2016 SABCS Review

prevention, biomarkers and genomics

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Disclosure

None

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Outline

- **Hereditary: S2-1-4**
- **Prevention: S2-04**
- **Biomarkers: S3-01, S4-04**
- **Genomics: S6-05**

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Breast cancer risks associated with mutations in cancer predisposition genes identified by clinical genetic testing of 60,000 breast cancer patients

Fergus J. Couch, Hermela Shimelis, Chunling Hu, Jenna Lilyquist, Jie Na, Eric C. Polley, Steven N. Hart, Rachel McFarland, Robert Huether, Holly LaDuca, David E. Goldgar, Jill S. Dolinsky.

Association studies to estimate breast cancer risks

- Nationwide testing of 121,197 individuals referred for hereditary cancer genetic testing by Ambry Genetics between March 2012 and June 2016
- 38,326 Caucasian breast cancer patients from 65,057 tested were eligible for analysis
- Pathogenic variants from germline genetic testing of 21 known and candidate breast cancer predisposition genes were evaluated
- Frequency of variants in breast cancer cases was compared with frequency of variants in ExAC Non-Finn European non-TCGA controls

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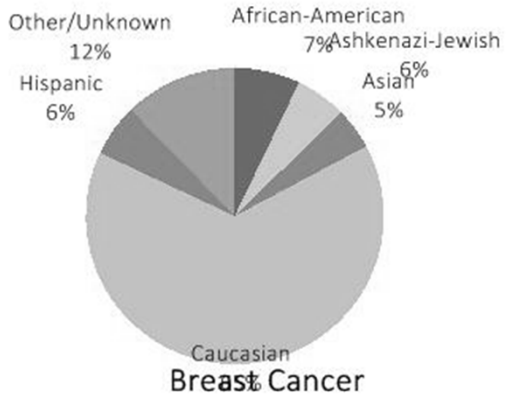
S2-01

Study Population: Ethnicity



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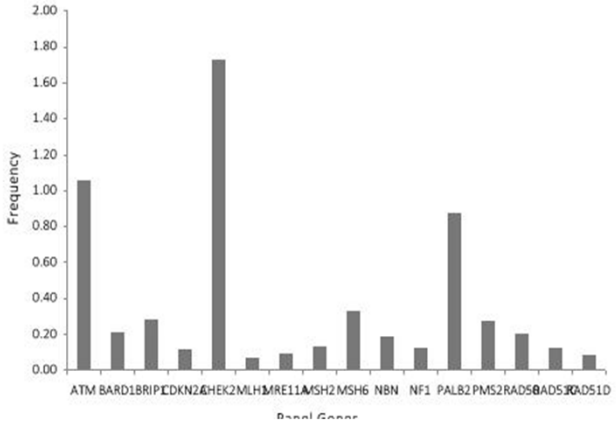
Frequency of pathogenic mutations by gene



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Associations of pathogenic variants with breast cancer Caucasian Cases vs. ExAC NFE-non-TCGA

Gene	CaseAC	CaseAN	ControlAC	ControlAN	OR	95%CI	p-value
ATM	279	58500	90	53288	2.83	2.23-3.64	3.66×10^{-25}
BRIP1	71	57114	41	53681	1.63	1.09-2.45	0.014
CHEK2	429	58222	163	50430	2.29	1.91-2.76	4.84×10^{-21}
CHEK2*	745	58222	424	50430	1.53	1.35-1.73	1.93×10^{-12}
PALB2	245	60092	29	53738	7.58	5.15-11.56	5.17×10^{-20}

Associations of pathogenic variants with breast cancer Caucasian Cases vs. ExAC NFE-non-TCGA

Gene	CaseAC	CaseAN	ControlAC	ControlAN	OR	95%CI	p-value
BARD1	52	57114	22	52157	2.16	1.29-3.73	0.0023
CDKN2A	6	16928	7	48624	2.46	0.68-8.56	0.11
RAD51D	18	51936	6	53110	3.07	1.17-9.44	0.014
MSH6	32	30978	28	52301	1.93	1.13-3.33	0.011
MRE11A	21	57114	23	53534	0.86	0.45-1.62	0.65
NBN	48	57114	39	52529	1.13	0.73-1.77	0.59
RAD50	46	57114	86	52948	0.50	0.34-0.72	0.00011
RAD51C	27	57114	31	53293	0.81	0.47-1.41	0.43

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Results

- Confirms ORs in known highly penetrant genes- *TP53, PTEN, CDH1*
- Supports recent shift of *PALB2* to high risk breast cancer gene
- Supports recent consideration of breast MRI for *ATM* and *PALB2*
- Moderate risk breast cancer genes
  - *BARD1*
  - *RAD51D*
  - *MSH6*
- Not associated with moderate of high risks
  - *NBN*
  - *RAD50*
  - *MRE11A*
  - *BRIP1*
  - *RAD51C*

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Implications for Medical Management

Consider/Recommend Breast MRI	Discuss Option of RRM/ Consider based on family history	Unknown or insufficient evidence for BC risk	No increased BC Risk
ATM	*ATM* ←	*BARD1* ←	*BRIP1*
BRCA1	*BRCA1*	*MLH1*	
BRCA2	*BRCA2*	*MSH2*	
CDH1	*CDH1*	*MRE11A*	
CHEK2	*PALB2* ←	*MSH6* ←	
NBN	*PTEN*	*PMS2*	
NF1	*TP53*	*RAD50*	
PALB2		*RAD51C*	
PTEN		*RAD51D* ←	
STK11			
TP53			

Ref: NCCN Guidelines Version 1.2017 - Sept 19, 2016

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The landscape of somatic genetic alterations in BRCA1 and BRCA2 breast cancers

Burke KA, Macedo GS, Piscuoglio GS, Ng CK, **Geyer FC**, Martelotto LG, Papanastatiou AD, De Filippo MR, Schultheis AM, Brogi E, Robson M, Wen YH, Weigelt B, Schnitt SJ, Tung N & Reis-Filho JS

Memorial Sloan Kettering Cancer Center.

What is the second hit?

- **29 BRCA1 and 10 BRCA2 cases**
- **High-indepth sequencing and mutational and copy number analysis**

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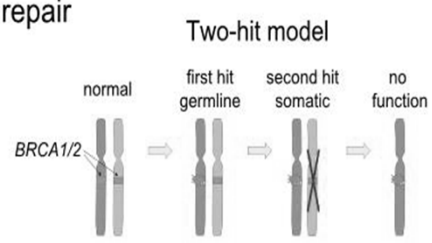
S2-02

BRCA1 and BRCA2 tumorigenesis

What do these genes have in common?

1) Key players in homologous recombination (HR) DNA repair

2) HR deficiency (HRD) is caused by two 'hits'
   - Second hit:
     - LOH
     - Somatic mutation



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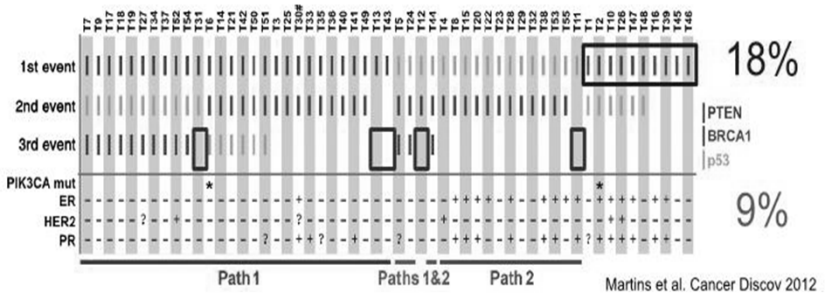
Chronology of somatic genetic events

1. Somatic loss of *BRCA1* WT allele is considered to be crucial for BRCA1 oncogenesis.

2. Complete loss of *BRCA1* is embryonically lethal.



Martins et al. Cancer Discov 2012

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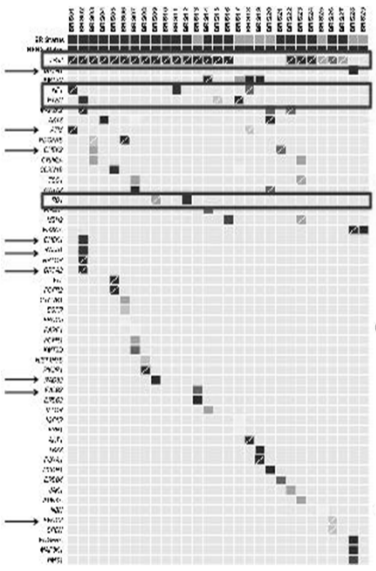
Results - 29 BRCA1 tumors



TP53 – 76%
NF1 – 10%
PTEN – 10%
RB1 – 7%

BRCA1 – 3%
ATM – 7%
CHEK2 – 7%
CHEK1 – 3%
RAD51 – 3%
BRCA2 – 3%
RAD52 – 3%
PALB2 – 3%
ERCC3 – 3%

DNA repair-related genes

Cancer Cell Fraction
0 < CCF < 5%
5% < CCF ≤ 20%
20% < CCF ≤ 40%
40% < CCF ≤ 60%
60% < CCF ≤ 80%
80% < CCF < 100%
Amplification
Heterozygous Deletion
Loss of Heterozygosity
Clonal Mutation (ABSOLUTE)

