Abstract #: 553626

Influence of *TONSL* on tumor suppressor function of *RAD51* and resistance to CDK4/6 inhibitors in ER+ breast cancer.

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BACKGROUND

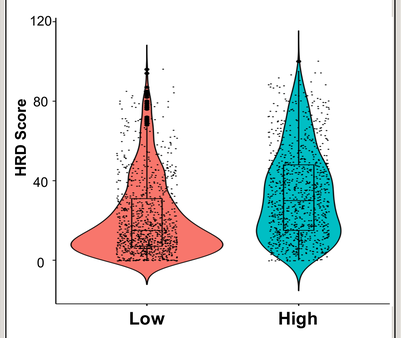
- Homologous Recombination (HR) and Replication Stress (RS) resolution mechanisms help to maintain genomic stability. TONSL, MMS22L, RAD51, BRCA1/2 are the few proteins that cooperatively coordinate HR and RS resolution.
- Therefore, genes in these pathways are expected to function as tumor suppressors.
- However, only *BRCA*1/2 function as tumor suppressors as their germline mutations increase cancer susceptibility.
- *TONSL* and *RAD51* are frequently overexpressed in tumors.
- Here, we investigated the relationship between *TONSL-RAD51* axis in breast cancer (BC) outcome and response to CDK4/6 inhibitors (CDK4/6i) and endocrine therapy (ET).

METHODS

- 10,980 ER+ BC samples tested by NGS (592, NextSeq; WES/WTS, NovaSeq, Caris Life Sciences).
- Dual expression group *TONSL/RAD51*-high (H), *TONSL*-H/*RAD51*-low (L), *TONSL*-L/*RAD51*-H, and *TONSL*/*RAD51*-L were classified by RNA expression above or below median.
- HRD score and *TONSL*/HRD-H/L survival was analyzed using METABRIC (ER+ BC, n = 1904) dataset.
- *TONSL-RAD51* role in sensitivity to CDK4/6i was determined using *TONSL*-amplified ER+ MCF-7 cells.
- Real-world median overall survival (mOS) of *TONSL/RAD51* groups was derived from insurance claims and calculated from biopsy, start of ET or CDK4/6i to last contact using Kaplan-Meier. Statistical significance was assessed using chi-square and Mann-Whitney U with multiple comparison adjustments ($q < 0.05$).

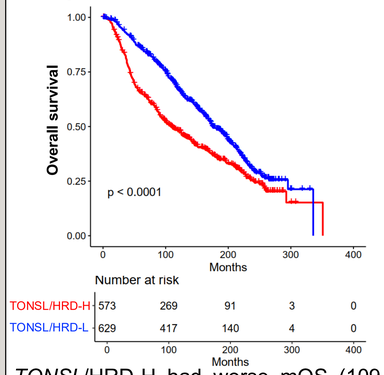
RESULTS

Figure 1. HRD score in *TONSL*-high (H) compared to to *TONSL*-low (L)



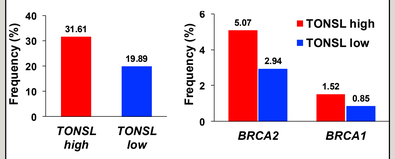
TONSL-H had higher HRD score (33.7 vs 21.1) compared to *TONSL*-L (n = 952 each), p<0.05.

Figure 2. TONSL/HRD-H is associated with poor outcome in ER+ patients



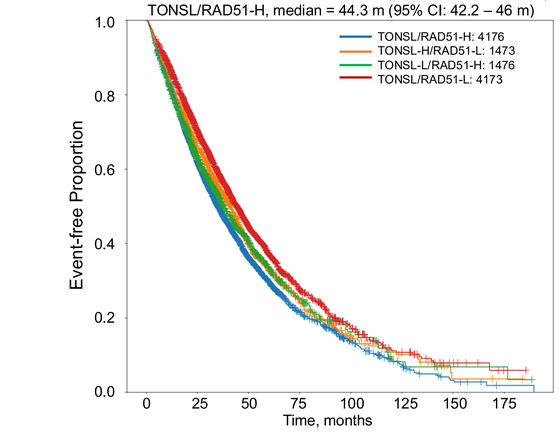
TONSL/HRD-H had worse mOS (109 m vs 138 m, p<0.05) compared to *TONSL*/HRD-L

Figure 3. Frequency of LOH and *BRCA2/1* mutation

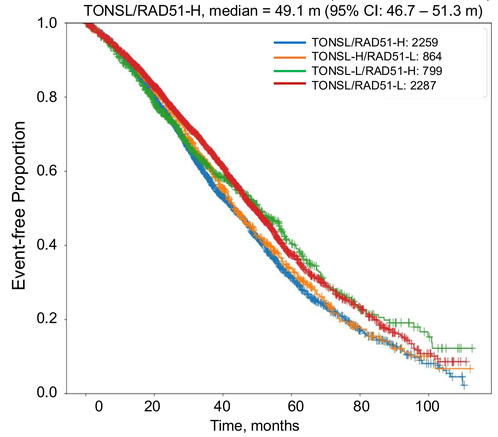


TONSL-H had higher frequency of LOH (31.6% vs 19.8%), *BRCA2* (5.1% vs 2.9%) and *BRCA1* (1.5% vs 0.8%) mutation, all $q < 0.05$.

