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Novel *ESR1* fusion gene identified from matched primary and recurred breast cancers by RNA-sequencing

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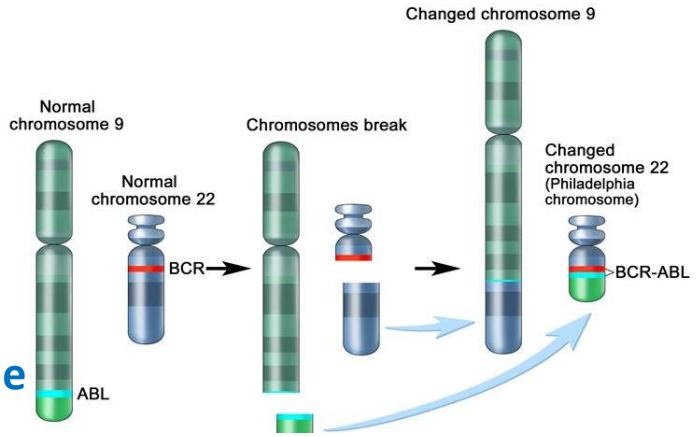
Fusion genes

- **Joining of a two independent , unrelated genes**
  - Result of a **structural rearrangement** of the genome
  - Creates a **chimeric proteins** unique in cancers



- ***BCR-ABL1*, t(9;22)(q34;q11), Philadelphia chromosome**
  - Present in hematologic malignancies
  - Diagnosis and monitoring response to Gleevec

- **Numerous fusion genes in solid tumors** identified owing to RNA-sequencing
  - ETS fusions, eg. *TMPRSS2-ERG* fusion among 50% of prostate cancers in 2005
  - ALK fusions in 6.7% of non-small cell lung cancer (ALK inhibitor)
  - Abundant in epithelial tumors eg. lung, ovary, prostate etc.
  - *Gain of function by overexpression, loss of function by early truncation*

Edwards et al. BCR 2011
Robinson et al. Nat Med 2011
Soda et al. Nature 207
Wang et al. Mol med rep 2017

Aim

- **To discover fusion genes which drive tumor progression and metastasis**

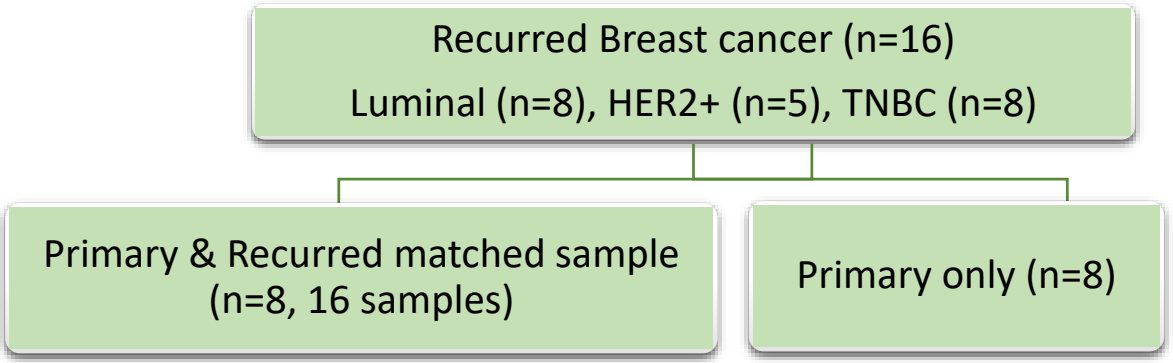
- **By RNA-sequencing of matched primary and recurred breast cancer samples**

Method

- **Sixteen recurred** breast cancer cases
- **RNA-sequencing** from **primary/recurrent** tumor tissues using FFPE tissue

