# Fusion Analysis of Solid Tumors Reveals Novel Rearrangements in Breast Carcinomas

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PRESENTED AT: ASCO ANNUAL MEETING '16

# Disclaimers

Dr. Igor Astaturov

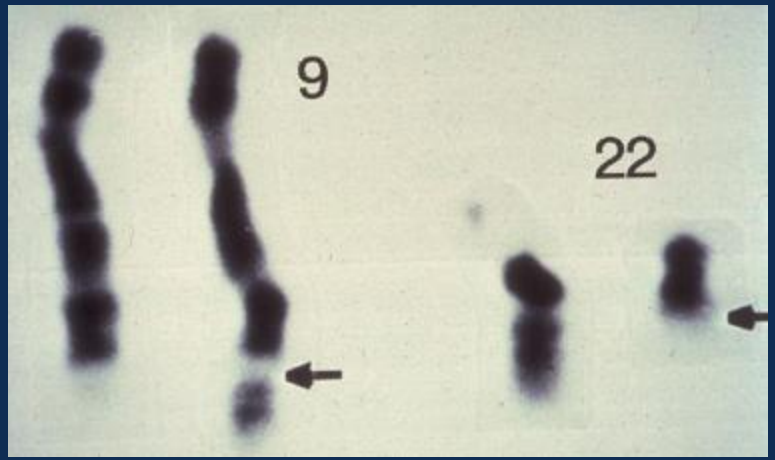
- Consultant (Caris Life Sciences)

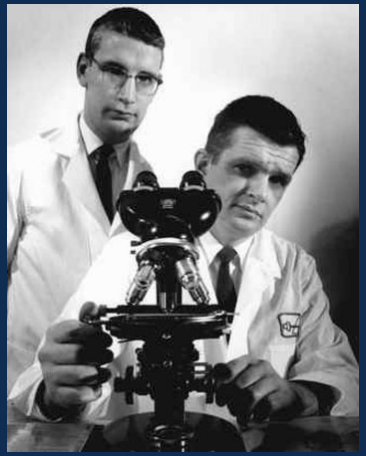
# Outline

- 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**

↓

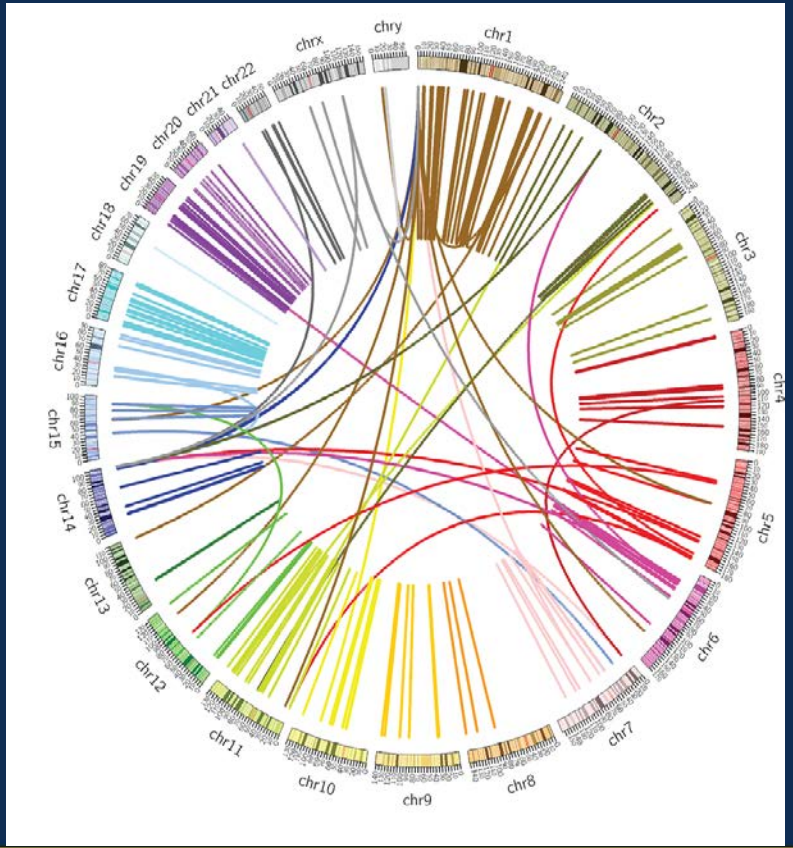
**NGS**
**Bioinformatics filtering and Sanger validation**