Receptor Status
Positive
Negative

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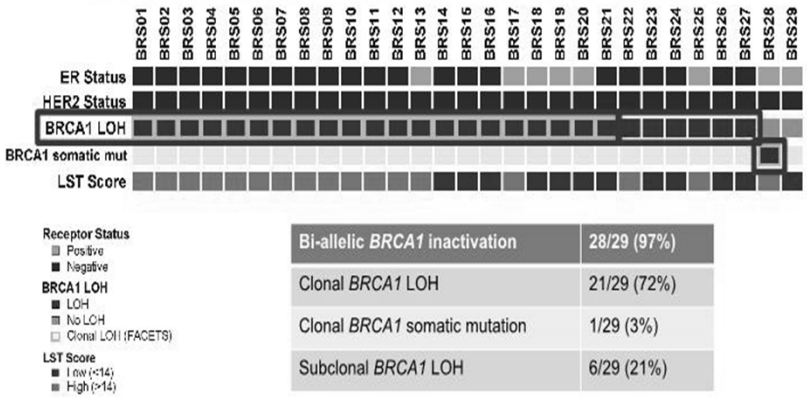
Second *BRCA1* hit in BRCA1 tumors



Bi-allelic *BRCA1* inactivation	28/29 (97%)
Clonal *BRCA1* LOH	21/29 (72%)
Clonal *BRCA1* somatic mutation	1/29 (3%)
Subclonal *BRCA1* LOH	6/29 (21%)

No differences in the frequency of bi-allelic *BRCA1* inactivation according to ER status

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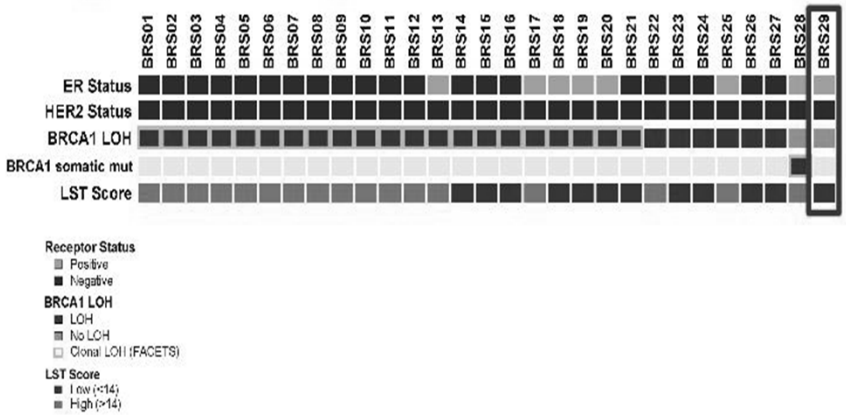
Single case lacking bi-allelic *BRCA1* inactivation



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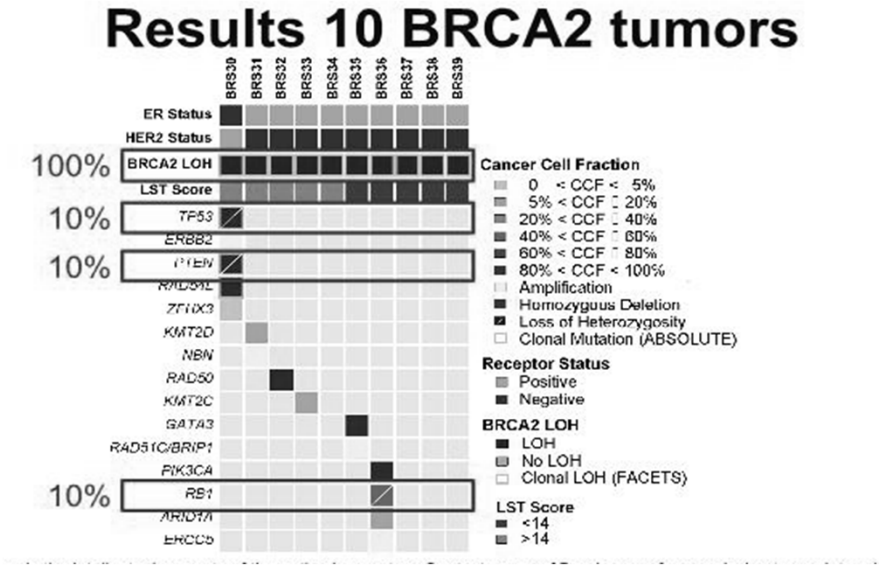
Results 10 BRCA2 tumors



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Main conclusion

- **BRCA1**
  - **TP53 mutation most common**
  - **Second hit**
    - » **Present in most ER+/ER- tumors**
    - » **Mainly by LOH of the WT allele**
    - » **Clonal in the majority of cases (75%)**
- **BRCA2**
  - **Genetically heterogeneous, without a highly recurrently altered gene**
  - **Second hit**
    - » **All cases had clonal LOH of the WT allele**

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Does BRCA status affect outcome in young breast cancer patients? Results from the POSH prospective study

Diana Eccles, Ellen Copson, Tom Maishman, Will Tapper, Ramsey Cutress, Stephanie Greville-Heygate, Bryony Eccles, Sue Gerty, Louise Jones, Douglas G Altman, Lorraine Durcan, Peter Simmonds, Jamie Allen, Craig Luccarini, Doug Easton, Alison Dunning, POSH study steering group and collaborators

- **126 UK NHS clinics 2000-2008**
- **Invasive BC diagnosed <40 yrs old**
- **2759 pts included in the analysis**
- **379/2759 (14%) had BRCA1 or 2 mutations, or both**

S2-03

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Pre-specified Statistical Analysis Plan

- Early stage disease known BRCA status
- Primary outcome – OS in (BRCA+) versus (BRCA-)
- Secondary outcomes – OS and DDFS including
  - All patients
    - BRCA1+ versus BRCA1-
    - BRCA2+ versus BRCA2-
  - TNBC patients only
    - BRCA+ versus BRCA-

Adjusted for age, grade, stage, ER, HER2, BMI

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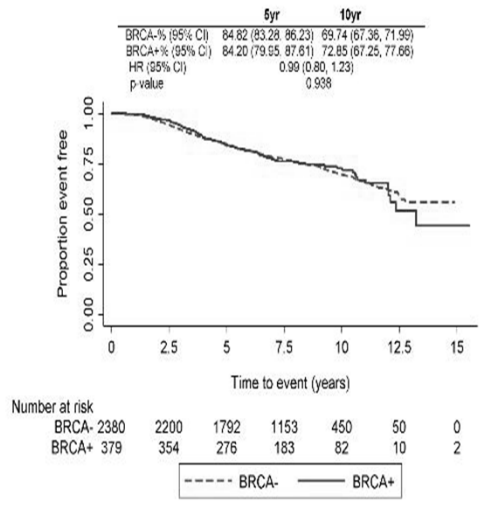
Primary outcome (all) BRCA+ v BRCA-



Overall Survival

UVA HR 0.99 (0.80 to 1.23)
p=0.938

MVA HR 0.96 (0.77 to 1.21)
p=0.742

Median follow up time (years) = 8.2 (0.4-15.6)

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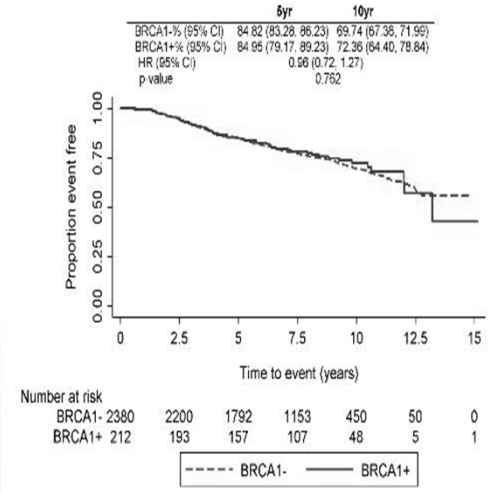
All cases - BRCA1 versus non-carriers



Overall Survival

UVA HR 0.96 (0.72 to 1.27)
p=0.762

MVA HR 0.93 (0.69 to 1.25)
p=0.608

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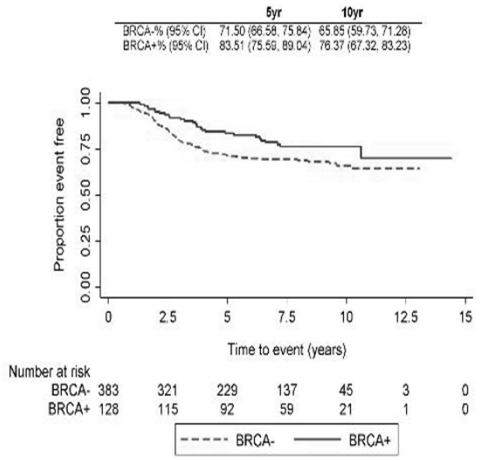
TNBC - overall survival



~11% difference at 10 years

MVA:
2 years 0.45 (0.26 to 0.79), p=0.006
5 years 1.04 (0.62 to 1.72), p=0.898

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Triple negative breast cancer cases

Treatment type	All TNBC n=511	BRCA+ n=128	BRCA- n=383	BRCA+ vs BRCA -
Breast conservation	301 (59%)	71 (55%)	230 (60%)	p=0.362
Unilateral mastectomy	206 (40%)	55 (43%)	151 (39%)	p=0.479
Anthracyclines	345 (68%)	90 (70%)	255 (67%)	p=0.435
A + T	151 (30%)	33 (26%)	118 (31%)	p=0.280
Bilateral mastectomy				
<1 year after diagnosis	**29 (6%)**	**19 (15%)**	**10 (3%)**	**p<0.001**

Percentages and tests performed on complete data

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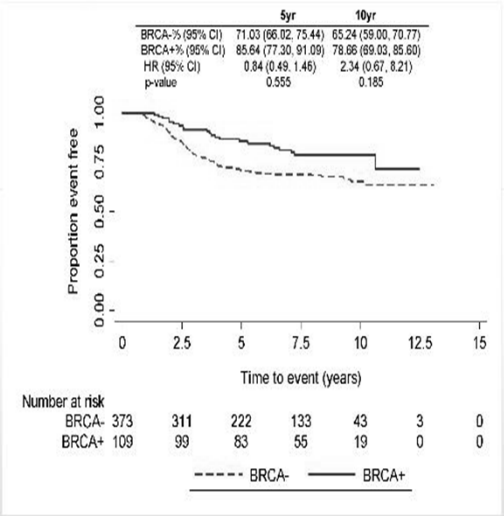
TNBC patients – no bilateral mastectomy



~13% difference at 10 years

MVA:
2 years 0.37 (0.20 to 0.70), p=0.002
5 years 0.86 (0.50 to 1.51), p=0.618

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Is there a difference?