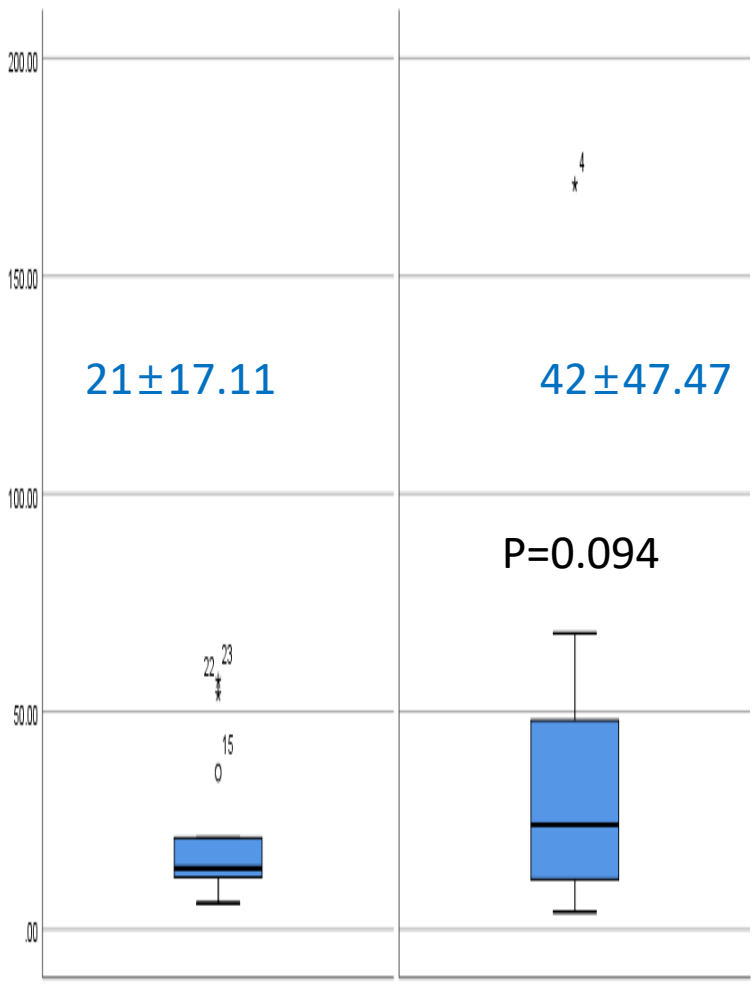
- Quality control and Sequencing successfully achieved from
  - 8 primary/recurred matched samples and 8 primary tumor only

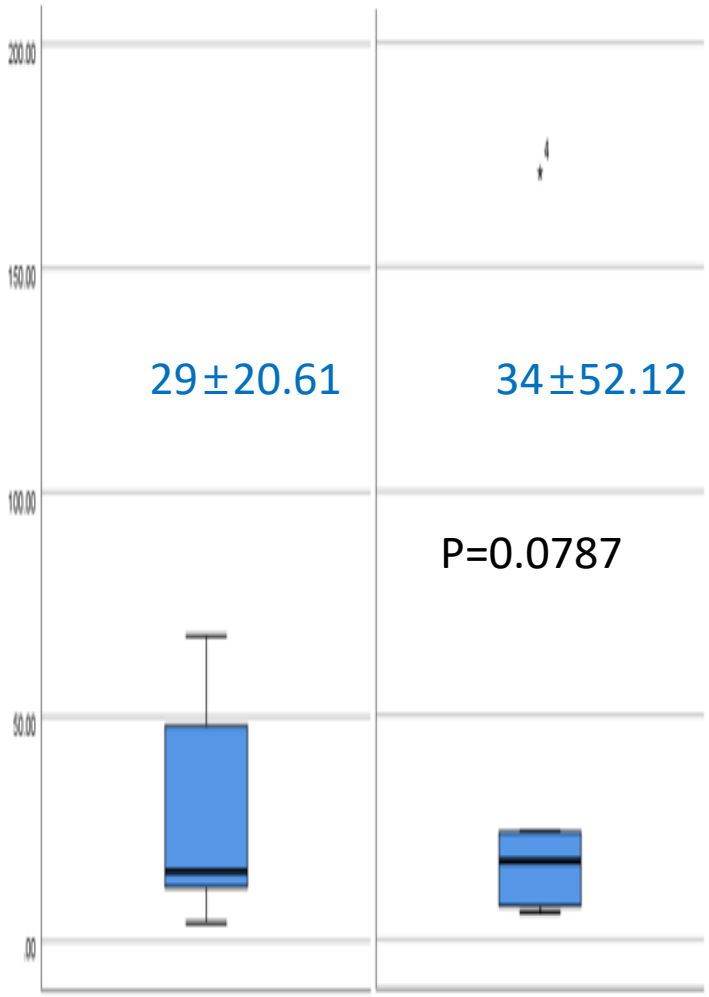
- Fusion detecting algorithm **DeFuse**
- Fusion transcripts and gene expression data
- Fusions with >0.5 probability chosen for analysis



