# TONSL, an immortalizing oncogene on Chr.8q24.3 amplicon, confers intrinsic resistance to CDK4/6 inhibitors in breast cancer.

Harikrishna Nakshatri[1], Aditi S. Khatpe[1], Urja R. Kamdar[1], George Sandusky[1], Sandra K. Althouse[1], Sachin Kumar Deshmukh[2], Sharon Wu[2], Ninad Kulkarni[2], Lisa A. Poole[2], Priscila Barreto Coelho[3], Maryam B. Lustberg[4], George W. Sledge Jr[2], Kathy D Miller[1] and Poornima Bhat-Nakshatri[1]

[1]Indiana University School of Medicine, Indianapolis, IN, [2]Caris Life Sciences, Phoenix, AZ, [3]Sylvester Cancer Center, University of Miami, FL, [4]Yale School of Medicine, Yale University, New Haven, CT

## BACKGROUND

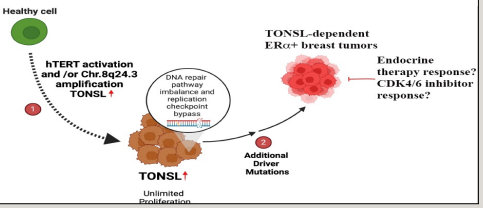
- Advanced Estrogen receptor-positive (ER+) breast cancers benefit from the inhibitors of cell-cycle kinases *CDK4* and *CDK6*. However, 20% of breast cancers (BC) show intrinsic resistance to these inhibitors.
- Although the mechanisms of acquired resistance to CDK4/6 inhibitors have been studied extensively, little is known about mechanisms of intrinsic resistance.
- We had recently reported a gene called *TONSL*, a DNA repair and chromatin organization-associated gene located on chromosome 8q24.3 amplicon, as an immortalizing oncogene and its overexpression in ER+ breast cancer is associated with poor outcome.
- In preclinical models, *TONSL*-immortalized breast epithelial cell lines generated ER+ adenocarcinomas upon transformation.
- Here we investigated whether *TONSL* amplification confers intrinsic resistance to CDK4/6 inhibitors. Here we compare the survival of *TONSL* amplified vs not amplified ER+ breast cancer patients treated with CDK4/6 inhibitors.

## METHODS

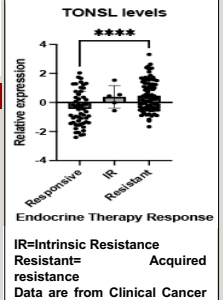
- 10,980 ER+ breast cancer samples (*TONSL* amplified, n=313; Not amplified, n=10,667) were analyzed by next-generation sequencing (WES, NovaSeq) and Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- Copy number variations were determined using CNVKit.
- A tissue microarray comprising ~200 ER+ breast cancers was examined for *TONSL* and phospho-RB expression levels by immunohistochemistry.
- The role of *TONSL* in sensitivity to abemaciclib was examined *in vitro* in a *TONSL* amplified ER+ breast cancer cell line (generated from MCF-7 derived tumor in nude mice) with and without shRNA-mediated *TONSL* knockdown using bromodeoxyuridine (BrdU) incorporation ELISA.
- Real-world median overall survival (mOS) was derived from insurance claims and calculated from the biopsy to last contact or start of CDK4/6 inhibitor (abemiciclib/palbociclib/ribociclib) to last contact or first to last dose of CDK4/6 inhibitor treatment (time on treatment) using Kaplan-Meier.
- Statistical significance was assessed using chi-square and Mann-Whitney U and p<0.05 was considered significant.

## RESULTS

### TONSL is an immortalizing oncogene that can initiate ER+ breast cancer (Cancer Research 83:1345-1360)

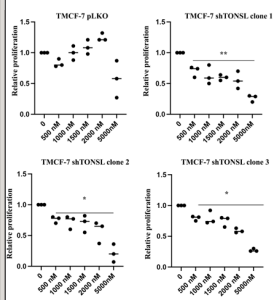



### Endocrine resistant BC express higher levels of TONSL



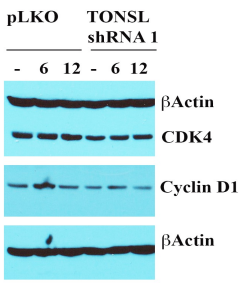

IR=Intrinsic Resistance
Resistant= Acquired resistance
Data are from Clinical Cancer Research 28:3618-3629

### TONSL knockdown reverses abemaciclib resistance of TMC-7, an MCF-7 variant intrinsically resistant to abemaciclib




Cells were treated with indicated concentration of the drug and cell proliferation was measured using BrDU ELISA after 72 hours.