- **There is no significant difference in survival between BRCA gene carriers and non-carriers amongst all young breast cancer patients.**

- There is a consistent 11% difference in survival in favour of BRCA gene carriers presenting with a TNBC
- Since the survival benefit is only apparent in TNBC cases, we would have needed **1,116** patients with TNBC for an 11% difference to reach statistical significance.
- Bilateral mastectomy soon after diagnosis does not improve survival in young BRCA gene carriers.

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Worse Breast Cancer Prognosis of BRCA1/BRCA2 Mutation Carriers: What's the Evidence? A Systematic Review with Meta-Analysis

- 66 studies were included
- In contrast to currently held beliefs of some oncologists, current evidence does not support worse breast cancer survival of BRCA1/2 mutation carriers in the adjuvant setting; differences if any are likely to be small.

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van den Broek AJ, Schmidt MK, van 't Veer LJ, Tollenaar RAEM, van Leeuwen FE (2015) PLoS ONE 10(3): e0120189. doi:10.1371/journal.pone.0120189

Hereditary BC summary

- Large cohort of genetic testing from Ambry Genetics supports the inclusion of TP53, PTEN, CDH1 and PALB2 as high risk genes, MRI screening and RRM may be considered based on family hx
- BRCA mutation second hit is caused mainly by LOH of the WT allele, with TP53 mutation being the most common in BRCA1 BC while there is no recurrently mutated gene in BRCA2 BC
- BRCA mutation status does not affect the survival of young women with EBC

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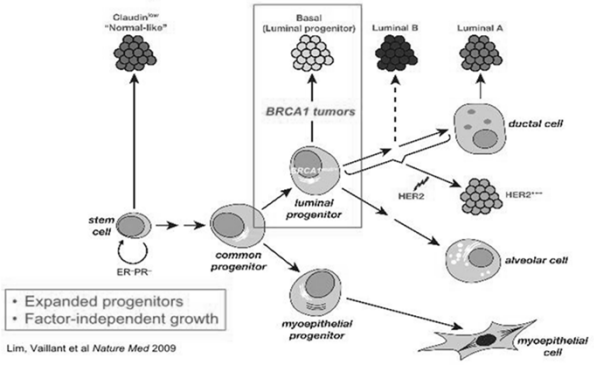
RANK ligand as a target for BC prevention in BRCA1 mutation carriers

Aberrant luminal progenitors are the likely target for tumorigenesis in *BRCA1* mutation carriers



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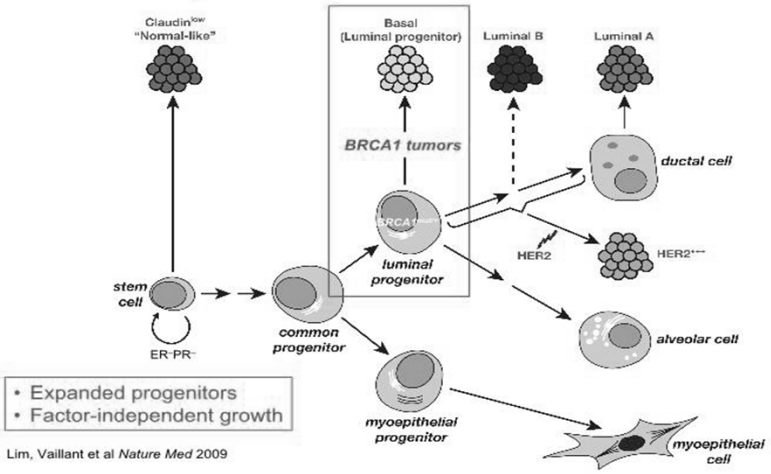
Emma Nolan et al, S2-04

Aberrant luminal progenitors are the likely target for tumorigenesis in *BRCA1* mutation carriers



Lim, Vaillant et al *Nature Med* 2009

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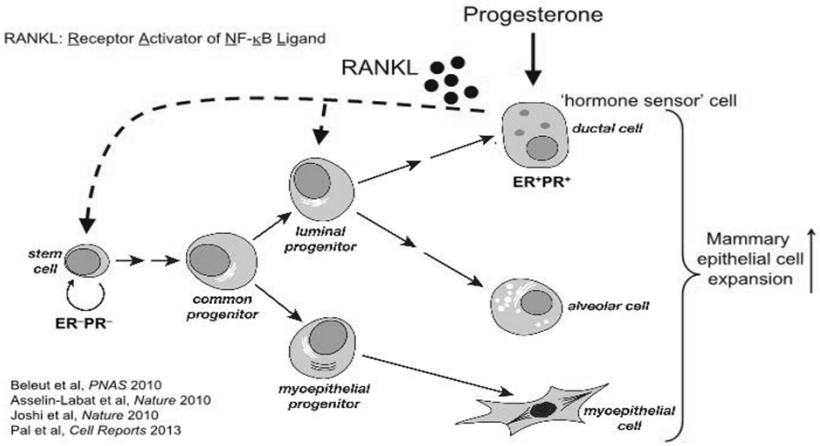
RANKL has emerged as a key effector of progesterone signaling to stem/progenitor cells in mice



Beleut et al, *PNAS* 2010
Asselin-Labat et al, *Nature* 2010
Joshi et al, *Nature* 2010
Pal et al, *Cell Reports* 2013

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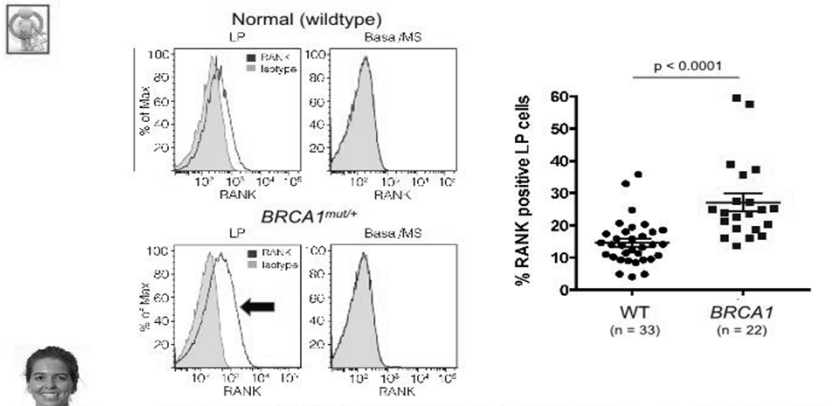
The receptor for RANKL, RANK, is expressed in the luminal progenitor subset



Emma Nolan

Prominent RANK expression in progenitors from BRCA1 mutation carriers

kConFab

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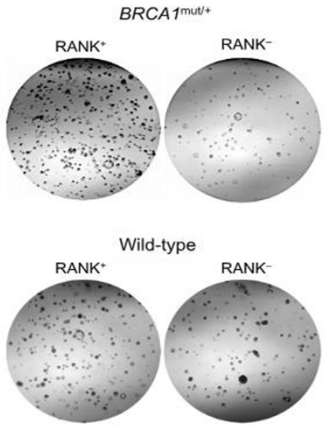
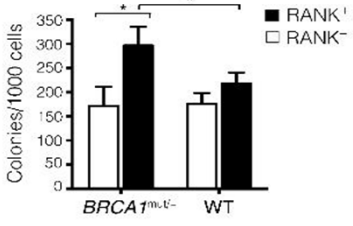
RANK+ progenitors from ostensibly normal (preneoplastic) tissue exhibit enhanced clonogenic activity





Mean ± s.e.m; (n = 6)

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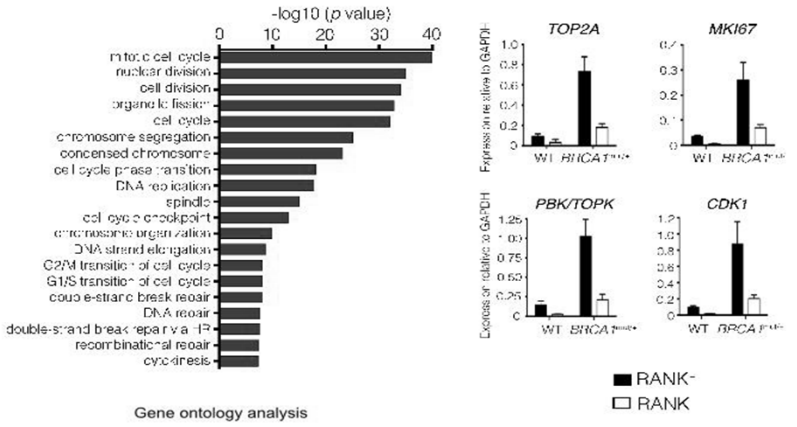
RANK⁺ luminal progenitors have enhanced mitotic activity