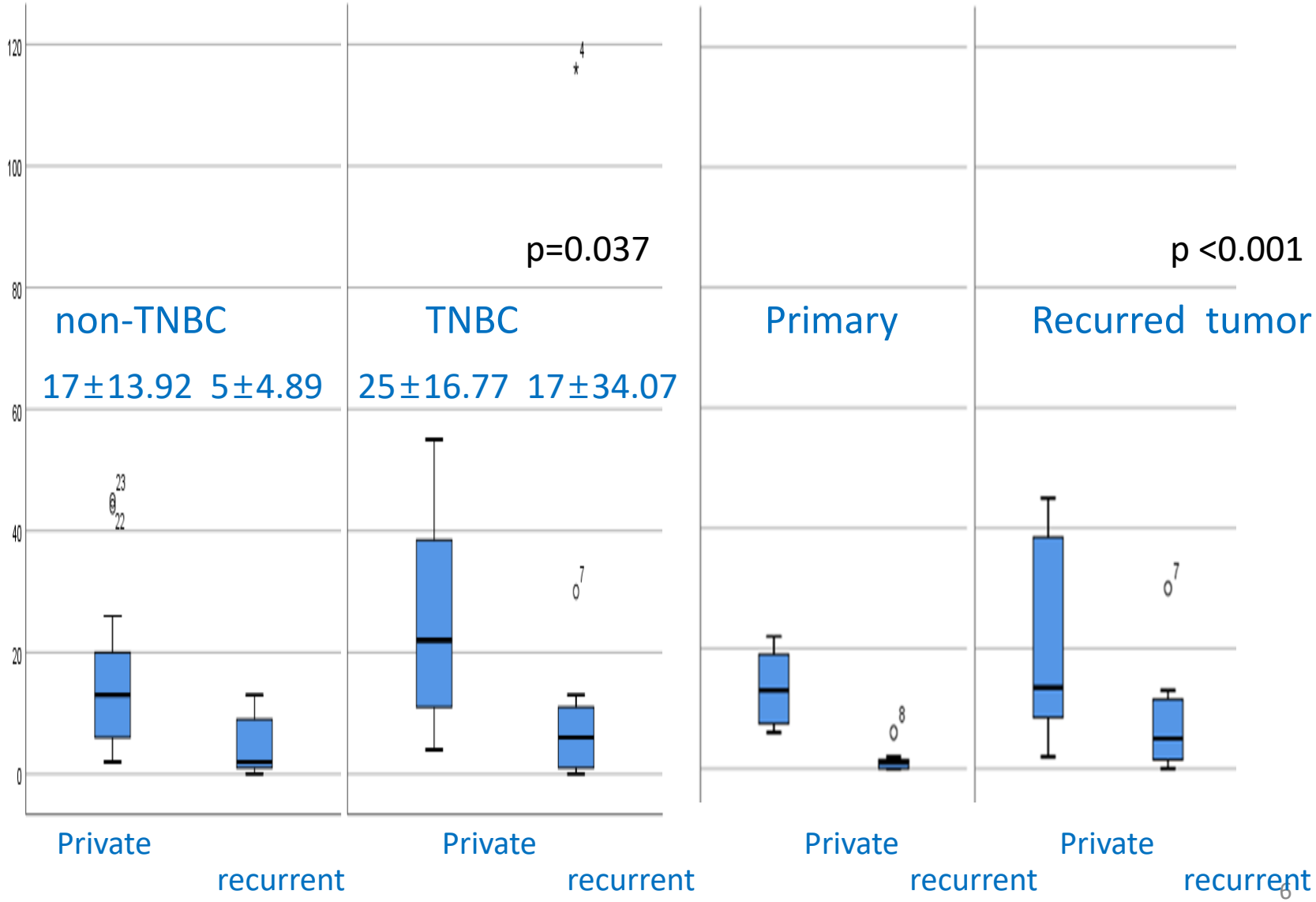
Detecting and visualizing gene fusions. Germany Methods