### TONSL regulates CDK4 and Cyclin D levels.



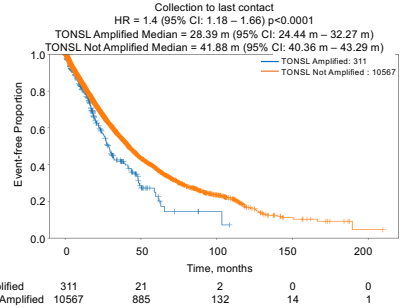

Cells were treated with $10^{-10}$M E2 for indicated time. CDK4 and Cyclin D1 levels were measured by Western blotting. CDK6 was undetectable with tested antibodies.

### Demographic details of ER+ breast tumors in Caris database analyzed for TONSL amplification

| | | Not Amplified | Amplified |
|---|---|---|---|
| Count (N) | | 10667 | 313 |
| Median age [range] | | 64 [20 -> 89] | 62 [27 -> 89] |
| Race | White | 77.77% (6499/8357) | 68.67% (171/249) |
| | Black | 14.08% (1177/8357) | 20.08% (50/249) |
| | Asian/Pacific Islander | 3.89% (325/8357) | 5.62% (14/249) |
| | Other | 4.26% (356/8357) | 5.62% (14/249) |
| Ethnicity | Not Hispanic or Latino | 89.58% (7709/8606) | 90.73% (225/248) |
| | Hispanic or Latino | 10.42% (897/8606) | 9.27% (23/248) |
| Tumor site | Primary | 36.95% (3941/10667) | 33.23% (104/313) |
| | Metastatic | 63.05% (6726/10667) | 66.77% (209/313) |

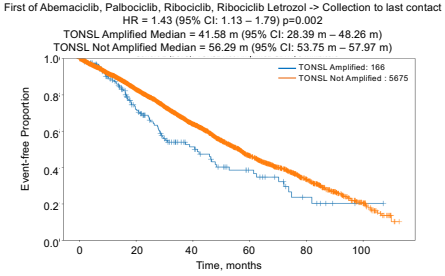
- Race and ethnicity data is self reported

### TONSL amplification is associated with poor outcome in ER+ patients



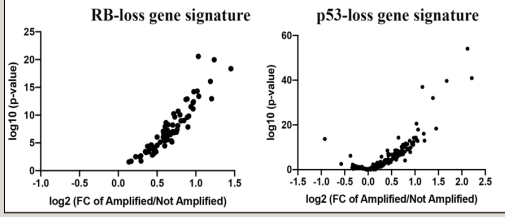

| | | | | | |
|---|---|---|---|---|---|
| TONSL Amplified | 311 | 21 | 2 | 0 | 0 |
| TONSL Not Amplified | 10567 | 885 | 132 | 14 | 1 |

### TONSL amplified ER+ tumors had worse survival with CDK4/6 inhibitors

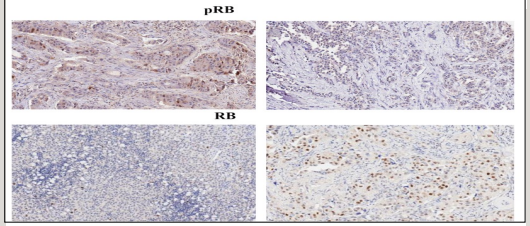



| | | | | | | |
|---|---|---|---|---|---|---|
| TONSL Amplified | 166 | 89 | 40 | 20 | 7 | 1 |
| TONSL Not Amplified | 5675 | 3131 | 1498 | 643 | 255 | 48 |

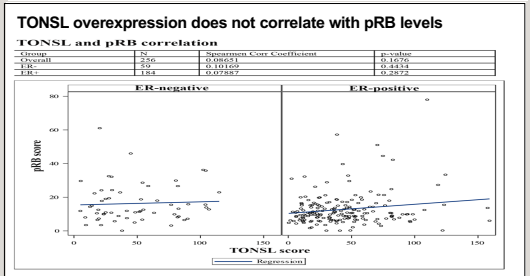
### TONSL amplification correlates with RB-loss and p53-loss gene signatures




### Representative pRB (S807/811) and RB IHC of TMA with 256 breast tumors with ~15 years follow up




### TONSL overexpression does not correlate with pRB levels




## CONCLUSIONS

- **TONSL overexpression correlates with endocrine resistance.**
- **TONSL amplification is numerically higher in breast tumors of Black women than in White women .**
- **TONSL amplification is associated with intrinsic resistance to CDK4/6 inhibitors**
- **TONSL amplification/overexpression correlates with RB-loss and p53-loss signature but not pRB levels.**

## INDIANA UNIVERSITY MELVIN AND BREN SIMON COMPREHENSIVE CANCER CENTER

Funding: Susan G Komen for the Cure SAC232147