Fusion Analysis of Solid Tumors Reveals Novel Rearrangements in Breast Carcinomas

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Disclaimers

Dr. Igor Astaturov

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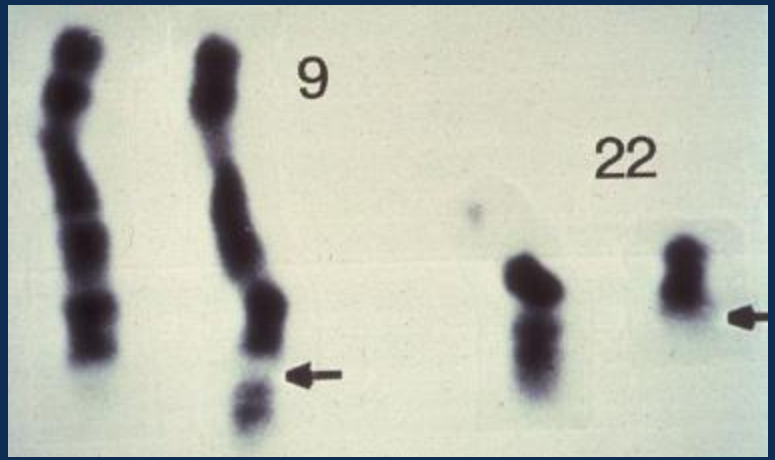
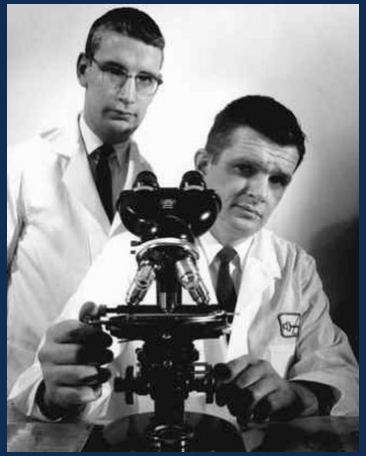
Outline

- Introduction
- Methods
- Overall results
- Breast CA results
- Clinical relevance

Introduction to gene fusion

- Fusions of genes are common events in epithelial cancers
- Recurrent fusions arise as the result of genomic rearrangements or abnormal processing of mRNA
- Proteins translated from gene fusions are potential drug targets





Methods

- Retrospective evaluation on 1,915 solid tumor specimens evaluated by fusion analysis (+/- NGS)
- **ArcherDx** fusion assay based on anchored multiplex PCR (AMP)
  - FusionPlex Solid Tumor Kit
  - 52 genes analyzed
- All assays performed by Caris laboratories

Reverse transcription:
RNA → DNA

↓

Adapter ligation

Nested PCR amplification of target regions

↓

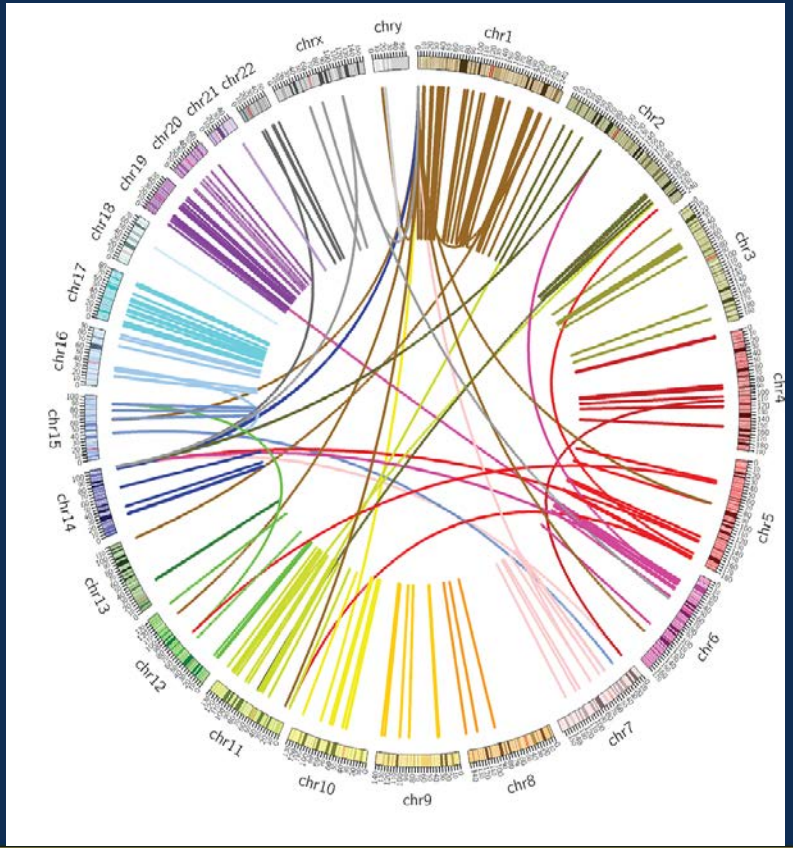
NGS
Bioinformatics filtering and Sanger validation