Number of fusion genes across Subtypes

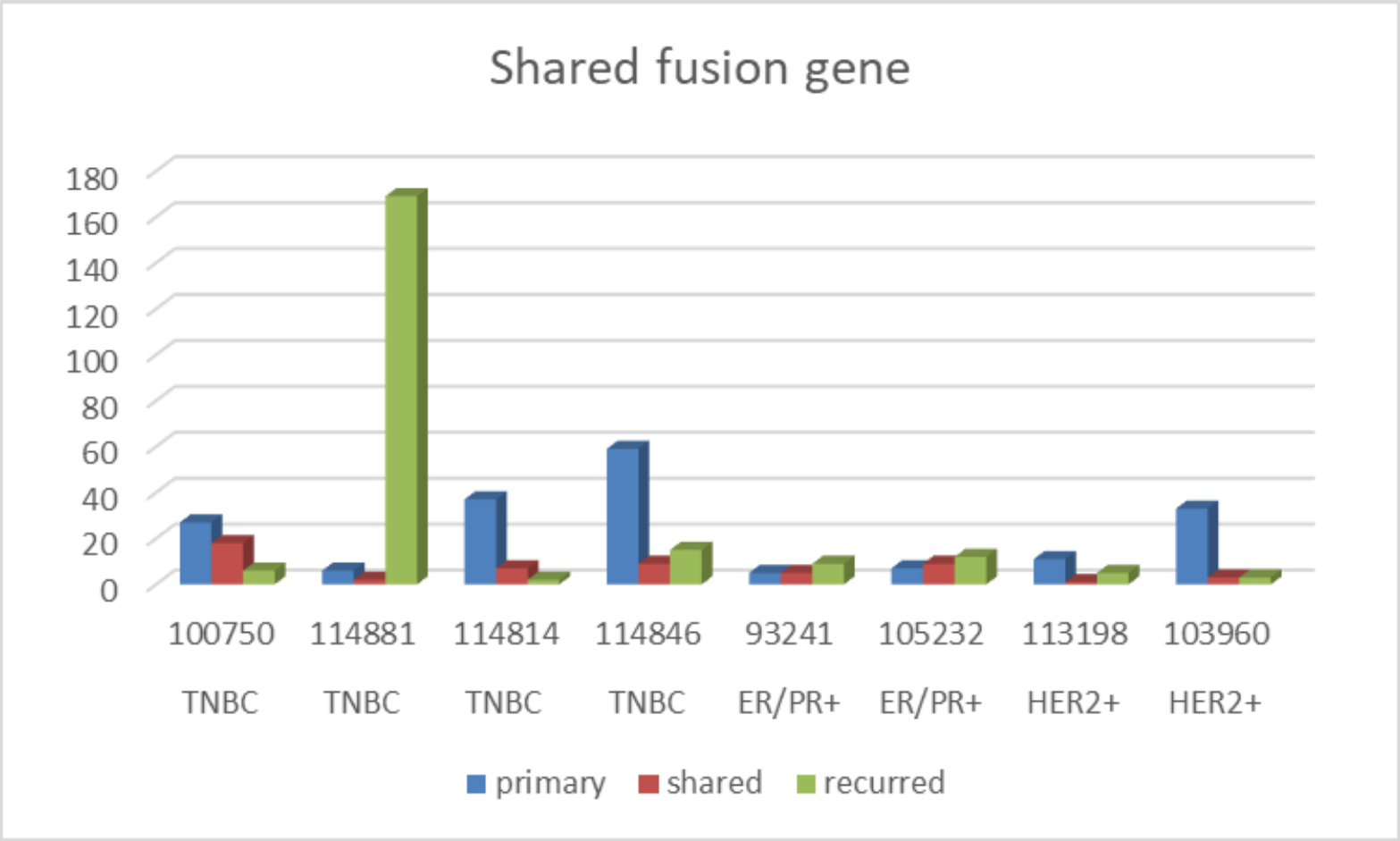




Recurrent fusions



Shared fusions between primary and recurred tumors



ESR1 gene, estrogen receptor coding gene

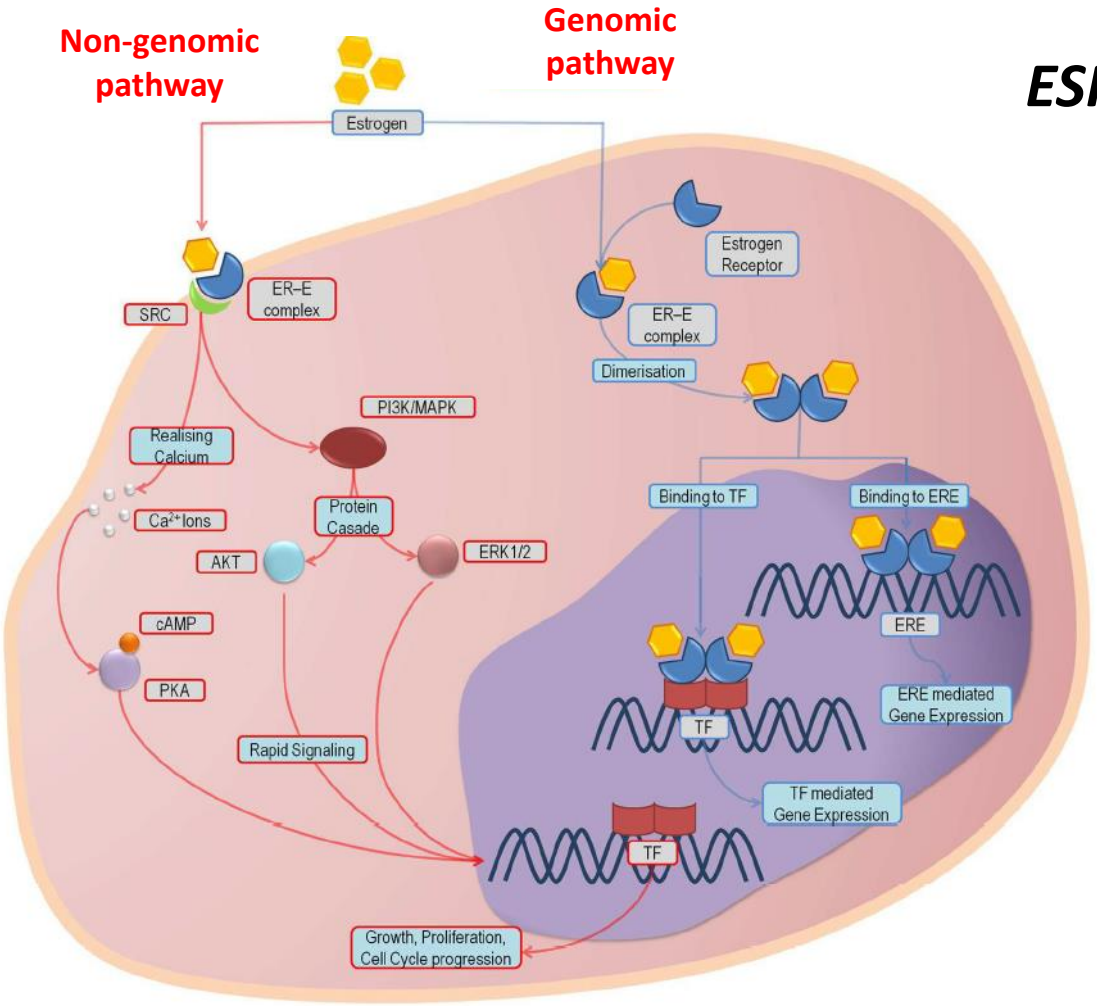
DBD : DNA-binding domain

LBD : Ligand-binding domain

AF1 AF2 : Activation function 1, 2 domain

- ***ESR1* gene**
  - Chr6:151,977,826-152,450,754 (472,929 bases, hg19)
  - 10 exons translated into Estrogen receptor protein
- **Estrogen receptor (ER) protein**
  - Transcription factor, DNA binding domain, Ligand binding domain
  - Target of antiestrogen therapy