# 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)

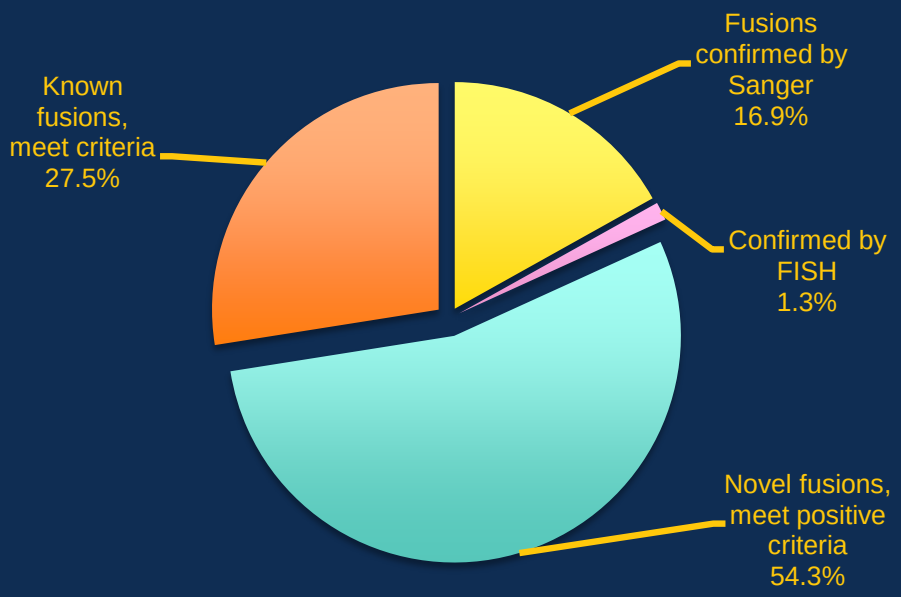
# Results: analysis of n=1915 cases

*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).