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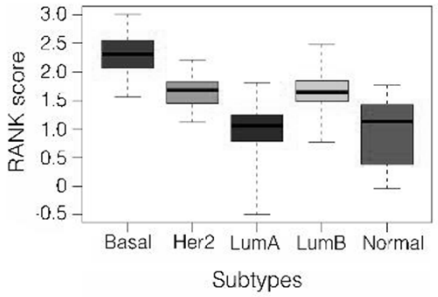
RANK⁺ gene signature correlates with basal-like cancers



RANK⁺ (not RANK⁻) luminal progenitors are the probable 'cell-of-origin' for basal-like cancers

RANK⁺ progenitors represent a key target population

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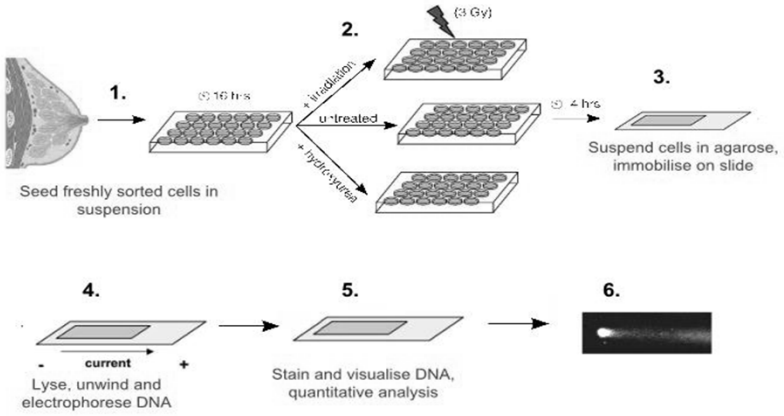
Are RANK⁺ cells prone to acquiring DNA damage?

- RANK⁺ and RANK⁻ luminal progenitors isolated from WT and *BRCA1*$^{mut/+}$ breast tissue
- **Comet assays** performed 4 hrs following γ-irradiation (IR; 3 Gy) or hydroxyurea (HU)



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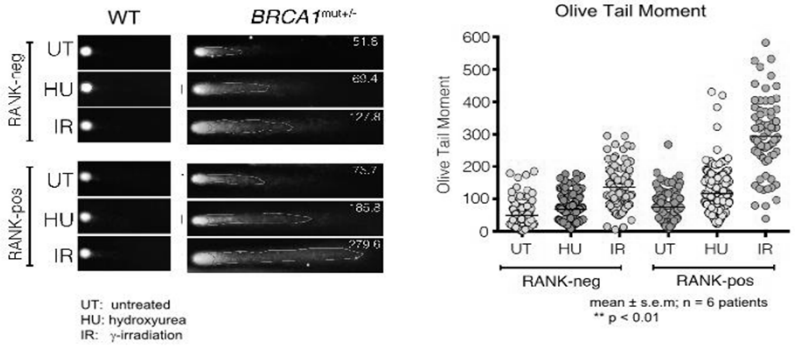
BRCA1$^{mut/+}$ RANK⁺ progenitors are deficient in DNA repair



UT: untreated
HU: hydroxyurea
IR: γ-irradiation

Heterozygous BRCA1 breast epithelium exhibits defective DNA repair, most prominent in RANK⁺ luminal progenitors

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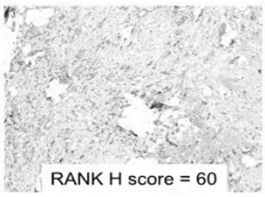
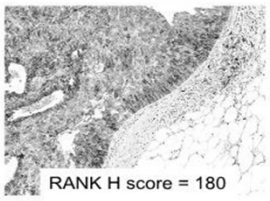
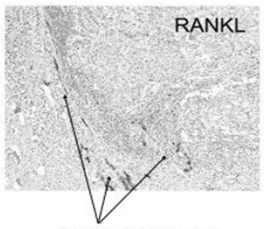
RANK expression remains prominent in *BRCA1*-associated tumors

WT | *BRCA1*-mutated







RANKL within normal breast adjacent to tumor

Genotype	RANK⁺
WT	**10%** (31/311)
BRCA1	**42%** (61/144)
BRCA2	15% (17/115)

H-score:
intensity x % RANK-positive cells

kConFab

Dan Branstetter
Bill Dougall

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transbcr

'BRCA-D' pilot study

Investigator-initiated study
ACTRN12614000694617

A pre-operative window study evaluating the biological effects of **D**enosumab on normal breast tissue from ***BRCA1*** and *BRCA2* mutation carriers **and high-risk women**

BRCA1 carrier
n = 20

BRCA2 carrier
n = 10

High Risk
n = 10

BASELINE

Denosumab 120 mg sc

C1D1 C2D1 C3D1

SURGERY

Prophylactic Mastectomy *or* biopsy

Breast tissue (luteal phase)
Blood & serum
MRI
MMG
Dental check

Breast tissue (luteal phase)
Blood & serum
MRI

Primary endpoint: Reduction in median Ki67% in *BRCA1* carriers
Secondary endpoint: Feasibility, safety, bone turnover, MRI BPE
Exploratory: Gene profiling, FACS, CFCs, SNP, serum RANKL/OPG




Sheau Wen Lok, Bruce Mann, Alice Bergin, Kylie Shackleton

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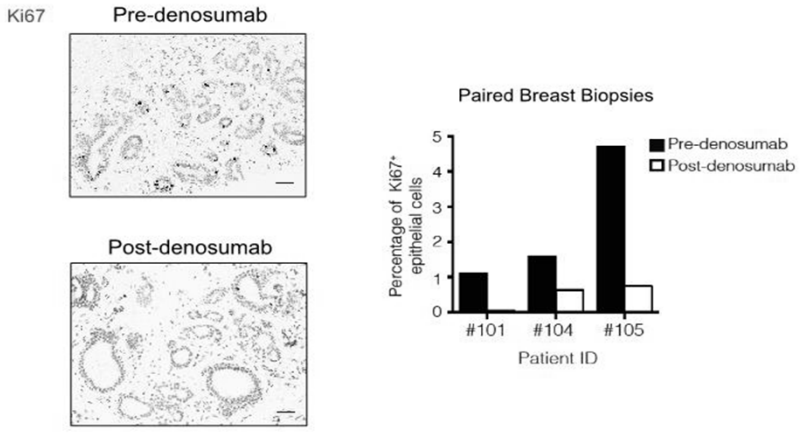
BRCA-D patient biopsies



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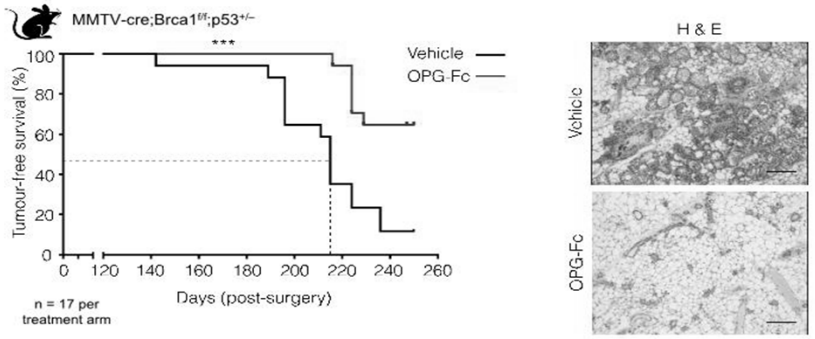
RANKL inhibition delays tumor development in mouse models



- Tumor onset delayed in mice treated with the RANKL inhibitor OPG-Fc (p = 0.0002)
- Median tumor onset not reached in OPG-Fc group, 11/17 (65%) mice tumor-free
- Dramatically reduced hyperplasia in OPG-Fc treated mice

Nolan et al, *Nature Med* 2016
& Sigl et al, *Cell Research* 2016

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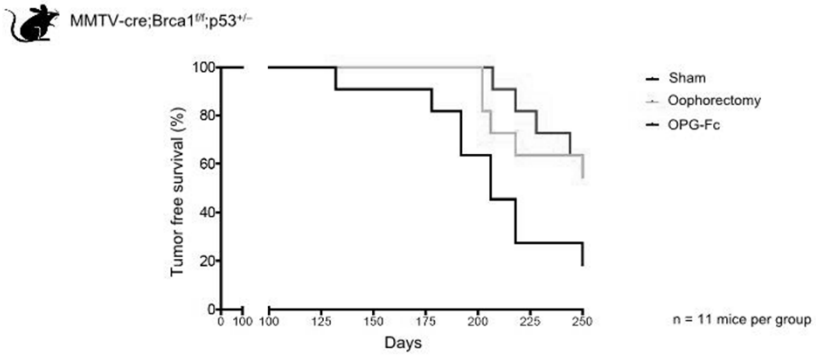
RANKL inhibition and oophorectomy curtail tumor development in a *Brca1* mouse model



RANKL inhibition and oophorectomy appear effective in attenuating tumor development in Brca1 mouse models

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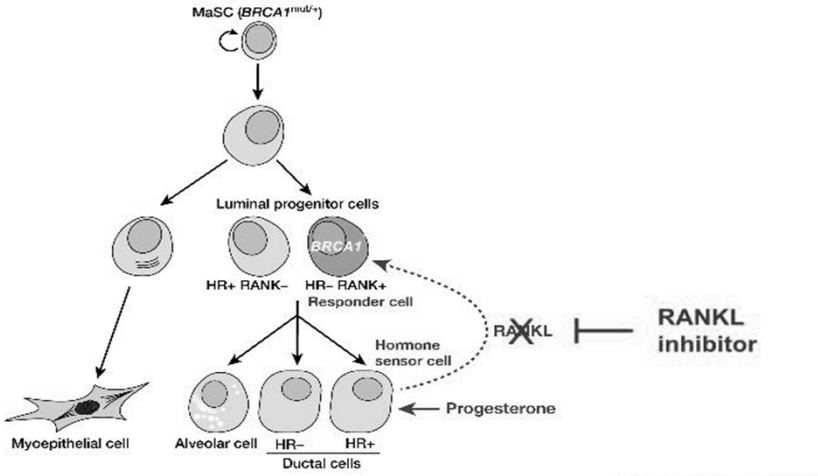
Switching off progenitor cells as a breast cancer prevention strategy in *BRCA1* mutation carriers