Jonathan T. Lei et al. Cell rep. 2018



ESR1 gene, Estrogen receptor

DBD : DNA-binding domain

LBD : Ligand-binding domain

AF1 AF2 : Activation function 1, 2 domain

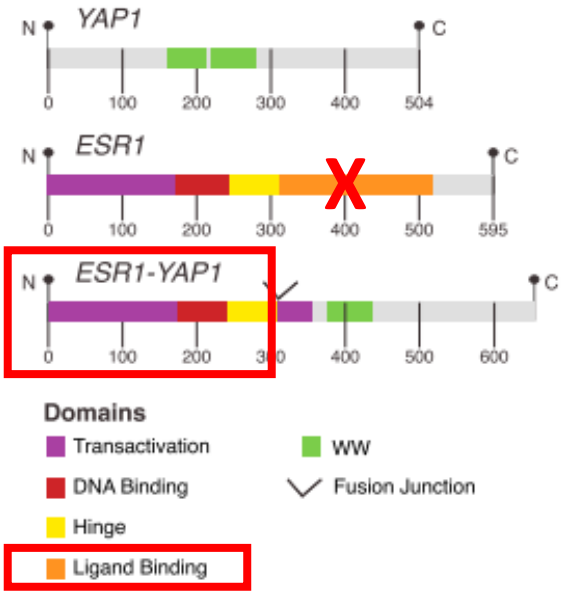
Jonathan T. Lei et al. Cell rep. 2018
Słowikowski et al. Mol Biol rep 2016

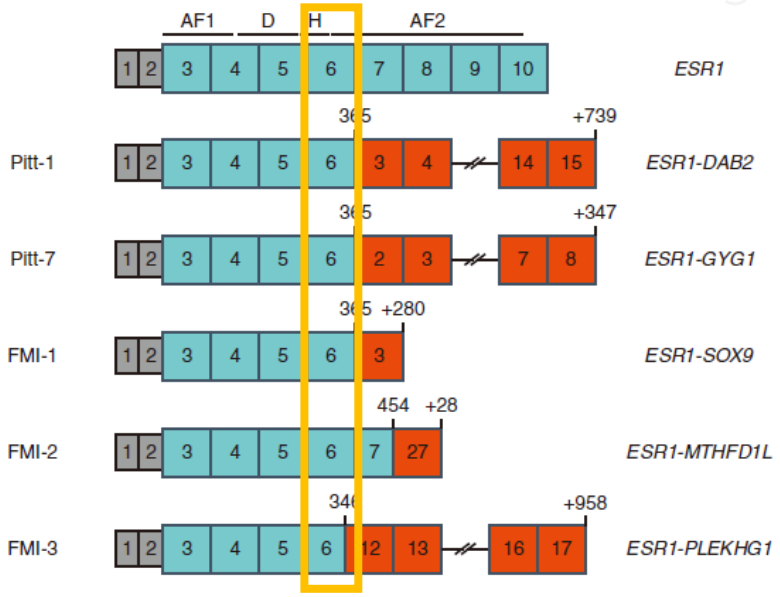
Previously reported *ESR1* fusions driving endocrine resistance

