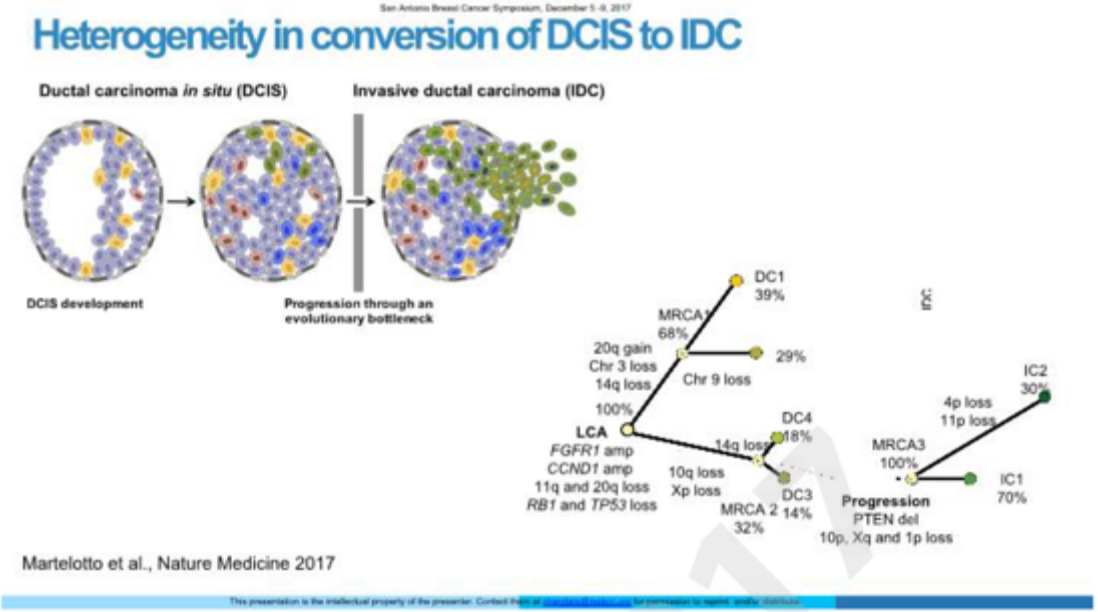
San Antonio Breast Cancer Symposium, December 5 -9, 2017

Intratumoral Heterogeneity

- Decades of research proving heterogeneity in cancer
- Origins are widely reported (local pressures, genomic instability, stem cells)
- Manifestations are numerous – focus here on genetic (transmittable)
- Major questions – (1) where is it observed in clinical breast cancer?
  (2) what are the implications of its presence?
  (3) can we easily detect heterogeneity in clinical practice?
  (4) are there implications for it in treatment decisions?

Intratumoral Heterogeneity

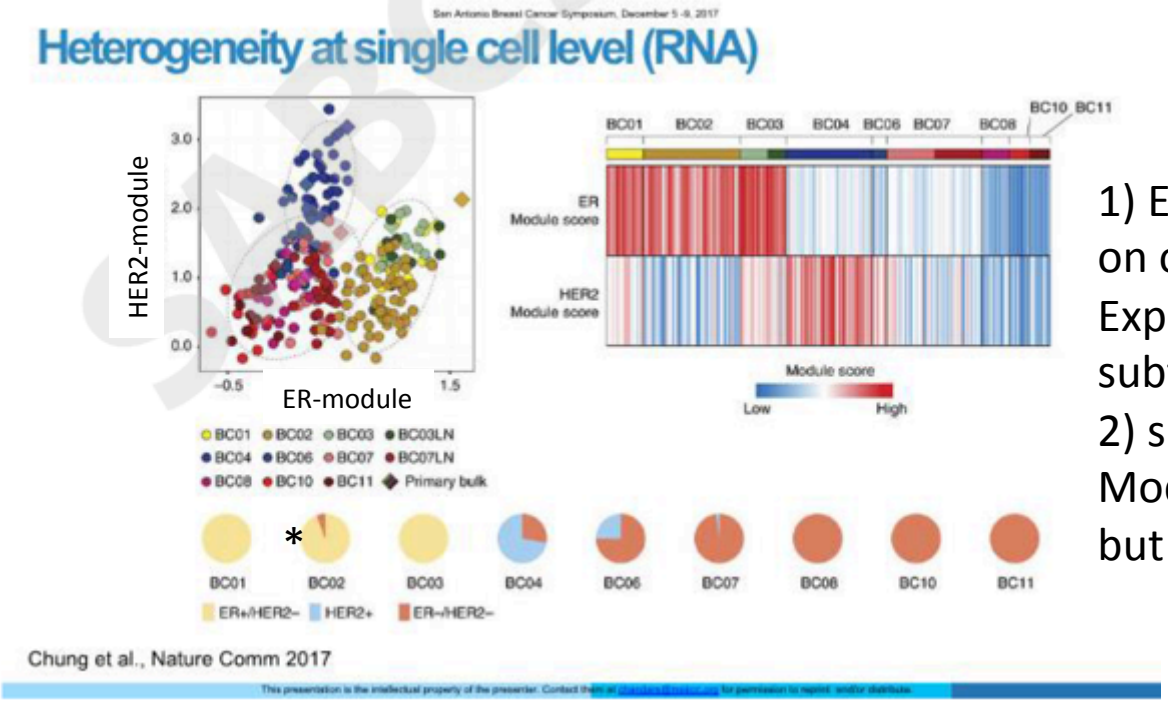
Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1