Nolan et al, *Nature Med* 2016

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'BRCA-P' Trial Design

A randomised double blind phase III breast cancer prevention study of denosumab vs placebo in *BRCA1* and *BRCA2* mutation carriers

Translational studies:
Bone density, Mammographic density, MRI Birads, SNPs, Tumor tissue, Serum biomarkers

Global Lead:
Christian Singer, ABCSG

ABCSG

Australia & New Zealand Breast Cancer Trials Group

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Bone modulating agents and survival in EBC

- **Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomized trials (EBCTCG, Lancet 2015)**
- **Denosumab Improves Disease-free Survival for Postmenopausal Patients With HR-positive Breast Cancer, ABCSG-18 (M. Gnant et al, SABCS 2015)**
- **Ongoing D-CARE trial in EBC**
- **Use of adjuvant bone modulating agents for survial gain warrants discussion with pts with EBC**

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Outline

- **Hereditary: S2-1-4**
- **Prevention: S2-04**
- **Biomarkers: S3-01, S4-04**
- **Genomics: S6-05**

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International MEta-analysis

of circulating tumor cell detection

in early breast cancer pts

treated by NEOadjuvant chemotherapy (IMENEO study)

FC Bidard*, S Michiels, V Mueller, S Riethdorf, LJ Esserman, A Lucci, B Naume, J Horiguchi, R Gisbert-Criado, S Sleijfer, M Toi, JA Garcia-Saenz, A Hartkopf, D Generali, F Rothé, J Smerage, L Muinelo, J Stebbing, P Viens, M Magbanua, CS Hall, O Engebraaten, D Takata, J Vidal-Martínez, W Onstenk, N Fujisawa, E Diaz-Rubio, FA Taran, MR Cappelletti, M Ignatiadis, C Proudhon, D Wolf, J Bowman Bauldry, E Borgen, R Nagaoka, V Carañana, J Kraan, M Maestro, SY Brucker, K Weber, F Reyal, D Amara, MG Karhade, RR Mathiesen, H Tokiniwa, A Llombart-Cussac, K d'Hollander, P Cottu, JW Park, S Loibl, JY Pierga, K Pantel

* Medical Oncology, Institut Curie, Paris, France

S 03-01

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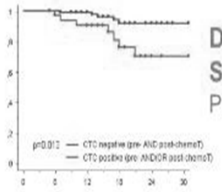
CTCs in neoadjuvant setting

First study with CellSearch®, N=118 pts (REMAGUS02)

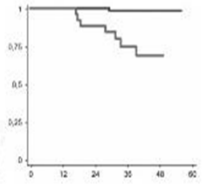
➔ No significant association between CTC detection and pCR

➔ Prognostic impact on:



Distant-Metastasis-Free Survival

Pierga *et al.*, Clin Cancer Res 2008



Overall Survival

Bidard *et al.*, Ann Oncol 2010

Numerous studies initiated worldwide
very few published / heterogeneous results

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Methods

- literature & abstracts search up to Dec 2014
- direct contact with all centers deemed to have eligible data:
  - ➔ **CTC count** by CellSearch®
  - ➔ **Early BC pts treated with neoadjuvant chemotherapy** (NCT)
  - ➔ **Survival** (published or not)

Non-overlapping **CTC time points:**
- [-5;0] weeks before NCT = baseline
- [1;8] weeks after start of NCT
- [-5;0] weeks before the surgery
- [1;52] weeks after surgery

Statistics

Cox regression models (stratified by study) & **landmark method**
Overfitting bias of multivariate prognostic models *(used to report average increases in likelihood ratio)* was limited by **resampling procedures**

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Data collection

Letter of intent

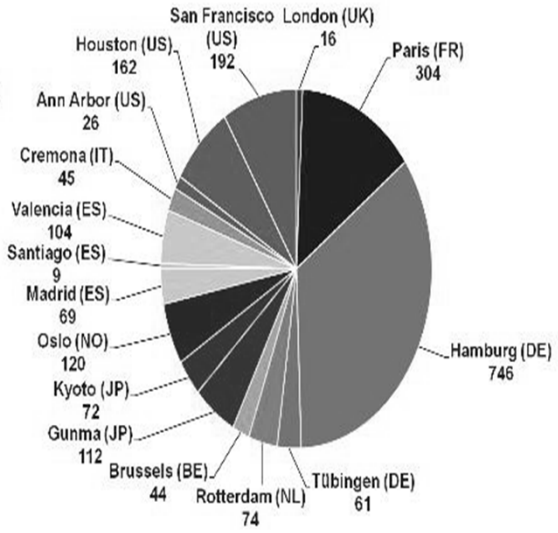
#2,000 potentially eligible pts from 18 centers

call for data → 2 centers off study

2,239 pts data received

data cleaning → 83 excluded

2,156 individual patients
21 studies
16 centers



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CTC detection

	N patients	≥1 CTC	≥2 CTC	≥5 CTC
Before NCT	1574	25.2%	12.6%	5.9%
Before surgery	1200	15.1%	5.3%	1.0%

Decrease during NCT: p<.0001

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CTC detection & baseline characteristics

	N patients	≥1 CTC	≥2 CTC	≥5 CTC	continuous
Before NCT	1574	25.2%	12.6%	5.9%	
cT size		**p<.0001**	**p<.0001**	**p<.0001**	**p<.0001**
cT1	122 (7.9%)	18.9%	8.2%	3.3%	
cT2	770 (49.8%)	22.3%	10.3%	3.5%	
cT3	343 (22.2%)	24.2%	12.2%	6.1%	
cT4a-c	108 (7.0%)	28.7%	16.7%	8.3%	
cT4d	204 (13.2%)	41.2%	24.5%	15.7%	
cN status		**p=.051**	**p=.021**	**p=.009**	**p=.024**
cN0	656 (41.9%)	22.7%	10.4%	4.1%	
cN+	911 (58.1%)	27.1%	14.4%	7.2%	
Subgroup		**p=.23**	**p=.028**	**p=0.54**	**p=.12**
HER2+	365 (23.2%)	24.1%	11.0%	4.7%	
HR+	800 (51.0%)	24.1%	11.5%	5.3%	
Triple Neg.	405 (25.8%)	28.4%	16.5%	8.4%	

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CTC detection: association with pCR

pCR was defined as ypT0/isN0 in 92.5% of patients (N=1916/2072)

pCR was observed in 24.3% of patients (N=503/2072)

	N patients	≥1 CTC	≥2 CTC	≥5 CTC	continuous
CTC before NCT		p=.076	p=.65	p=.90	p=.10
pCR	374 (24.0%)	21.7%	12.0%	6.1%	
No pCR	1183 (76.0%)	26.3%	13.0%	5.9%	
CTC before surgery		p=.45	p=.13	p=.53	p=.52
pCR	300 (26.3%)	13.7%	7.0%	1.3%	
No pCR	841 (73.7%)	15.7%	4.6%	1.0%	

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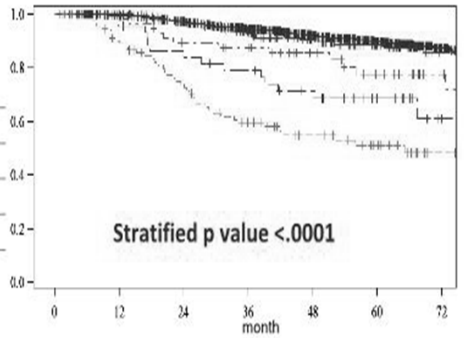
CTC before NCT & Overall Survival

	N pts	% Events	Hazard Ratio
0 CTC	1175	9.8%	1
1 CTC	199	10.6%	1.09 [0.65-1.69]
2 CTC	59	23.7%	2.63 [1.42-4.54]
3-4 CTC	47	29.8%	3.84 [2.08-6.66]
≥ 5 CTC	93	46.2%	6.25 [4.34-9.09]



Same HR observed without T4d tumors
No interaction found with tumor subtype

s

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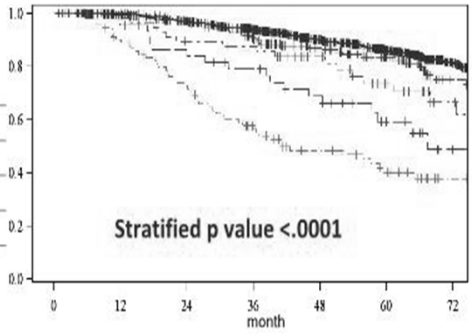
CTC before NCT & Distant Disease-Free Survival

	N pts	% Events	Hazard Ratio
0 CTC	1175	14.6%	1
1 CTC	199	18.1%	1.19 [0.81-1.69]
2 CTC	59	33.9%	2.44 [1.47-3.84]
3-4 CTC	47	38.3%	3.44 [1.96-5.55]
≥ 5 CTC	93	58.1%	5.00 [3.57-7.14]



Same HR observed without T4d tumors
No interaction found with tumor subtype

s

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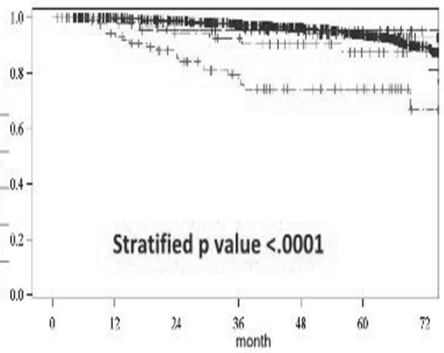
CTC before NCT & Locoregional Relapse-Free Interval