Methods (cont'd.)

Inclusion criteria:

- >20,000 total reads per sample
- Must be picked up >2 random primers (a.k.a. RNA start sites)
- Exon in open reading frame
- No sequence similarity between the two fusion partners (prone to artifacts)
- Novel isoform or fusion >10% of the reads for the targeted region
- Fusion is found in a database of known fusions (e.g. Archer Quiver database)
- Not detected among >11,000 fusions in normal tissues (Babicenau, Nucleic Acid Res. 2016)

Recurrent fusions in normal human tissues



Methods (assay validation)

- Validated gene fusions: *ALK*, *BRAF*, *cMET* (exon 14 skipping), *EGFRvIII*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1*, and *RSPO3*
- 140 total samples were used for assay validation

  (93 positives and 47 negatives)

  – Confirmatory assay: FISH, RT-PCR/Sanger sequencing, and RT-PCR/fragment analysis (for EGFRvIII)

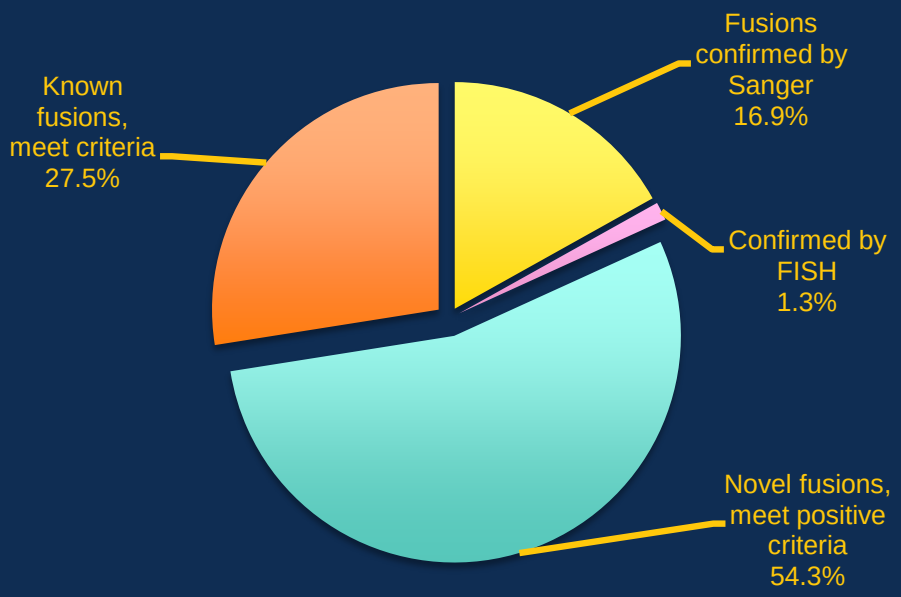
Results: analysis of n=1915 cases

Important to determine functional vs. non-functional fusion genes

Interpretation challenges:

- Does the fusion make biological sense?
- Is an open reading frame present?
- Low level fusions may not be reproducible (e.g. TMPRSS2-ERG in uveal melanoma).
- Many unique and previously unknown fusions (e.g. ETS family, CFTR-BRAF in a pancreatic CA).