Figure 4. ER+ breast cancer patients' survival



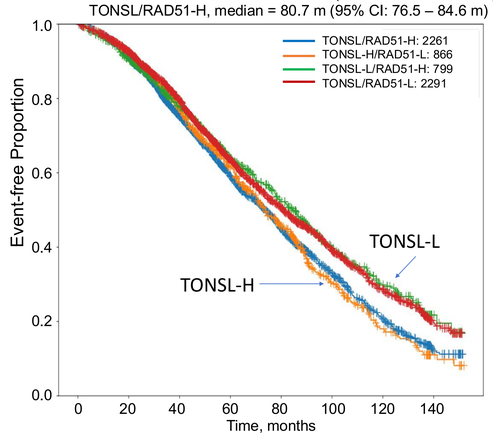
TONSL/RAD51-H	4176	2134	671	206	78	26	7	1
TONSL-H/RAD51-L	1473	886	328	99	35	16	2	1
TONSL-L/RAD51-H	1476	740	227	68	24	6	2	2
TONSL/RAD51-L	4173	2450	818	199	70	26	8	1



TONSL/RAD51-H	2259	1624	762	303	93	17
TONSL-H/RAD51-L	864	644	332	133	37	3
TONSL-L/RAD51-H	799	550	282	122	40	11
TONSL/RAD51-L	2287	1667	904	307	117	19

CDK4/6i/Endocrine therapy

First of Abemaciclib, Palbociclib, Ribociclib, Anastrozole, Exemestane, Fulvestrant, Letrozole, Tamoxifen to last contact
p<0.0001
TONSL/RAD51-H, median = 73.8 m (95% CI: 74.4 – 77 m)
TONSL-H/RAD51-L, median = 73.8 m (95% CI: 70.1 – 79 m)
TONSL-L/RAD51-H, median = 86.6 m (95% CI: 77.8 – 90.1 m)
TONSL/RAD51-H, median = 80.7 m (95% CI: 76.5 – 84.6 m)



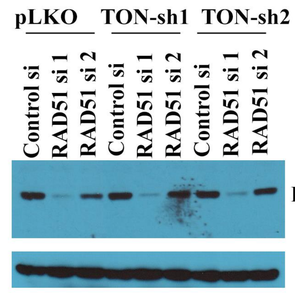
TONSL/RAD51-H	2261	1971	1415	947	602	339	164	29
TONSL-H/RAD51-L	866	781	584	388	243	109	45	10
TONSL-L/RAD51-H	799	689	510	348	246	138	74	11
TONSL/RAD51-L	2291	2016	1504	985	659	384	211	40

Table 1. Survival by TONSL and RAD51 expression

	TONSL/ RAD51-H	TONSL-H/ RAD51-L	TONSL-L/ RAD51-H	TONSL/ RAD51-L	p-value
Overall	35 m (n=4176)	41.4 m (n=1473)	37.9 m (n=1476)	44.3 m (n=4173)	<0.01
ET	63.4 m (n=1361)	58 m (n=461)	65.2 m (n=508)	76.1 m (n=1430)	<0.01
ET+ CDK4/6i	73.8 m (n=2261)	73.8 m (n=866)	84.6 m (n=799)	80.7 m (n=2291)	<0.01

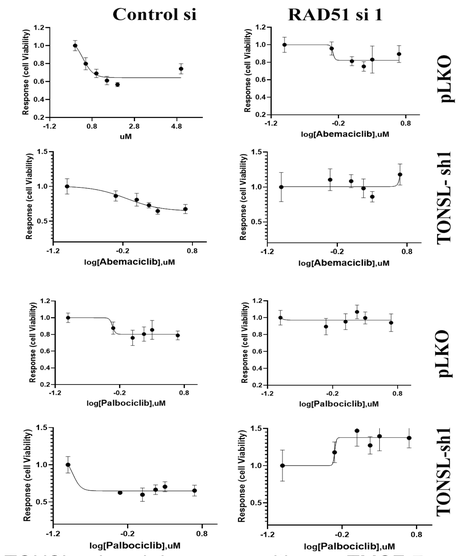
TONSL/RAD51-H had worse mOS compared to *TONSL*-H/*RAD51*-L, *TONSL*-L/*RAD51*-H or *TONSL*/*RAD51*-L. *TONSL*-H/*RAD51*-L had worse survival with ET compared to other groups. With ET+CDK4/6i, *TONSL/RAD51*-H and *TONSL*-H/*RAD51*-L had similar but worse mOS compared to *TONSL*-L/*RAD51*-H and *TONSL*/*RAD51*-L.

Figure 5. TMCF7-RAD51 siRNA effects confirmation



RAD51si1 reduced RAD51 expression in TMCF-7 cells with and without knockdown of TONSL.

Figure 6. RAD51 is required for the activity of Abemaciclib and Palbociclib



TONSL knockdown sensitizes TMCF-7 cells to Palbociclib. RAD51 is required for Palbociclib and Abemaciclib-mediated growth inhibition of TMCF-7 pLKO and TMCF-7 TONSL-sh cells. These results are consistent with better response of TONSL-L/RAD51-H tumors to ET+/CDK4/6i treatment (Table 1).

CONCLUSIONS

- ***TONSL*-H independent of *RAD51* expression is associated with poor response to ET or ET+CDK4/6i.**
- **Therapeutic targeting of TONSL to create TONSL-L/RAD51-H status may improve response to ET+CDK4/6i. Activity of TONSL is regulated by various protein complexes, destabilizers of TONSL-protein complexes could potentially help in sensitizing ER+ BC to ET or CDK4/6i.**

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This work is supported by Susan G Komen for the Cure SAC232147 to HN