- Known ***ESR1*** hotspot mutations at ligand binding domain

-> Estrogen independent growth driving endocrine resistance

- ***ESR1* Fusions with loss of ligand binding domain**

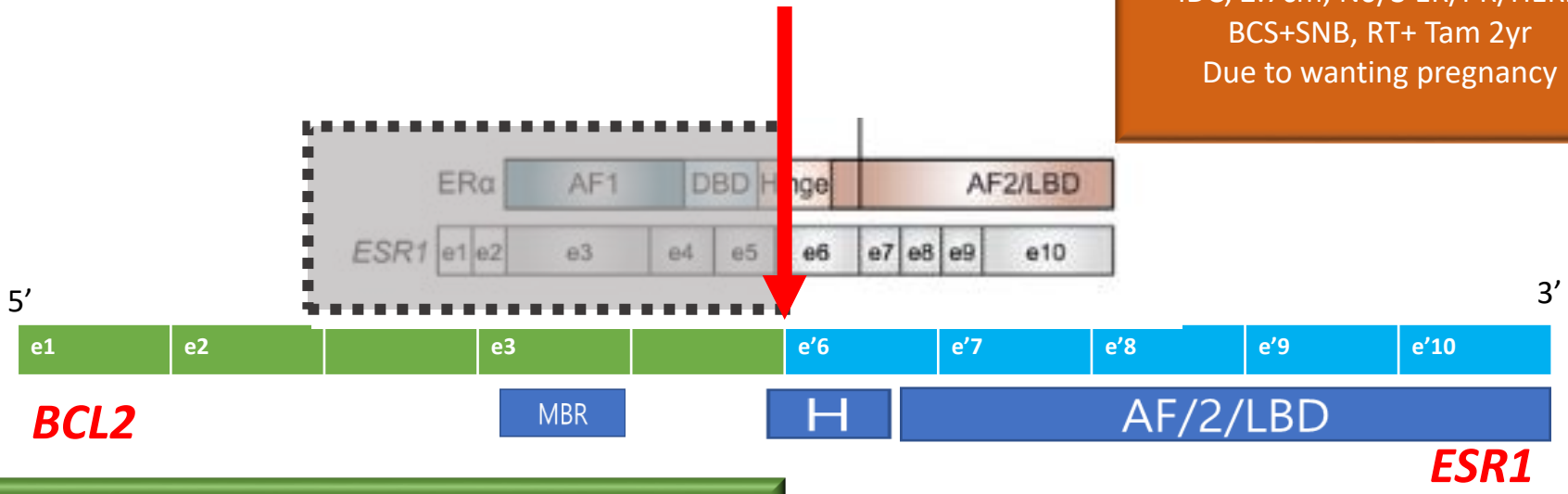




Li et al. Cell press 2013

Novel *BCL2-ESR1* fusion

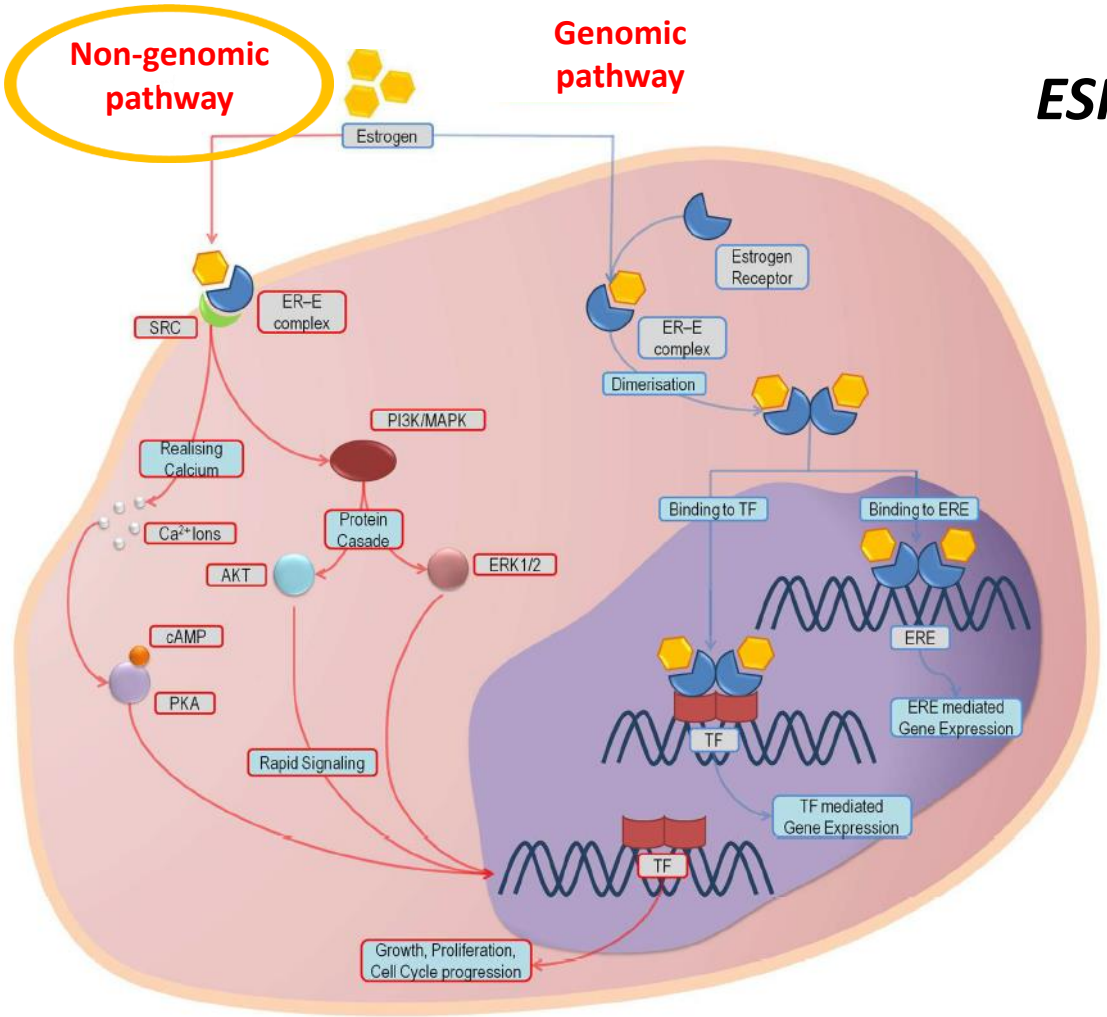
F/45 2015.4.3
IDC, 2.7cm, N0/3 ER/PR/HER2
BCS+SNB, RT+ Tam 2yr
Due to wanting pregnancy