	N pts	% Events	Hazard Ratio
0 CTC	1175	6.7%	1
1 CTC	199	6.0%	0.89 [0.46-1.61]
2 CTC	59	15.3%	2.43 [1.12-4.76]
3-4 CTC	47	4.3%	1.23 [0.20-4.00]
≥ 5 CTC	93	22.6%	4.16 [2.32-6.66]



Same HR observed without T4d tumors
No interaction found with tumor subtype

s

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Overall clinical validity (threshold ≥2 CTC)

Multivariate analyses

Time point		OS	
		HR	p
CTC at baseline *(landmark analysis)*		4.19 [2.97-5.88]	<.0001
cT	T3-T4	1.49	.0023
	T4d	2.94	
cN	cN1	1.65	.0045
Subgroup	HER2+	1.69	<.0001
	Triple Neg	5.24	
pCR	No	5.88	<.0001

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Overall clinical validity (threshold ≥2 CTC)

Multivariate analyses

Time point	OS		DDFS		LRFI	
	HR	p	HR	p	HR	p
CTC at baseline *(landmark analysis)*	4.19 [2.97-5.88]	<.0001	3.79 [2.84-5.03]	<.0001	3.20 [1.93-5.19]	<.0001
CTC [-5;0]w before surgery *(landmark analysis)*	2.56 [1.45-4.23]	.0020	2.69 [1.67-4.12]	<.0001	1.05 [0.32-2.55]	.92

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Conclusion

- Post-neoadjuvant survival does not exclusively rely on breast cancer characteristics & pCR
- CTC number-dependent impact on OS, DDFS and LRFI
  significant above ≥2 CTC/7.5ml
- CTC complements *(but not dupplicates)* usual prognostic factors
- Next steps: - clinical utility trials *(e.g. post-neoadjuvant therapy)*
  - further biological characterization

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CTC in BC

- **MBC**
  - **prognostic before and during therapy (Cristofanilli et at, NEJM 2004; Bidard et al, Lancet Oncol 2014)**
  - **Predictive (SWOG S0500, Smerage et al, SABCS 2013)**
- **EBC**
  - **Prognostic before therapy (Lucci et al, Lancet Oncol 2012; Rack et alk, J Natl Cancer Inst 2014)**
- **LABC**
  - **Prognostic for survival but not predictive for pCR (SABCS 2016)**
- **Ready for prime time? Not yet**

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S 4-4

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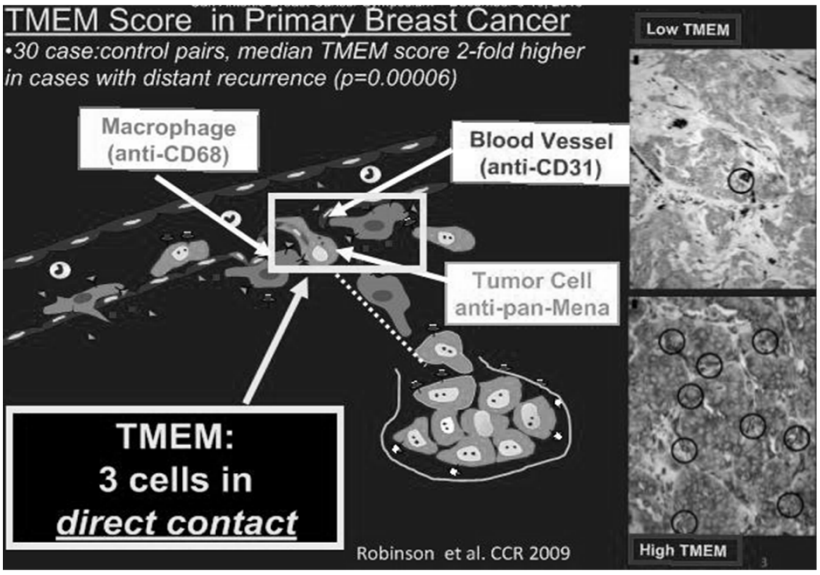
TMEM Score in Primary Breast Cancer

•30 case:control pairs, median TMEM score 2-fold higher in cases with distant recurrence (p=0.00006)



Robinson et al. CCR 2009

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TMEM Score is Associated with Distant Recurrence in Operable Breast Cancer

- 259 case:control pairs from population-based cohort
- 53% node negative, 46% received adjuvant chemotherapy
- No correlation with IHC4, nodal status, or tumor size
- TMEM score prognostic in ER+, HER2-neg disease in multivariate model including nodal status, size, and grade

TMEM Score Tertile	Odds ratio (95% CI)
≤6	1.00 (referent)
7–22	1.32 (0.70 to 2.52)
≥23	2.70 (1.39 to 5.26)
P trend	0.004
Continuous TMEM Score (per 10 units)	1.16 (95% CI 1.03 to 1.30)

Rohan et al. JNCI 2014

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Methods: Study Population, Endpoints & Objectives

- Methodology:
  - REMARK guidelines for biomarker development
  - Statistical plan: 80% power, 2-sided 5% significance level to detect at least a 2.2-fold higher risk of distant recurrence between highest vs. lowest tertile
- Study Population:
  - 600 women with stage I-III breast cancer in E2197 (2603 consented to research)
  - Treated with adjuvant AC or AT in E2197 (plus endocrine therapy if HR+)
- Clinical Endpoints:
  - Distant relapse free interval (DRFI): distant recurrence
  - Relapse free interval (RFI): distant or local/regional recurrence
- Study Objectives:
  - Aim 1: Association between (TMEM) MetaSite Score & DRFI (& RFI) by subtype
  - Aim 2 (HR+/HER2-): Correlation between MetaSite Score & Oncotype RS
  - Aim 3 (HR+/HER2-): Compare association between RS & MetaSite Score score with recurrence

Goldstein et al. J Clin Oncol 2008 ; Badve et al. J Clin Oncol 2008; McShane et al. JNCI 2005

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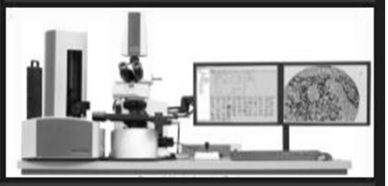
Methods: MetaSite *Breast*™ Assay and Data Analysis

MetaStat, Inc Laboratory (Boston, MA)
•(CLIA ID No. 22D2094085)
Triple immunostain - 3 cells in direct contact
•Endothelial cells: anti-CD31 (AbCam/Epitomics Clone EP3095)
•Macrophages: anti-CD68 mouse ab (Thermo Scientific Clone PGM1)
•Mena: anti-Pan-Mena mouse ab (Gertler laboratory, MIT)

Imaging & Automation:
•Perkin Elmer Vectra 2
•Up to 100 20X images acquired in areas of invasive tumor
•InForm (PE) & VisioPharm software



MetaSite Score
•Sum of TMEM from the top 3 highest density 20X fields of-view
Data transfer & analysis
•TMEM score data sent to ECOG-ACRIN, merged with clinical dataset
•Statistical analysis done by ECOG-ACRIN biostatistician, using weighted analysis methods (Gray R. Lifetime Data Analysis, 2009)

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Results – Aim 1: Continuous MetaSite Score by Breast Cancer Subtype & Recurrence

Weighted partial likelihood models were fit using cox proportional hazard function with variances adjusted for sampling, and time-varying coefficient models were examined

- Weighted mean MetaSite Score
  - Significantly lower in HR+, HER2- (16.1) than TNBC (23.8, p=0.01) and HER+ disease (26.2, p=0.03)
- Prognostic association with recurrence
  - No significant association in proportional hazards model in any breast cancer subtype over entire period
  - Not proportional over time in HR+, HER2- (p=0.01)
  - Significant association in years 0-5 for DRFI (p=0.001) and RFI (p=0.006) in HR+, HER2-, not years 5-10

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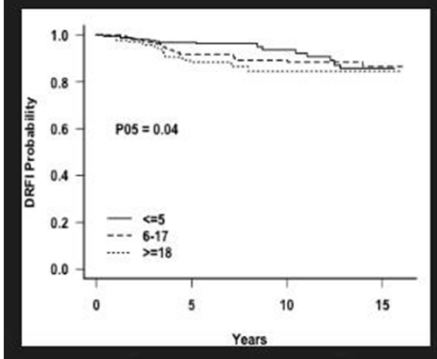
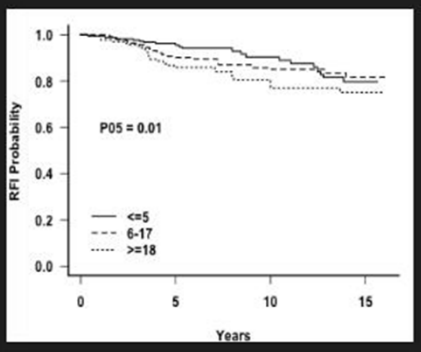
Results – Aim 2 : Outcomes for MetaSite Score (by empirical tertiles for entire cohort) in HR+/HER2- Disease in Years 0-5





DRFI (p=0.04)

RFI (p=0.01)

