Proteomic subtyping of stage II/III TNBC from the I-SPY2 TRIAL reveals a druggable steroid hormone receptor signature (SRS) that is associated with poor outcome and enriched in non-pCR patients' residual disease

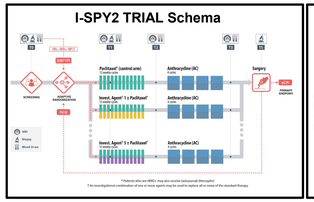
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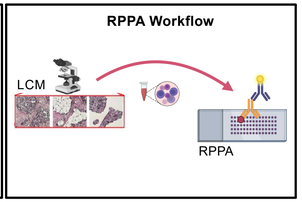
Background & Methodology

- I-SPY2 TNBC Response Predictive Subtypes (RPS) predict for benefit from immuno-therapy and DNA-damaging agents in Immune+ and DNA Repair Deficient (DRD)+ subgroups
- We have previously described a subset of TNBC that express elevated levels of Estrogen Receptor alpha (ERα).
- These ERα "high" tumors express a steroid receptor signature (SRS) characterized by coincident elevation of ERα, Androgen Receptor (AR) and activated Glucocorticoid Receptor (pGR S211) as determined by Reverse Phase Protein Array (RPPA) analysis of laser capture microdissection (LCM)-enriched tumor epithelium.
- We have explored the drug target activation architecture of SRS+ TNBC and the associations of SRS+ status with patient outcome in the I-SPY2 TRIAL in patients who did not respond to neoadjuvant therapy, ie, did not achieve a pCR.

I-SPY2 TRIAL Schema



RPPA Workflow

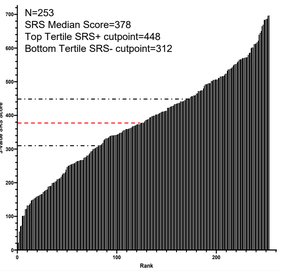


- SRS score was determined based on the equally weighted rank-sum addition of the 3 SRS component analytes
- SRS scores above and below the population median were considered SRS+ and SRS-, respectively
- For survival status and signaling pathway analyses, SRS scores were divided into tertiles and the top and bottom tertiles (N=84 each) were used as the SRS+ and SRS- populations.
- Statistical analyses were performed using GraphPad Prism v10.6 and JMP software v14. Uncorrected p-values <0.05 were used for determination of significance and results are descriptive.
- Clinical information was provided by Quantum Leap Healthcare Collaborative

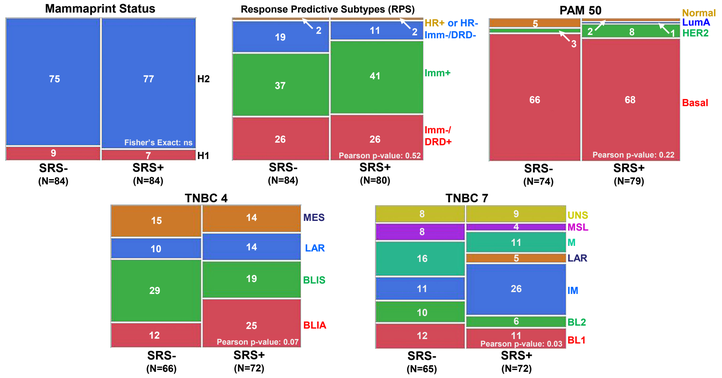
Dataset Characteristics

	N
Full pre-treatment RPPA data set	**948**
TNBC	**336**
ER-/PR+/HER2-	**26**
Complete RPPA SRS component data available	**253**
TNBC	**231**
ER-/PR+/HER2-	**22**
I-SPY2 arms represented	**10**
pCR YES	**77**
pCR NO	**176**
Matched surgical timepoint specimens	**48**

SRS Scoring Plot with Median and Tertile Cutpoints



Association of SRS Status with Various Gene Expression Classifiers in Pre-Treatment Specimens

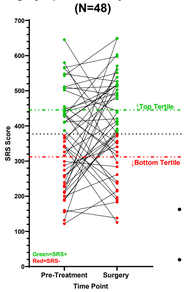


- **SRS status does not associate with the Mammaprint H1/H2, I-SPY2 RPS or PAM50 expression signatures**
- **SRS- status has a trending association with the TNBC4 BLIS subtype (p=0.07) and SRS+ status has a significant association with the TNBC7 Immunomodulatory (IM) subtype (p=0.03)**

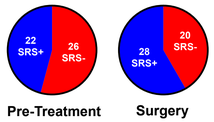
The protein expression-based SRS is not directly related to other TNBC-related gene expression signatures and may provide unique information regarding prognosis and therapeutic response.