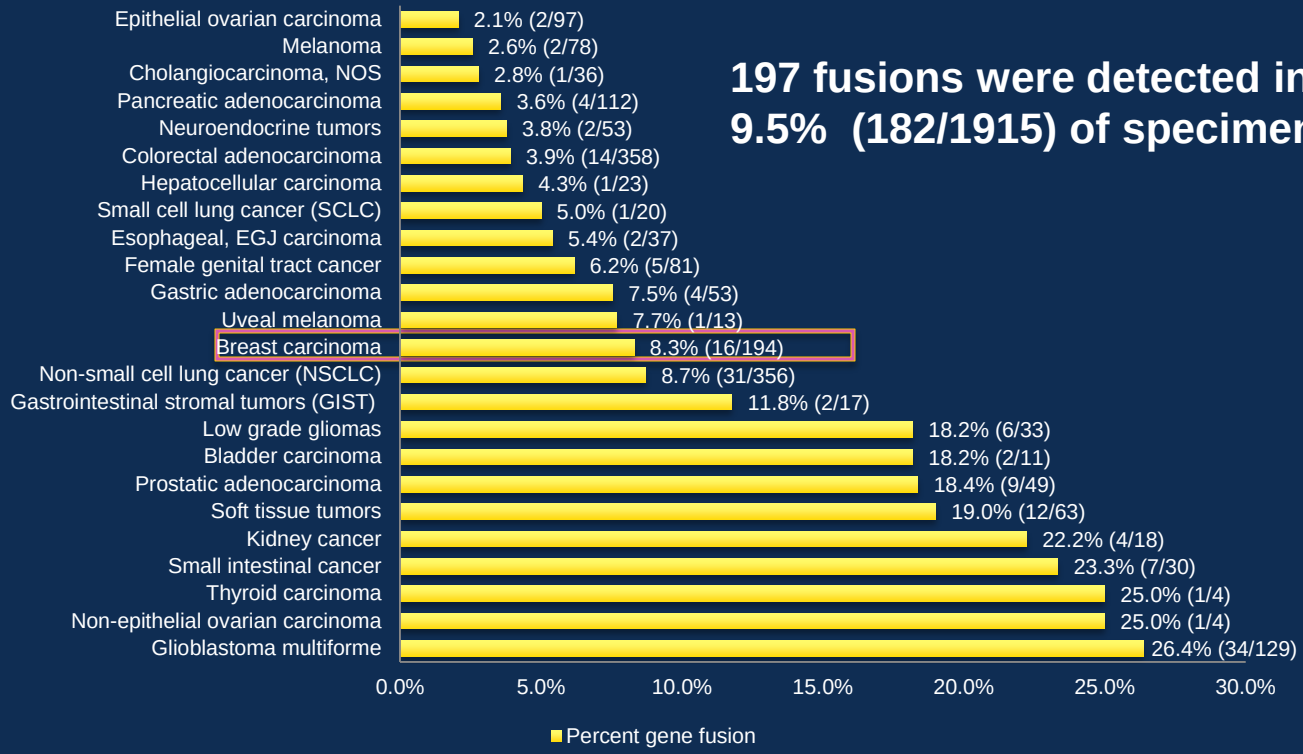
## Detected fusions in cohort



# 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

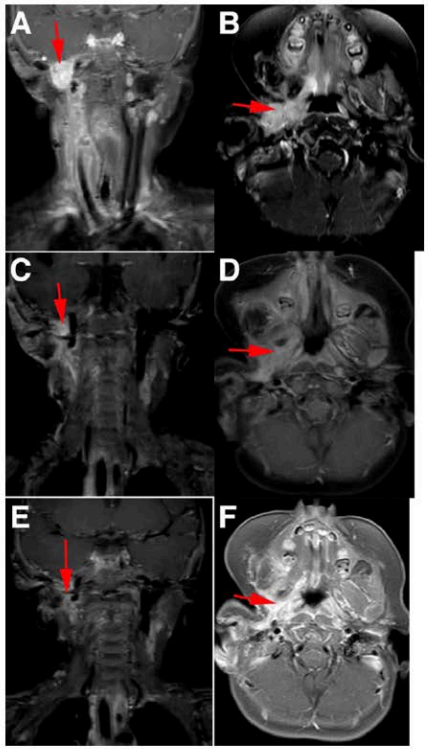



Fig. 1. Magnetic resonance imaging (MRI) of baseline disease assessment of the (A) neck and (B) oral cavity, with areas of interested highlighted with red arrows. Magnetic resonance imaging demonstrating >90% reduction in tumor masses of the (C) neck and (D) oral cavity following completion of the first 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

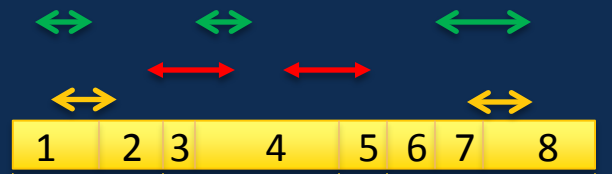
| Case | Fusion | ER | PR | HER2 | Other mut. | Age | Hormonal Rx |
|---|---|---|---|---|---|---|---|
| 1 (sub-clav. LN) | ***ESR1-ATP2B2*** | **Positive** (2+, 60%) | Negative | Negative | *BRCA2* TP53** | **57** | Anastrozole, Fulvestrant |
| 2 (axillary LN) | ***ESR1-MKL1, ESR1-TNRC6B*** | **Positive** (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)