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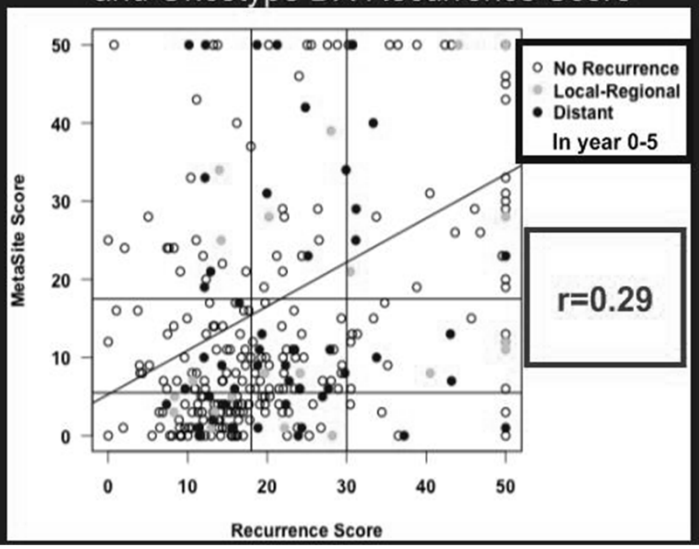
Results – Aim 2: Correlation between MetaSite Score and Oncotype DX Recurrence Score



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Conclusions: MetaSite Score-E2197 Clinical Trial Cohort

- Novel metastasis biomarker:
  - ✓ Interaction between breast cancer and microenvironment
  - ✓ Not driven by genes reflecting proliferation and ER signaling
- Analytic validity:
  - ✓ High degree analytical precision (AUC=0.97-0.99) and overall assay performance (AUC=0.91-0.96) in a CLIA lab (2016 SABCS - P2-05-06 12/8/16 7:30-9:00 AM)
- Clinical validity:
  - ✓ This is 3rd study showing association with recurrence
  - ✓ Prognostic independent tumor size, grade, nodal status, and Recurrence Score in HR+/HER2 disease
  - ✓ Complementary prognostic information to low/mid-range RS
  - ✓ Prognostic for early recurrence within 5 years

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Outline

- **Hereditary: S2-1-4**
- **Prevention: S2-04**
- **Biomarkers: S3-01, S4-04**
- **Genomics: S6-05**

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Comprehensive Comparison of Prognostic Signatures for Breast Cancer Recurrence in TransATAC

Ivana Sestak[1]
Richard Buus[2], Jack Cuzick[1], Peter Dubsky[3], Ralf Kronenwett[4], Sean Ferree[5], Dennis Sgroi[6], Catherine Schnabel[7], Rick Baehner[8], Elizabeth Mallon[2], Mitch Dowsett[2]

1. Centre for Cancer Prevention, Queen Mary University of London, London, UK
2. Ralph Lauren Centre for Breast Cancer Research, Royal Marsden, London, UK
3. Klinik St. Anna, Luzern, Switzerland
4. Sividon Diagnostics, Cologne, Germany
5. NanoString Technologies, Seattle, USA
6. Massachusetts General Hospital, Boston, USA
7. bioTheranostics, San Diego, USA
8. GenomicHealth, Redwood City, USA

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Aims

1. Prognostic performance of six signatures for distant recurrence in N- and N+ separately in transATAC:

In years 0-10 (chemotherapy)
In years 5-10 (extended endocrine therapy)

2. Added prognostic value of signatures to clinical variables

3. Clinically useful risk groups

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Prognostic signatures

Signature	Information included
Clinical Treatment Score **(CTS)**	Nodal status, grade, tumour size, age, treatment
Immunohistochemical markers **(IHC4)**	ER, PgR, Ki67, HER2
Oncotype Recurrence Score **(RS)**	21 genes (oestrogen, proliferation, invasion, HER2 genes)
Breast Cancer Index **(BCI)**	H/I and 5 proliferation genes (Molecular Grade Index)
Prosigna **(ROR)**	46 genes, proliferation score, tumour size (EU cut-offs from transATAC for N- and N+)
EndoPredict **(EPclin)**	12 genes (proliferation, differentiation, oestrogen); nodal status and tumour size

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Statistical analysis

- 818 postmenopausal women with ER+/HER2-negative disease
- 5 years of tamoxifen or anastrozole, NO chemotherapy
- 10 year median follow-up
- Distant recurrence (DR) primary endpoint
- Cox regression models used to determine prognostic value (LR-χ^2)
- Commercial cut-offs used to determine 10 year DR risk

→All results presented for node-negative and node-positive patients separately

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Patient characteristics

	Node-negative (N=591)	Node-positive (N=227)
Mean age, years (SD)	63.4 (7.9)	67.2 (8.2)
Mean BMI, kg/m² (SD)	27.3 (4.9)	27.1 (5.0)
Grade		
1	23.2%	18.9%
2	59.7%	61.2%
3	17.1%	19.8%
Mean tumour size, mm (SD)	17.6 (8.5)	25.7 (13.6)
Distant recurrence		
0-10 years	60 (10.2%)	66 (29.1%)
5-10 years	34 (5.7%)	31 (13.7%)

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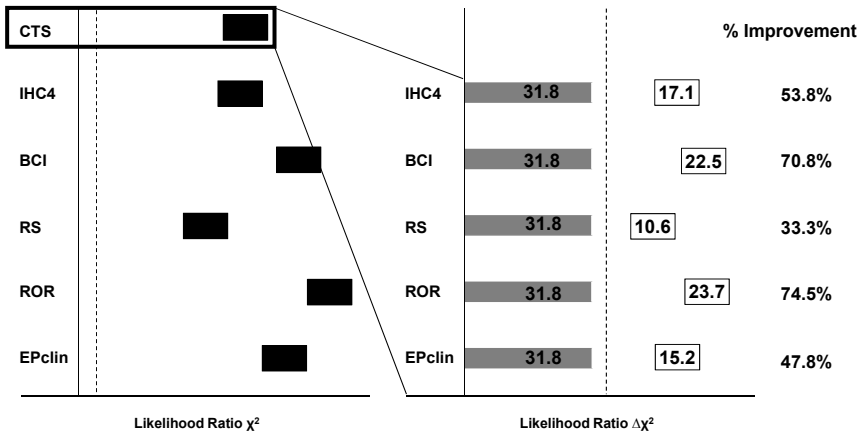
Prognostic value years 0-10 – node-negative



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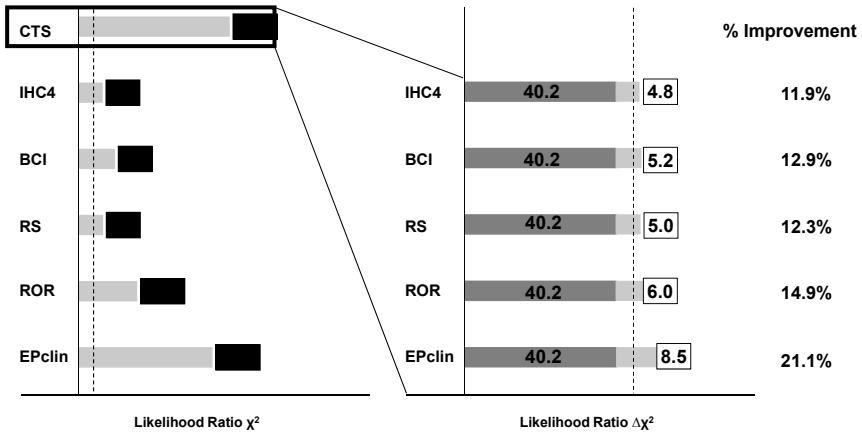
Prognostic value years 0-10 – node-positive



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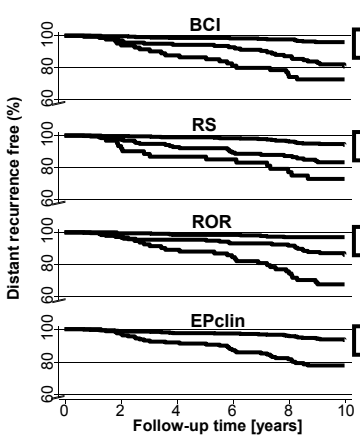
DR free (%) in years 0-10 – node-negative



	DR risk %	Patients % in risk groups
BCI	3.9%	**61.8**% patients
	19.3%	**24.2**% patients
	27.3%	**14.0**% patients
RS	5.9%	**63.3**% patients
	16.7%	**26.4**% patients
	27.2%	**10.3**% patients
ROR	3.0%	**53.8**% patients
	14.1%	**30.1**% patients
	33.4%	**16.1**% patients
EPclin	6.6%	**72.6**% patients
	22.1%	**27.4**% patients

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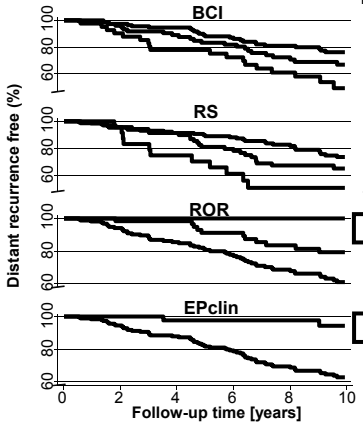
DR free (%) in years 0-10 – node-positive



	DR risk %	Patients % in risk groups
BCI	23.8%	**49.3**% patients
	33.1%	**32.6**% patients
	50.6%	**18.1**% patients
RS	26.2%	**57.7**% patients
	34.7%	**31.7**% patients
	48.8%	**10.6**% patients
ROR	0.0%	**6.6**% patients
	20.7%	**25.6**% patients
	39.1%	**67.8**% patients
EPclin	5.6%	**18.9**% patients
	37.2%	**81.1**% patients