Clonal evolution
What does it mean
What is relevant
clinical actionable

Intratumoral Heterogeneity

Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1



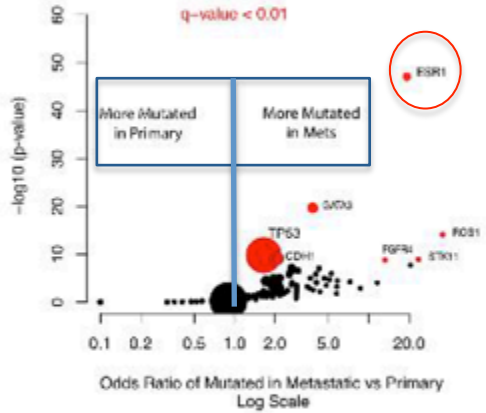
1) ER- and HER-module on overall gene Expression recapitulate subtypes, but
2) single cells could be Module ER (yellow), but by single genes TN (*)

Intratumoral Heterogeneity

Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1

Heterogeneity in MBC: ESR1

Primary and Mets



Razavi et al., AACR 2016

Primary and cfDNA

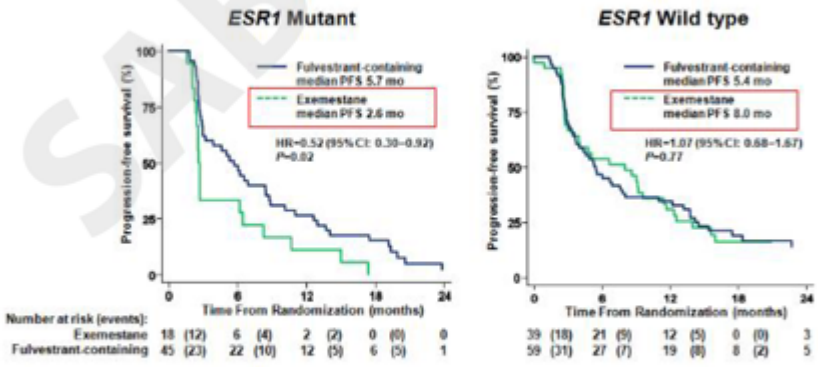
Gene	Mutation	Pre-tx tumor (06-FEB-2015)	Pre-tx cfDNA (10-FEB-2015)
AKT1	E17K	127/341 (37%)	87/331 (26%)
KEAP1	L153dup	179/561 (32%)	80/674 (12%)
NCOR1	Splice site	216/356 (61%)	26/149 (17%)
ESR1	**D538G**	**0/325 (0%)**	**16/313 (5%)**
NKX2-1	A102V	0/121 (0%)	12/241 (5%)

Smyth et al., manuscript in prep.

Intratumoral Heterogeneity

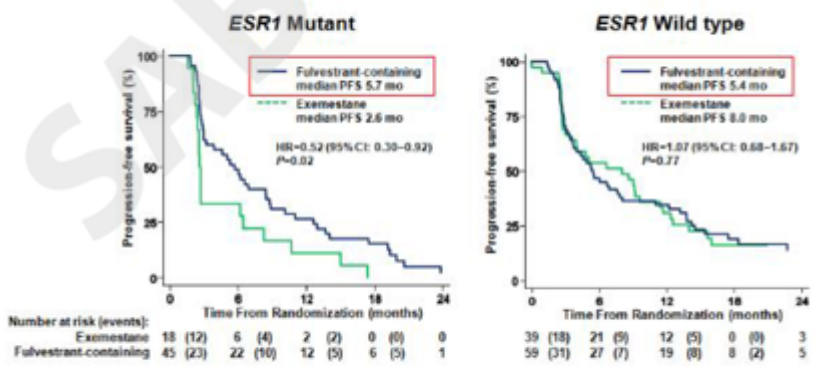
Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1