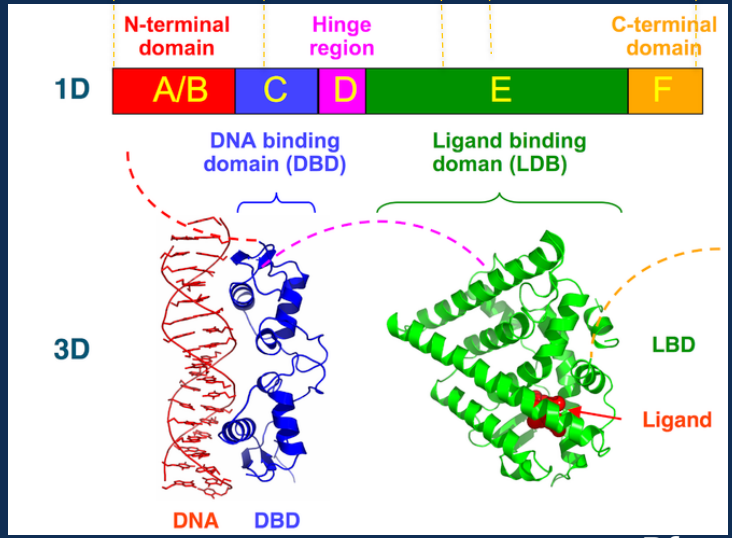
# *ESR1* fusions retain function

Coactivator binding
Dimerization
Transcription activation



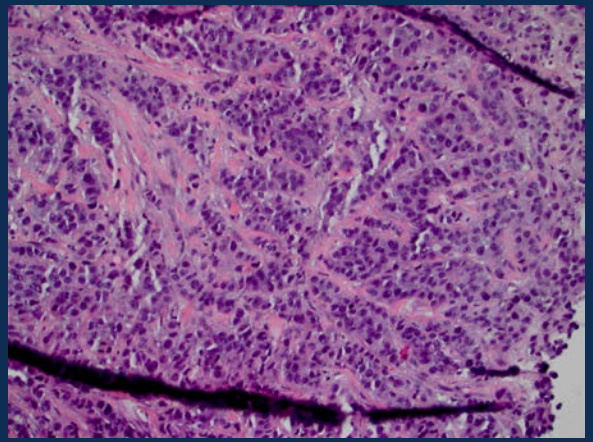

| ESR1 | Fusion partner | |
|---|---|---|
| 1 2 | 4 5 | ATP2B2 |
| 1 2 3 4 | 7 8 | MKL1 |
| 1 2 3 4 | 14 15 | TNRC6B |
| 1 2 3 4 5 | 9 10 | ARNT2 |
| 1 2 3 4 5 | 3 4 | C6ORF211 |

same specimen

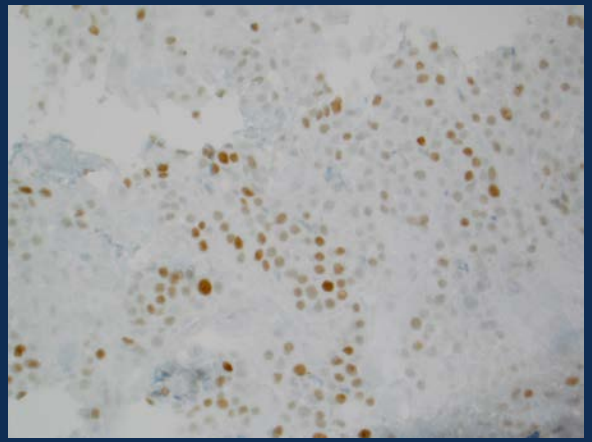



Pfam.xfam.org

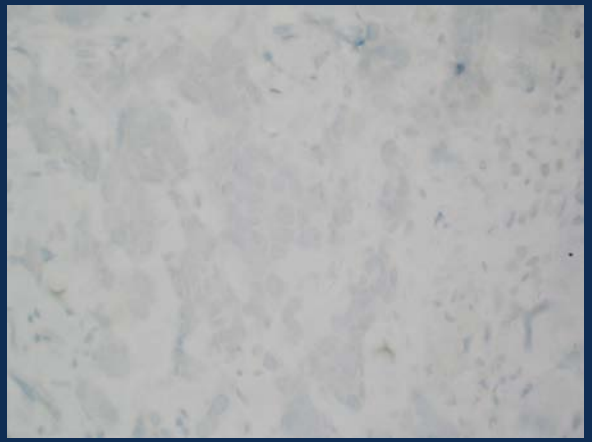
# *ESR1-ATP2B2* fusion in invasive breast CA – Case #1