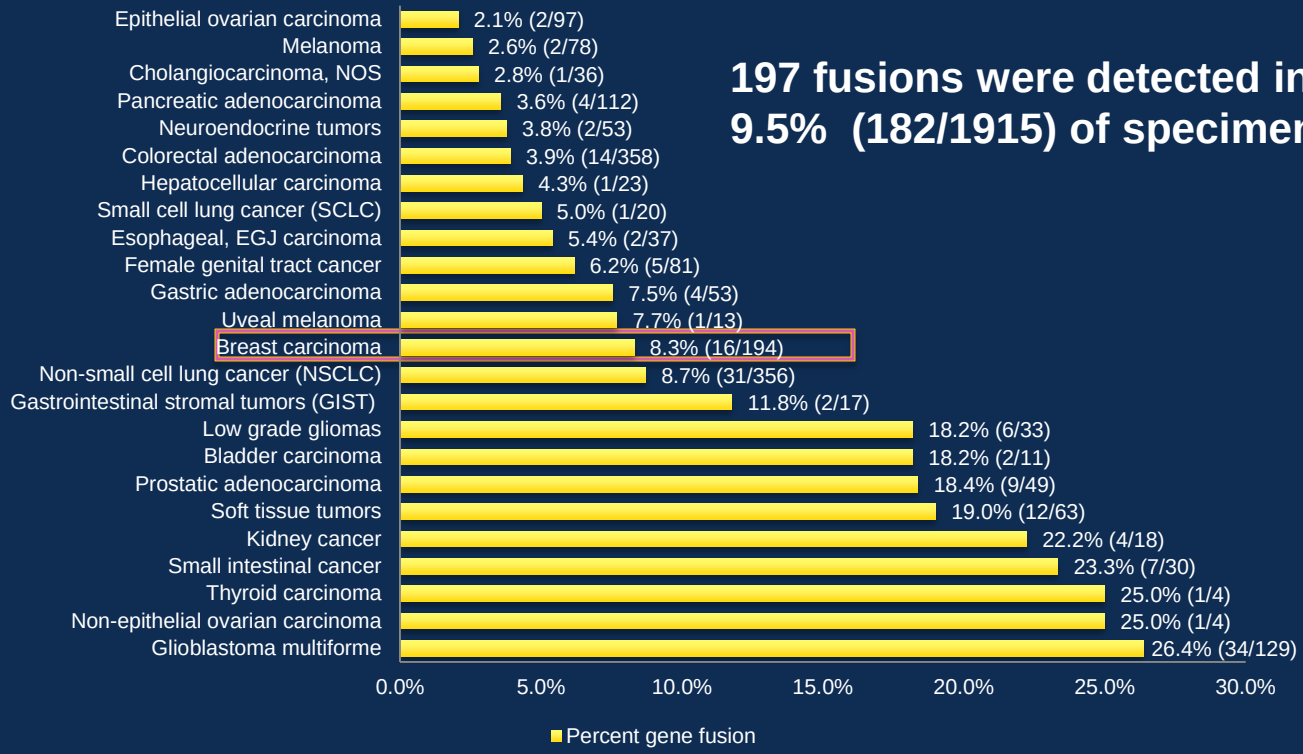
Detected fusions in cohort



Overall Results

21 unique fusions detected in
16 cases of breast adenocarcinoma:

- 5 fusions of ESR1
- 3 fusions of RAF1
- 2 fusions of FGFR2, PPARG, RET
- 1 fusion of EGFR, FGFR3, MAST2, PRKCA, PRKCB, ERG, ETV6



197 fusions were detected in 9.5% (182/1915) of specimens.