Subclonal alterations and resistance



Fribbens et al., JCO 2016

Subclonal alterations may predict response

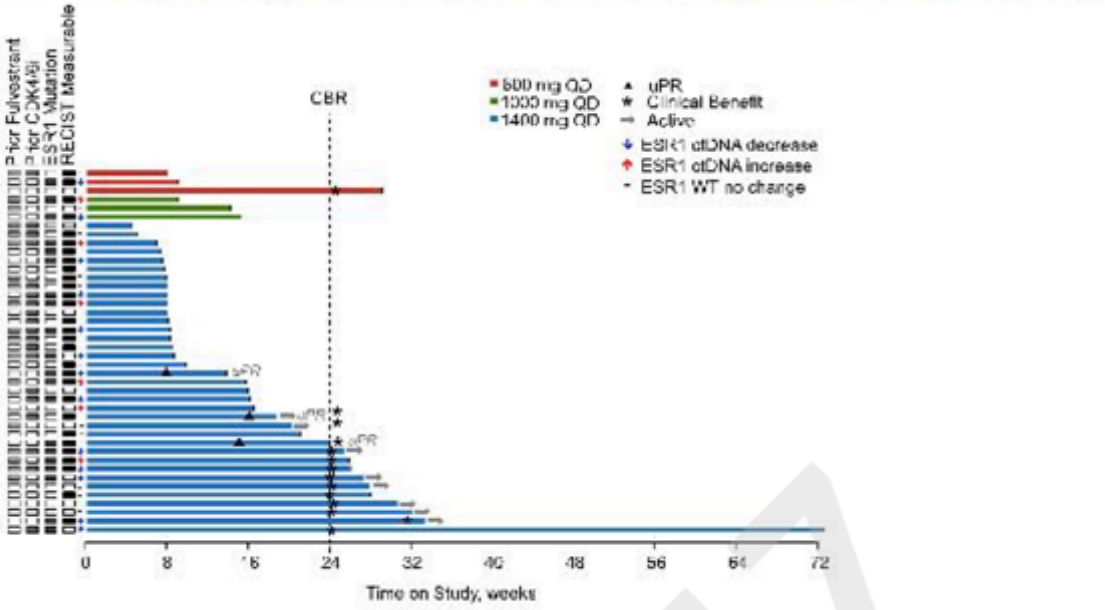


Fribbens et al., JCO 2016

Intratumoral Heterogeneity

Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1

Response of ESR1 mutants to oral SERDs in clinic: GDC0927



Dickler et al. SABCS 2017

Intratumoral Heterogeneity

Sarat Chandarlapaty, MSKCC; Basic Science BSF03-1

Conclusions/Discussion

(1) Where is it observed in clinical breast cancer?

(2) What are the implications of its presence?

(3) Can we easily detect heterogeneity in clinical practice?

(4) Are there implications for it in treatment decisions?

Early days for heterogeneity in practice
Treatment benefit worth pursuing
Clinical Trials – Translational science

BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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

BRCA1/2 Variants of Uncertain Significance (VUS)

- Missense, intronic and in-frame deletion and insertion mutations
- Many identified through predisposition panel testing
  - ~2-5% of all BRCA1/2 variants detected in U.S.A.
  - >3,000 individual BRCA1/2 VUS identified to date
- Many additional germline and somatic VUS identified through tumor sequencing

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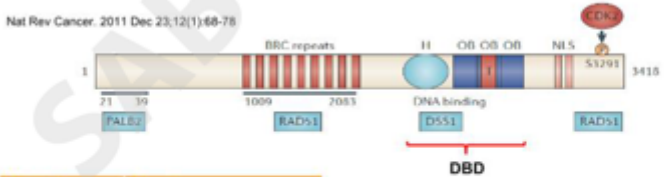
BRCA2 VUS



Mutation	Count
Missense	2940
Frameshift	1991
Nonsense	585
Splice site	159
Indels	139

- Many missense VUS in ClinVar
- Only 10 classified as deleterious by ENIGMA expert panel

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BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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Why are VUS problematic

Problematic for clinical management of BRCA1/2 patients

- Risk assessment of patient and family members
- Mammography/MRI screening
- Prophylactic surgery
- Treatment options (PARP inhibitors/platinum)