H&E

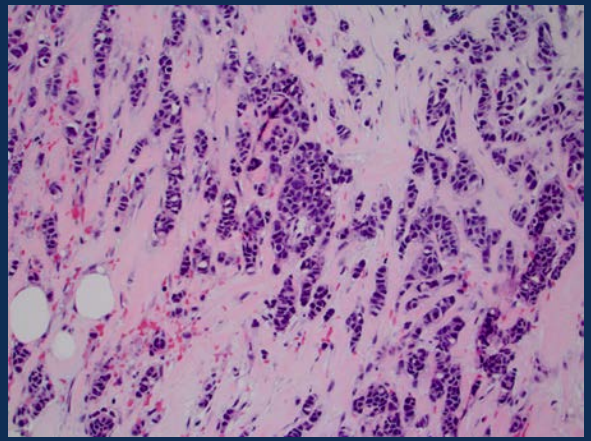


ER (2+, 60%) Ab Clone SP1

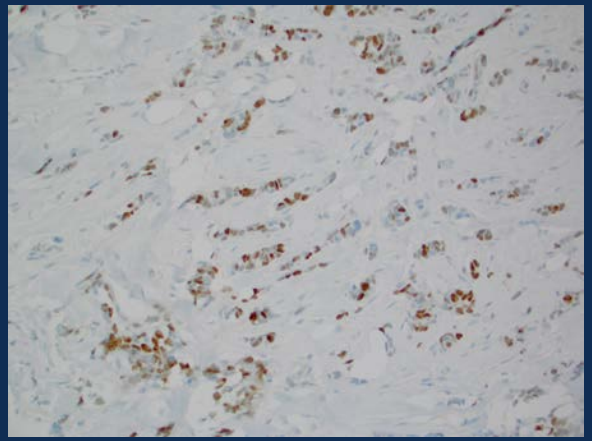


PR (0+, 100%)

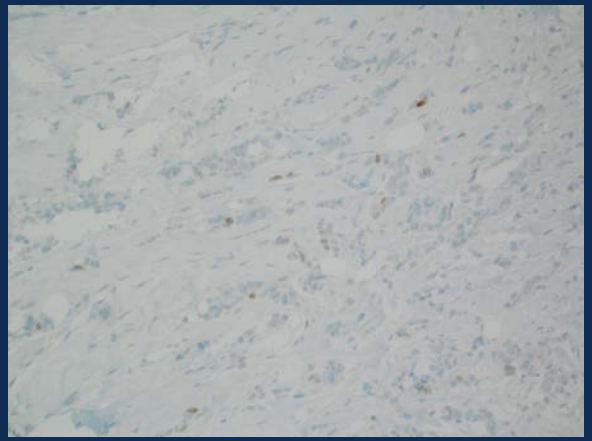
# *ESR1-MKL1 and ESR1-TNRC6B* fusions in Case #2



H&E



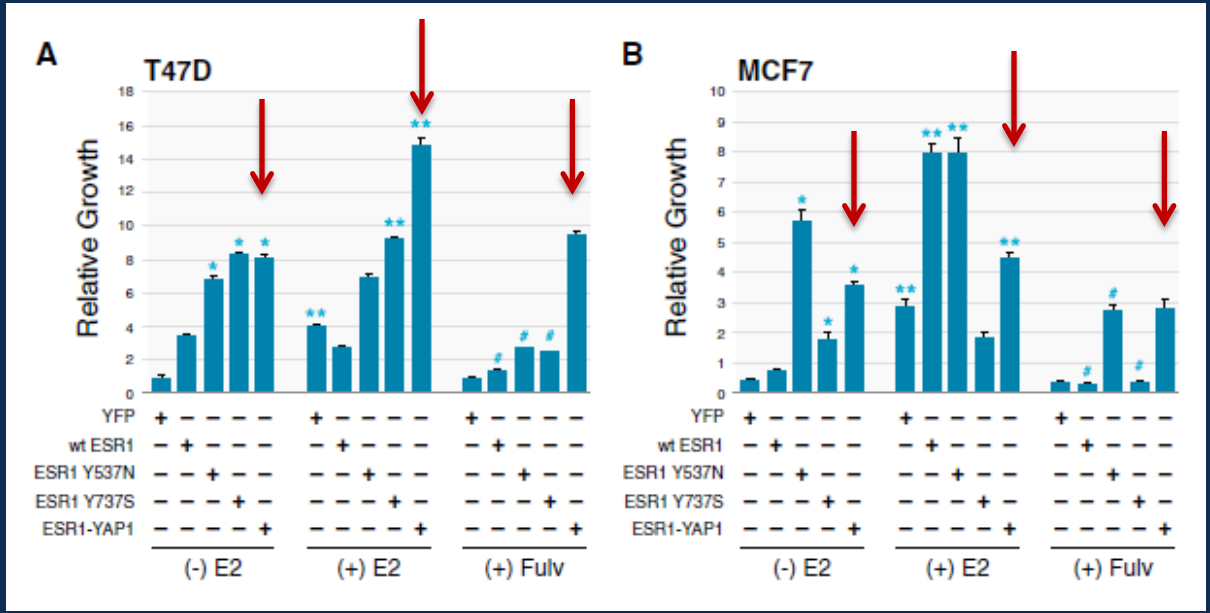
ER (2+, 90%) Ab Clone SP1



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