Fusions in overall cohort

Fusion Class	Fusion Gene of Interest
Kinases	**ALK, BRAF, *BRD3, BRD4,* EGFR, *FGFR1, FGFR2, FGFR3*,** *INSR* (insulin receptor), *MAST1, MAST2,* **MET**, *MUSK,* ***NTRK1, NTRK2, NTRK3, PKN1, PRKCA, PRKCB, RAF1,* RET, ROS1**
Transcription factors	*ERG, ESR1, EWSR1, ETV1, ETV5, ETV6, MAML2, MYB, PPARG, RELA, TFE3, NOTCH2*
GTPase-activator	*ARHGAP26*
Ligands	*MSMB,* ***NRG1*** *(ERBB3), RSPO3 (WNT pathway)*
Telomerase	*TERT*

Biomarkers in **bold** can be targeted with FDA-approved therapy or in the clinical trials setting.

Clinically important fusions – *NTRK1/2/3*

In our cohort:

GBM - 4

RCC - 1

GIST - 1

NSCLC - 1

Soft tissue sarcoma - 1

- *Nagasubramanian et al. (2016)* reported a pediatric patient with refractory IFS (infantile fibrosarcoma) with *TV6-NTRK3* fusion treated with LOXO-101, a pan-NTRK inhibitor

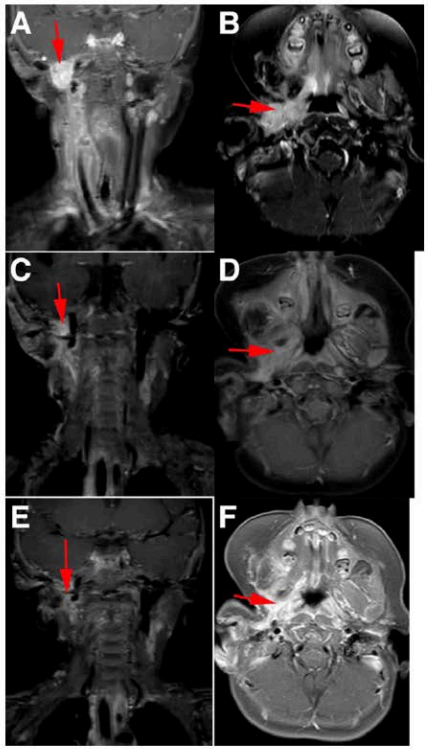


Fig. 1. Magnetic resonance imaging (MRI) of baseline disease assessment of the (A) neck and (B) oral cavity, with areas of interested highlighted with red arrows. Magnetic resonance imaging demonstrating >90% reduction in tumor masses of the (C) neck and (D) oral cavity following completion of the first month of therapy. Confirmation of the tumor response and decreased enhancement demonstrated by MRI of the (E) neck and (F) oral cavity following the second month of therapy.

Fusions in invasive breast carcinoma, n=16/194 (8.3%)

Fusion Category	Specific Fusion
Kinase fusions	*TIG1-**EGFR***
	***FGFR2**-CCDC3, **FGFR2**-PLEKHS1*
	***FGFR3**-TACC3*
	*FCGR2C-**MAST2***
	*BPTF-**PRKCA***
	*ANKRD28-**RAF1**, SH3BP5-**RAF1**, XPC-**RAF1***
	*CCDC6-**RET**, SPINT1-**RET***
Transcription factor fusions	***ESR1**-ATP2B2, **ESR1**-MKL1, **ESR1**-TNRC6B, **ESR1**-ARNT2, **ESR1**-C6ORF211*
	*HLCS-**ERG***
	*NUP210-**PPARG**, LSM14A-**PPARG***
	***ETV6**-RUNX1*