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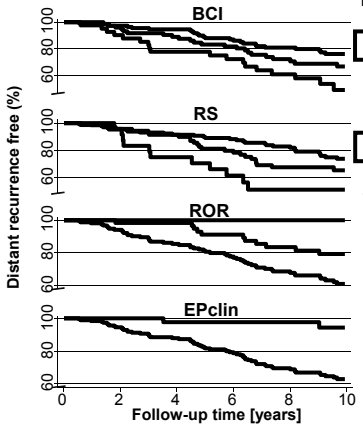
DR free (%) in years 0-10 – node-positive



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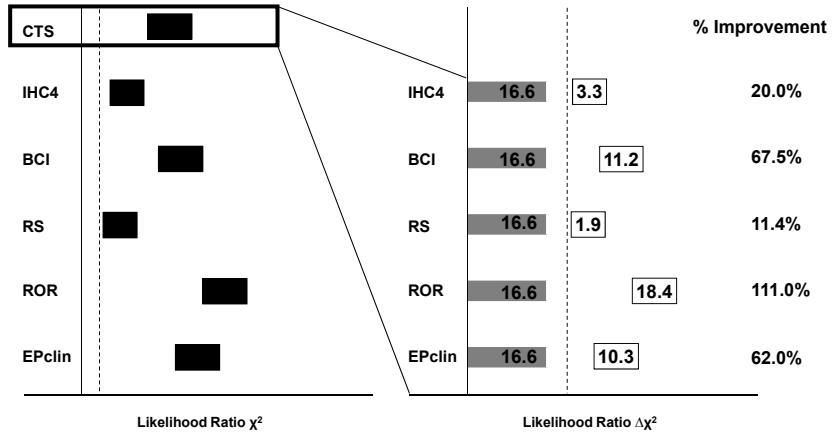
Prognostic value years 5-10 – node-negative



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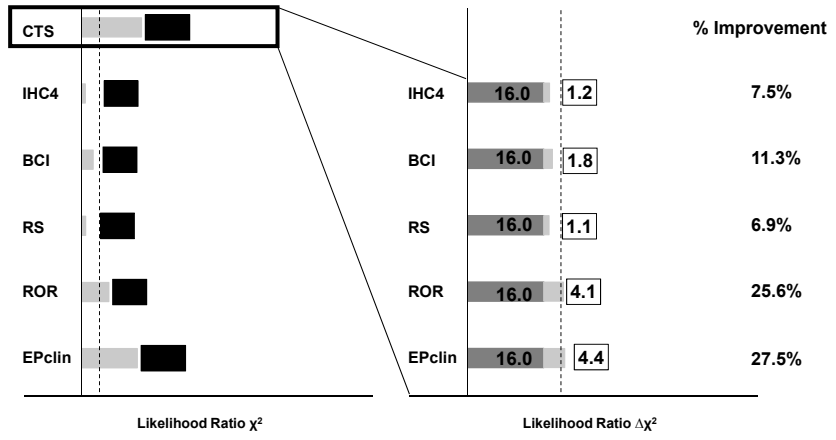
Prognostic value years 5-10 – node-positive



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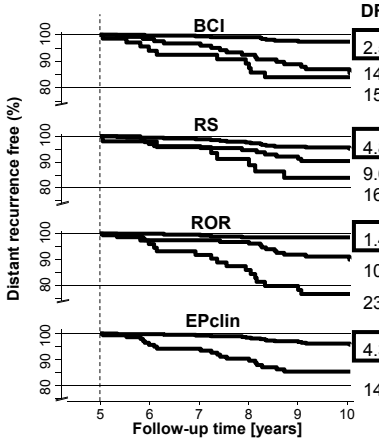
DR free (%) in years 5-10 – node-negative



	DR risk %	Patients % in risk groups
BCI	2.5%	**63.6**% patients
	14.4%	**23.6**% patients
	15.9%	**12.9**% patients
RS	4.8%	**65.6**% patients
	9.6%	**25.1**% patients
	16.1%	**9.4**% patients
ROR	1.4%	**54.6**% patients
	10.0%	**30.8**% patients
	23.2%	**14.6**% patients
EPclin	4.3%	**73.5**% patients
	14.6%	**26.5**% patients

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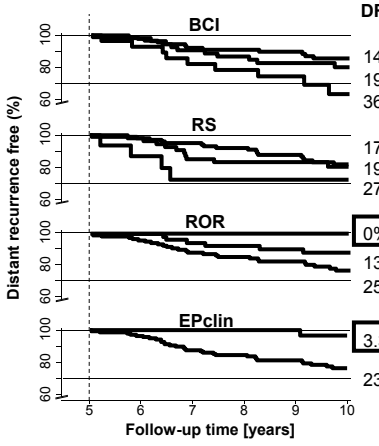
DR free (%) in years 5-10 – node-positive



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ROR	0%	**8.2**% patients
	13.0%	**28.0**% patients
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EPclin	3.3%	**22.0**% patients
	23.6%	**78.0**% patients

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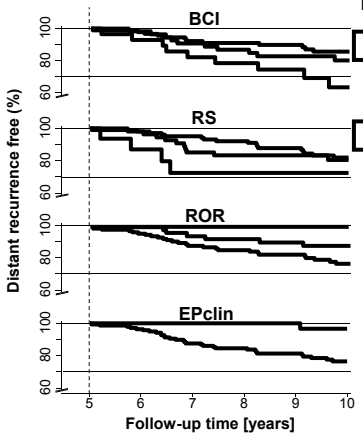
DR free (%) in years 5-10 – node-positive



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Conclusions

- Unique cohort with well annotated samples, mature clinical outcome, and prognostic information for six signatures

- Prediction of recurrence in years 0-10:
  - *Node-negative:*
    - All signatures good predictors and identify patients with a low DR risk
      → value of chemotherapy limited
  - *Node-positive:*
    - ROR/EPclin identify patients with low DR risk
      → value of chemotherapy limited

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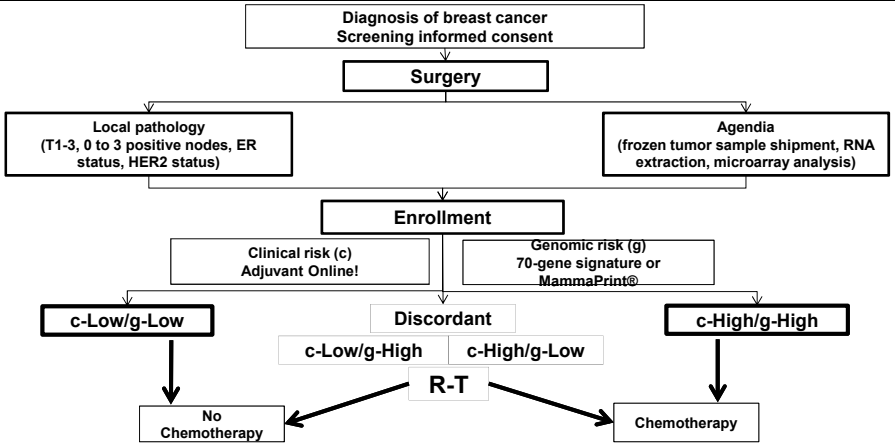
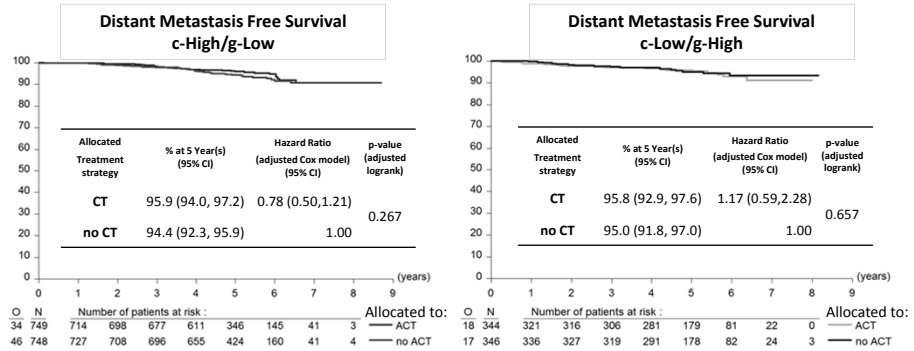
Conclusions II

- Prediction of recurrence in years 5-10:
  - *Node-negative:*
    - BCI, ROR and EPclin good predictors for late DR (above and beyond CTS)
    - All signatures identify patients with low risk of late DR
      → extended endocrine therapy not justified
  - *Node-positive:*
    - ROR/EPclin identify patients at low risk of late DR
      → extended endocrine therapy not justified
- Limitation:
  - CTS/IHC4 trained and ROR cut-off points estimated in transATAC
- Incorporation of certain clinical variables important

The MINDACT study design



Efficacy: CT vs no CT in discordant risk groups
Intent-to-treat analysis



EORTC The future of cancer therapy

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Other studies to compare different prognostic signatures

- **PAM50 is better differentiation of intermediate- and higher-risk groups than oncotype DX and IHC4 (M. Dowsett et al, J Clin Oncol. 2013)**
- **EPclin is more prognostic than RS (R. Buus et al, JNCI, 2016)**
- **MP vs ODX concordance: low risk (66%), high risk (78%), intermediate ODX (MP 52% low, 48% high), (R. Maroun et al, McGill, ASCO 2015)**
- **PAM50 vs ODX: moderate concordance (CM Kelly et al, Oncologist, 2012)**
- **PAM50, MammaPrint and Oncotype DX concordance, each test had significant prognostic value but individual risk assignments were often discordant (A Prat et al, Ann Oncol. 2012)**

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Prognostic signatures in EBC

- All signatures identify low risk pts with node-EBC in whom chemotherapy can be avoided
- Prognosis years 1-5: Oncotype DX or Mammaprint
- Prognosis years 6-10: BCI
- Ongoing RxPONDER trial (SWOG1007) will determine the utility of ODX in node+ setting

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Thank you!

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