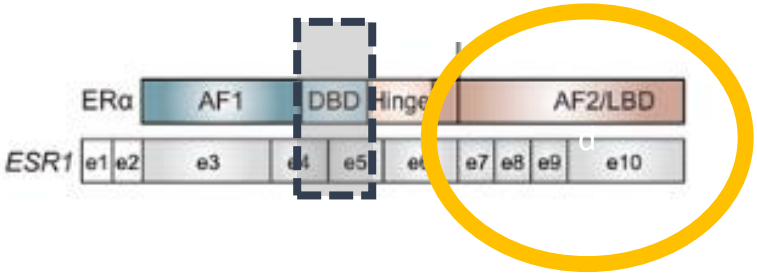
BCL2 - apoptosis-regulatory genes promoting cellular survival
ESR1 - gene coding estrogen receptor

Overexpression of both genes	
BCL2	*ESR1*
2221 FPKM	7101 FPKM
Out of frame	

Jonathan T. Lei et al. Cell rep. 2018 Agust



ESR1 gene, Estrogen receptor



- *BCL2-ESR1* fusion gene lead to overexpression of both genes
- Estrogen receptor protein without DNA binding domain
- May lead to activation of non-genomic pathway

Jonathan T. Lei et al. Cell rep. 2018
Słowikowski et al. Mol Biol rep 2016

Discussion

- **Triple negative breast cancer** displayed greater number of fusion transcripts.
- Greater proportion of private fusions, correlates with extensive heterogeneity of TNBCs and genomic instability
- Recurred tumors compared to its' primary tumors harbored less fusion transcripts across 8 matched cases
- Novel *ESR1* fusion gene found in hormone receptor positive breast cancer sample
- ***BCL2-ESR1* fusion** transcript encompassing ligand binding domain with loss of DNA binding domain displaying overexpression of both *BCL2* and *ESR1* gene
- May result in activation of non-genomic pathway of estrogen receptor, leading to *PIK3/MAPK/AKT* activation

Limitations and further plan

- **Small series of analysis** with 8 matched primary/recurred, 8 primary only

- Fusion transcript calling with **Defuse software only**. Comparison of other fusion calling algorithm may lower risk of false positivity

- Novel *BCL2-ESR1* fusion transcript identified **should be validated** considering high false positivity in RNA-sequencing data
- **Functional experiment** of *BCL2-ESR1* fusion necessary whether it subsequently lead to overexpression of non-genomic pathway, and gain oncogenicity

Summary

- RNA-sequencing revealed numerous fusion transcripts among primary and recurred breast cancer samples
- Triple negative breast cancer samples showed tumor heterogeneity with greater number of fusion transcripts and greater proportion of private fusions compared to other subtypes
- Among them novel *BCL2-ESR1* fusion identified which may possibly lead to activation of non-genomic pathway of estrogen receptor pathway
- Further validation and functional annotation to confirm their role in tumor progression and metastasis

Thank you for your attention!