Not shown- PRKCG:PRKCB, although detected, probably benign

ESR1 fusions in invasive breast CA

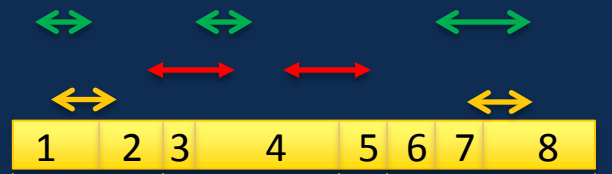
Case	Fusion	ER	PR	HER2	Other mut.	Age	Hormonal Rx
1 (sub-clav. LN)	***ESR1-ATP2B2***	**Positive** (2+, 60%)	Negative	Negative	*BRCA2* TP53**	**57**	Anastrozole, Fulvestrant
2 (axillary LN)	***ESR1-MKL1, ESR1-TNRC6B***	**Positive** (2+, 90%)	Low Positive (2+, 2%)	Negative	--	**52**	Fulvestrant Letrozole Leuprolide
3 (liver)	***ESR1-ARNT2***	**Positive** (2+, 98%)	Negative	Equivocal (2+, 70%), CISH Negative	--	**74**	N/A
4 (liver)	***ESR1-C6ORF211*****	**Positive** (2+, 95%)	Negative	Negative	--	**37**	Tamoxifen

* Molecular alterations were presumed pathogenic/tumorigenic

** Previously reported intra-chromosomal rearrangement at 6q25.1 (SABCS 2014)

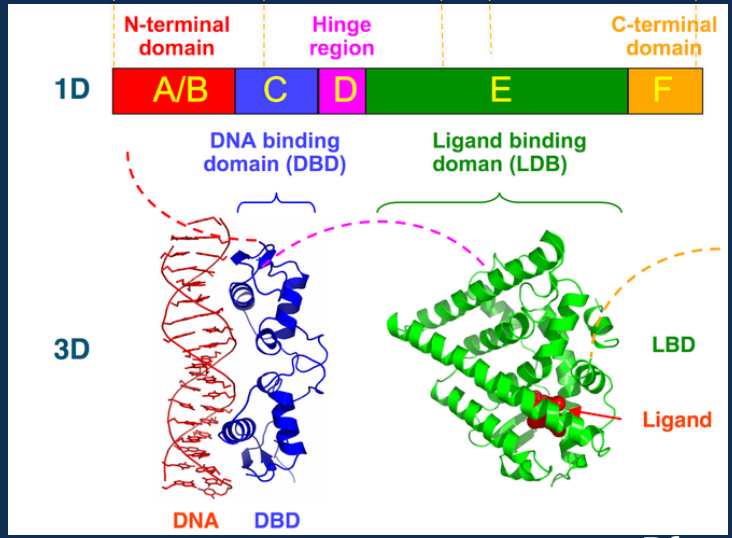
ESR1 fusions retain function

Coactivator binding
Dimerization
Transcription activation



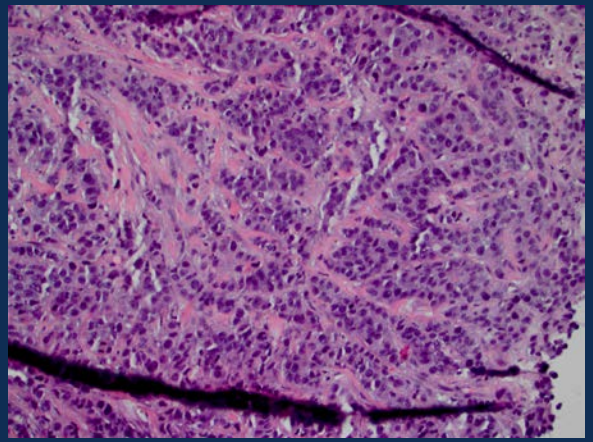
ESR1	Fusion partner	
1 2	4 5	ATP2B2
1 2 3 4	7 8	MKL1
1 2 3 4	14 15	TNRC6B
1 2 3 4 5	9 10	ARNT2
1 2 3 4 5	3 4	C6ORF211