Most VUS will not ultimately be pathogenic

VUS should NOT be used to guide care

- Inappropriate interventions on basis of VUS
- Inappropriate reassurance

Anxiety about unresolved uncertainty

"VUS exist in all hereditary breast cancer genes"

BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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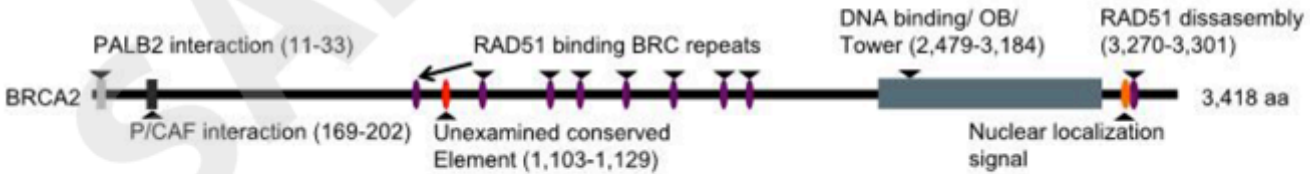
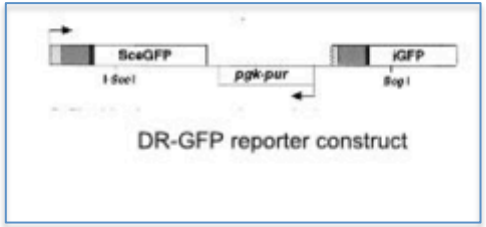
BRCA2 protein and Homology Directed Repair Assay





DR-GFP reporter construct

HDR Assay:

- functional BRCA2 repairs DNA break, resulting in green fluorescence
- non-functional > low fluorescence

BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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

Validation of an *in-vitro* HDR assay

Evaluated the use of an HDR functional assay to classify BRCA2 missense variants in the DNA binding domain (DBD)

Used 19 established non-pathogenic and 13 pathogenic missense variants in DBD

HDR assay showed 100% sensitivity (95% CI: 75.3%–100%) and 100% specificity (95% CI: 81.5%–100%) for pathogenic BRCA2 variants.

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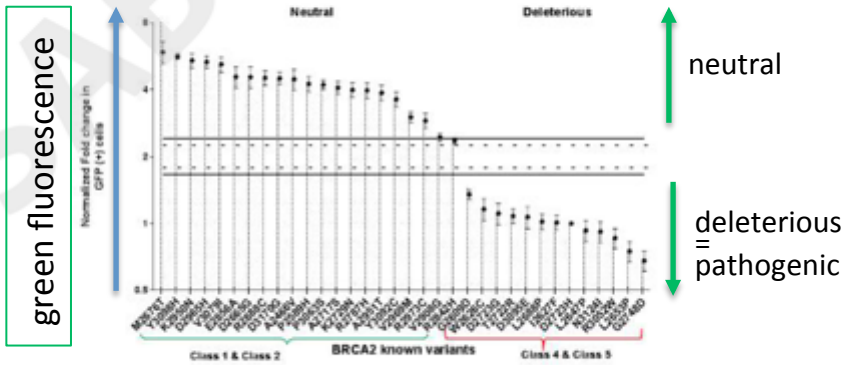
HDR assay sensitivity and specificity



BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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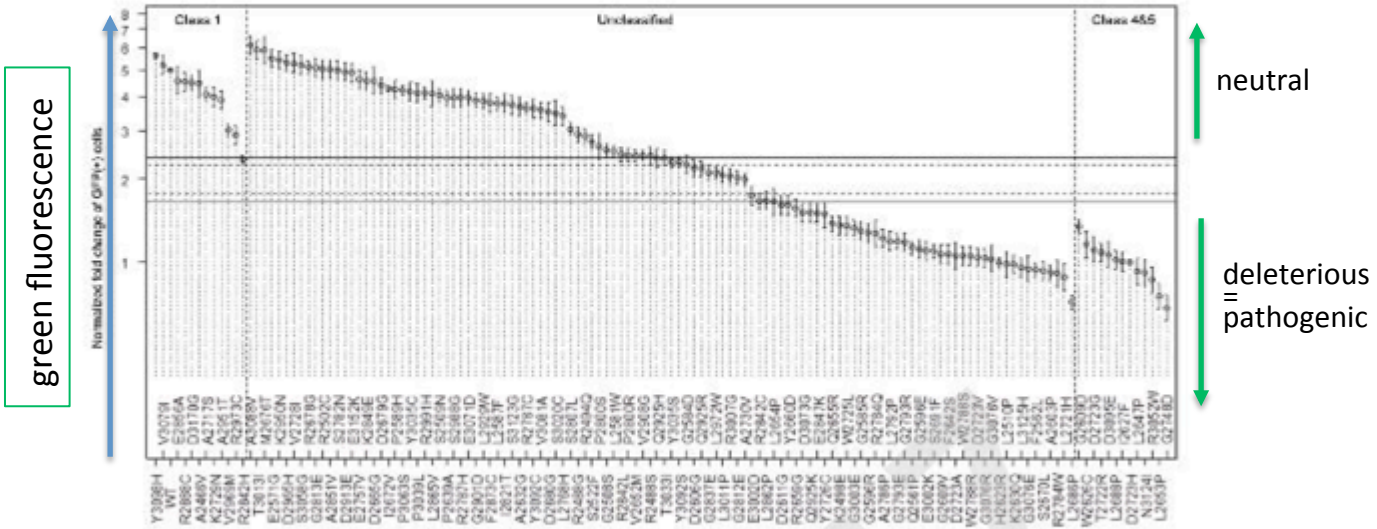
Evaluated 139 BRCA2 DBD missense variants



BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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Cancer risks for missense variants in BRCA2

	Ambry Genetics cases		gnomAD controls		Association with **breast cancer**			
BRCA2 variants	Mutated Alleles	# Cases	Mutated Alleles	# controls	OR	95%CI lower	95%CIUpper	p-value
Deleterious	62	49934	14	60010	5.32	2.97	9.73	$1.49x10^{-10}$
Neutral	79	49934	63	60010	1.51	1.07	2.12	0.0179

(excluding previously BRCA tested)

	Ambry Genetics cases		gnomAD controls		Association with **ovarian cancer**			
BRCA2 variants	Mutated Alleles	# Cases	Mutated Alleles	# controls	OR	95%CI lower	95%CI Upper	p-value
Deleterious	14	9064	14	60010	6.63	3.10	14.14	$2.91x10^{-6}$
Neutral	15	9064	63	60010	1.58	0.88	2.76	0.128

(excluding previously BRCA tested)

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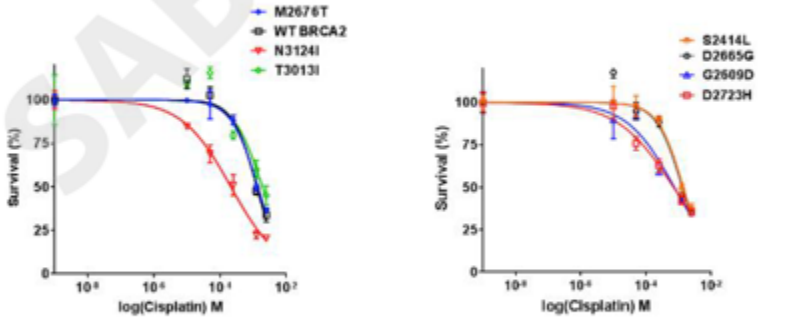
Influence of BRCA2 missense variants on response to cisplatin



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BRCA2 VUS implications for risk and treatment

Fergus Couch, Mayo Clinic; GS4-06

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Summary

- Quantitative methods and standards for classification of BRCA1 and BRCA2 VUS have been developed.
- Validated functional assays allow classification of the pathogenicity of many additional VUS
- Drug response assays allow prediction of the responsiveness of tumors to targeted therapy
- Public databases providing information on BRCA1 and BRCA2 variants have been developed (ClinVar and BRCA Exchange)

Summary of Presentations

- Liquid Biopsies in Breast Cancer
  Sarah Dawson, Melbourne; educational
  Nick Turner, Royal Marsden; AACR outstanding investigator award
    - > Role for ctDNA in follow-up/therapy de-escalation or escalation/clinical trials
- CTC's and Late Recurrence
  Joe Sparano, ECOG-ACRIN; GS06-3
    - > CTC positive associated with recurrence in ER+
- Intratumoral Heterogeneity
  Sarat Chandarlapaty, MSKCC; Basic Science Forum BSF03-1
    - > Still learning what heterogeneity is essential
- BRCA2 VUS implications for risk and treatment
  Fergus Couch, Mayo Clinic; GS4-06
    - > 'Variants of Unknown Significance', some are relevant for risk and therapy

"Here are my genes..."