SRS-Positivity is Enriched in the Surgical Samples from Non-Responding TNBC Patients

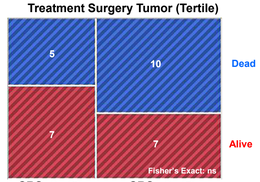
Patient-Matched Pre-Treatment & Surgery Specimens by SRS Score (N=48)



Distribution of SRS Status in Matched Pairs by Timepoint



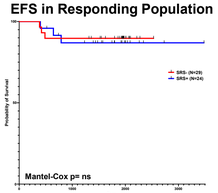
Survival Based on SRS Status on Post-Treatment Surgery Tumor (Tertile)



- **SRS+ status is enriched in the post-treatment surgical tumor (58% 28/48) compared to patient-matched pre-treatment tumors (45% 22/48) in patients who did not achieve a pCR**
- **Patients with SRS+ tumors at the surgery timepoint had decreased survival (41% alive, 7/17) compared to SRS- patients (58% alive, 7/12).**

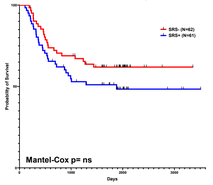
SRS Status at Pre-Treatment Associates with Event-Free Survival in Non-Responding TNBC Patients

EFS in Responding Population



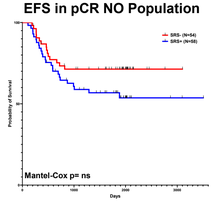
Pre-treatment SRS status has no effect on EFS in the patients who achieve a pCR

EFS in RCB II/III Population



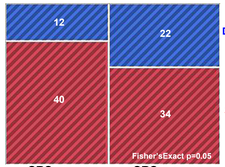
In the RCB II/III population, SRS+ patients have worse EFS than SRS-; difference is not significant

EFS in pCR NO Population



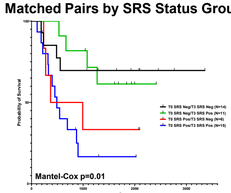
In the pCR NO population, patients who are SRS+ at pre-treatment have worse EFS than those who are SRS-; difference is not significant

Survival Status in pCR NO TNBC Pre-Treatment Population by SRS Status



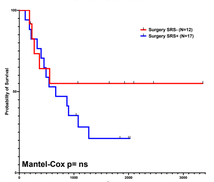
SRS+ status in pre-treatment tumor samples significantly associates with survival status in patients who did not achieve pCR.

EFS in Pre-Treatment/Surgery Matched Pairs by SRS Status Groups



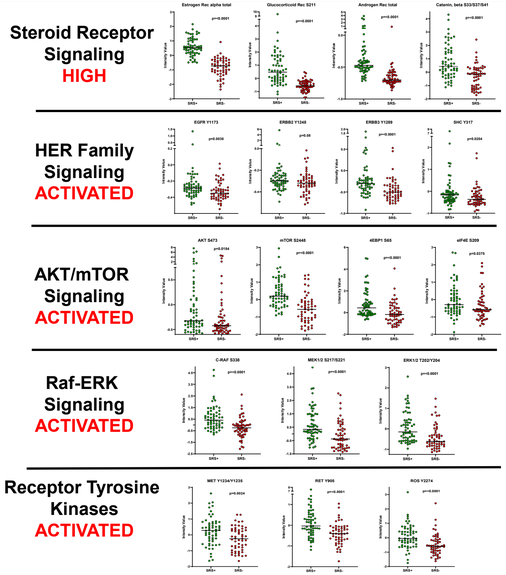
In matched pre-treatment and surgery pairs, pre-treatment SRS+ significantly associates with worse EFS while SRS status at surgery does not

EFS in Surgery Population by SRS Status



Patients with SRS+ tumors at surgery have worse EFS than patients with SRS- tumors at surgery; difference is not significant

Evaluation of Signaling Pathway Activation in Pre-Treatment Specimens Reveals Potential Therapeutic Targets Elevated in Non-Responding SRS+ Patients



Conclusions

- **TNBC patients with SRS+ tumors who do not achieve a pCR with preoperative therapy have a worse prognosis than those with SRS- tumors.**
- **Analysis of patient-matched pre-treatment/surgery specimens shows that SRS+ status at pre-treatment has stronger association with worse EFS than SRS status at surgery.**
- **Protein pathway activation mapping of the pre-treatment tumor biopsy samples from non-responding patients identified the activation of multiple actionable targets in SRS+ tumors**
- **We hypothesize that inhibiting the following protein drug targets and pathways are important data-driven strategies to prospectively assess in I-SPY 2.2 for TNBC patients:**
  - **1) Direct inhibition of AR-GR-WNT signaling**
  - **2) Direct inhibition of HER family signaling (which may also inhibit other RTKs and downstream signaling)**
  - **3) Direct inhibition of AKT-mTOR, and RAF-ERK signaling**
- **These strategies are supported by observations revealing interactions between steroid receptor activity and WNT/HER family signaling in breast cancer[1,2], and align with the goals of I-SPY 2.2 by focusing on the biology of non-response and *de novo* resistance.**

1. Ni M, et al. Cancer Cell. 2011 Jul 12;20(1):119-31.
2. Wulfkuhle et al. JCO Precis Oncol. 2018 Aug 16;2:PO.18.00024.

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