same specimen

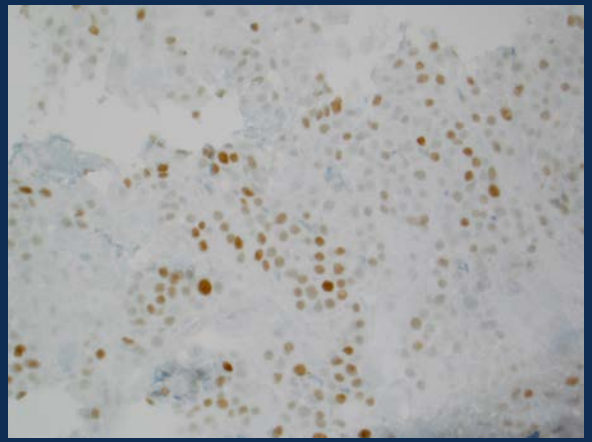


Pfam.xfam.org

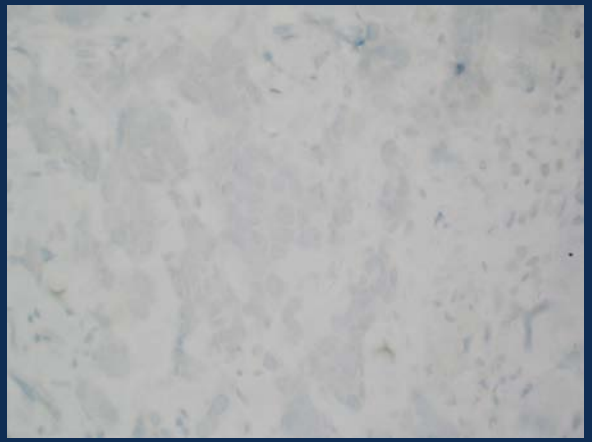
ESR1-ATP2B2 fusion in invasive breast CA – Case #1



H&E

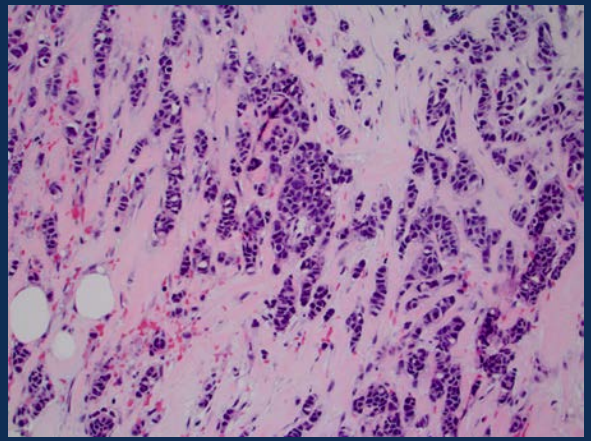


ER (2+, 60%) Ab Clone SP1

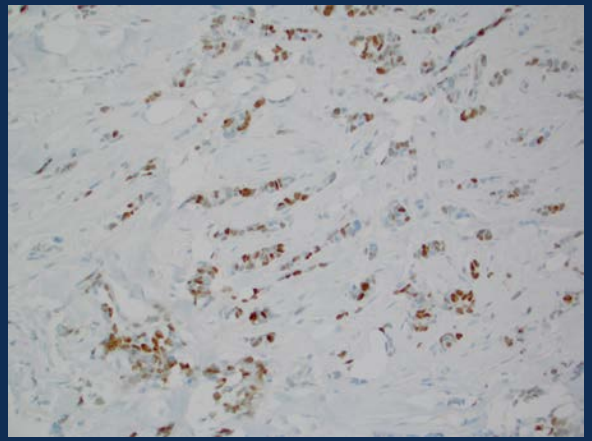


PR (0+, 100%)

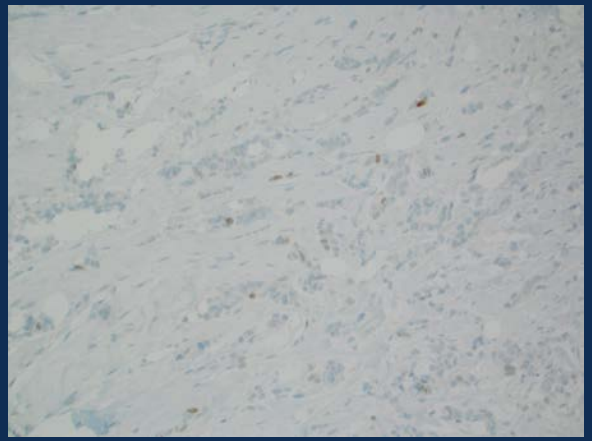
ESR1-MKL1 and ESR1-TNRC6B fusions in Case #2



H&E



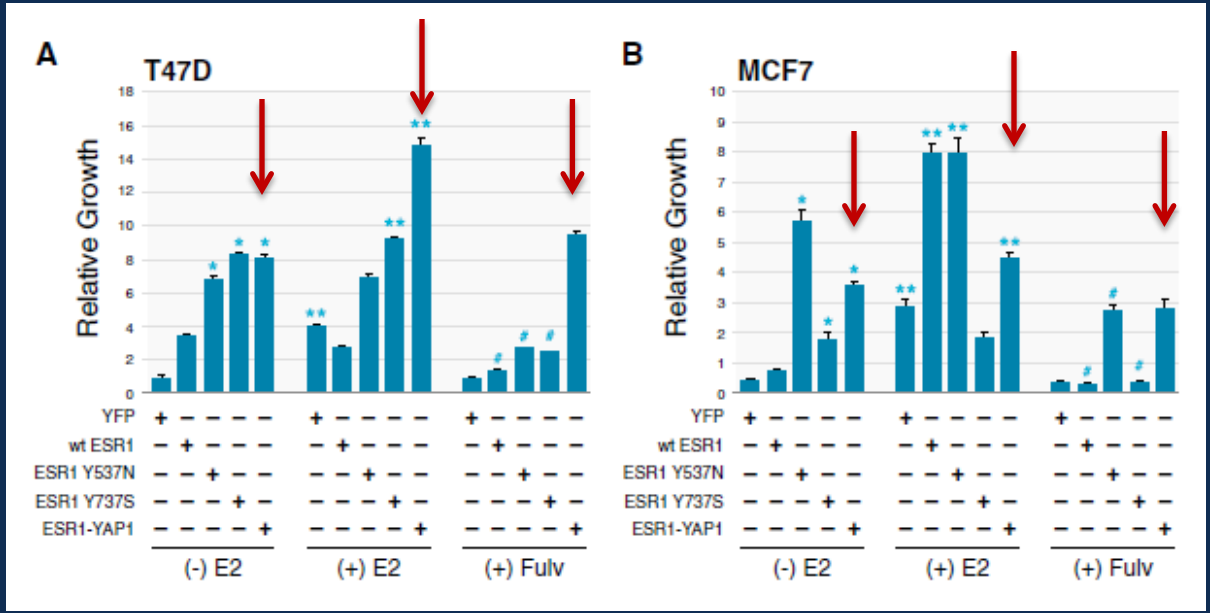
ER (2+, 90%) Ab Clone SP1



PR (2+, 2%)

ESR1 fusions in breast cancer: Mechanism

- C-terminus truncated *ESR1* fusions are recurrent and functional in breast cancer
- *ESR1* fusions involve many partner genes (here, ATP2B2, MKL1, TNRC6B, ARNT2, C6ORF211)
- ESR1 fusions (e.g. *ESR1-CCDC170, ESR1-YAP1*) are associated with anti-estrogen resistance



Li, S. et al. Cell Reports, 2013

Summary

- RNA assay for common gene fusions is now validated and available to guide the patients to clinical trials and drug treatment options
- Pathogenic gene fusions may be missed by DNA sequencing only
- Multiple ESR1 fusions should be prospectively evaluated for anti-estrogen resistance

Acknowledgements

- Jeff Kimbrough (for biostatistics)

Thank you