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Characterization of residual disease after neoadjuvant selective estrogen receptor degrader (SERD) therapy using tumor organoids in the I-SPY Endocrine Optimization Protocol (EOP)

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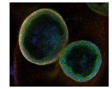

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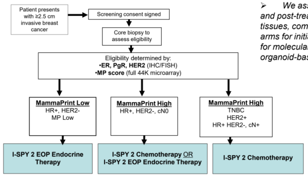
The I-SPY Trials

BACKGROUND

- Treatment of estrogen receptor (ER)-positive breast cancer with selective estrogen receptor degraders (SERDs) frequently results in the loss or reduction of ER expression. Whether these changes are due to on-target effects of SERDs degrading ER or arise as a mechanism of tumor resistance with associated changes in cellular phenotypes is unknown.
- It is critical to distinguish between these possibilities to assess treatment response and determine the most appropriate subsequent therapy.
- We created and conducted molecular analyses on patient-derived organoid cultures from post-treatment tissue in patients receiving neoadjuvant SERD therapy for early-stage ER+ breast cancer in the I-SPY2 Endocrine Optimization Protocol (EOP).

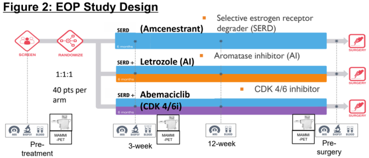
THE EOP STUDY

Figure 1: Overview of eligibility criteria for the I-SPY Endocrine Optimization Protocol (EOP)



- *We assessed pre- and post-treatment tissues, combining all arms for initial analyses for molecular and cell/ organoid-based assays.*

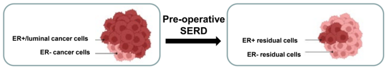
Figure 2: EOP Study Design



- **Primary Objectives for EOP Study**
  - To determine the feasibility of enrolling and treating molecularly-selected patients with early-stage HR+ breast cancer in a randomized neoadjuvant trial using an oral-SERD backbone.
- **Secondary Objectives for EOP Study**
  - To identify candidate biomarkers that can be used to assess response/sensitivity to endocrine therapy

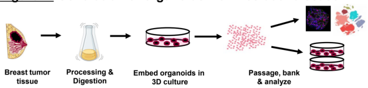
RESULTS

Figure 3: Decreased ER expression in residual disease



- Is the change in ER expression reflective of drug effect or of a shift in tumor subtype (e.g., from luminal to basal) that would warrant a change in adjuvant therapy?

Figure 4: Generation of organoids from residual disease

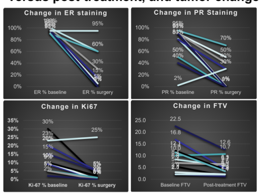


- Breast tumors were mechanically processed, banked and passaged in either Type 1 or Type 2 Organoid Medium as previously described [1].

Table 1: Number of patients on study who have gone to surgery by category

Grade		Age		Menopause		Nodal status	
G1	2	30-39	1	Pre-	8	+	2
G2	9	40-49	7	Post-	5	-	11
G3	2	50-59	1				
		60-69	3				
		70-79	1				

Figure 5: Immunohistochemistry results per case, pre-versus post-treatment, and tumor change on MRI



- Shown are the % of cells that are ER+, PR+, and Ki67+ in pre-treatment and post-treatment (surgical) samples for 13 patients on study, as well as Functional Tumor Volume (FTV) on MRI pre- vs. post-treatment.

Figure 6: Examples of organoids displaying solid or cystic morphologies (from post-treatment samples)

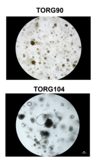


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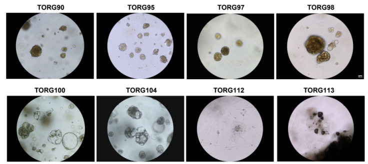
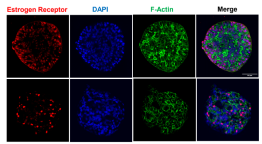
Figure 7: Patient-derived EOP organoid cultures



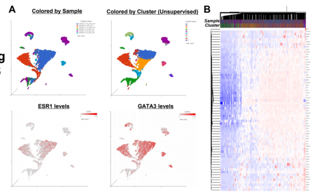
- Organoid growth was attempted from 10 cases, with subsequent growth from 8 cases. One of the two cases that did not grow appeared to be predominantly stromal cells at initial plating. The other case was thought to be due to transport issues with the tissue itself. Growth rates and organoid sizes varied significantly, as shown by bright field microscopy above (organoids at different stages).

Figure 8: Examples of ER protein expression in organoids.



- Confocal microscopy of organoids stained for the indicated markers. Six organoid cultures were assessed for ER expression by protein and/or RNA, with heterogeneous expression detected in all six. Two examples are shown from two different cases (post-treatment EOP organoids) grown in absence of amcenestrant for >1 mo.

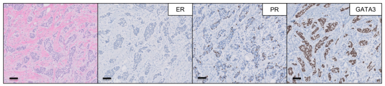
Figure 9: Single-cell RNA-sequencing results of 6 early passage organoid cultures from EOP post-treatment tissue.



- A) UMAPs colored by sample, unsupervised clustering result, or ESR1 (ER) or GATA3 RNA expression levels. B) Single-cell heatmap showing the expression of a curated list of ER target and associated genes.
- Preliminary results indicate one case exhibited a change in subtype from Luminal A (pre-treatment) to Luminal B (post-treatment). Additional subtype analyses are ongoing.

RESULTS

Figure 10. GATA3 expression in post-treatment tumors.



- Immunohistochemistry result showing GATA3 in a representative case with low ER and PR expression after SERD treatment. 5/5 cases stained for GATA3 were GATA3 positive at the time of surgery. Scale = 100 μm.

SUMMARY & FUTURE DIRECTIONS

Our anticipated results in different scenarios:

Sample showing "on-target effect":
- ER expression turned back on in organoid culture
- Breast cancer subtype unchanged post-treatment
- Other markers unchanged (e.g. GATA3, ER, PR)

Sample showing "acquired resistance due to subtype switching":
- ER not expressed in organoids
- Breast cancer subtype changed post-treatment
- Other markers changed pre- vs. post-treatment

Sample showing "resistance due to outgrowth of a subclone":
- ER not expressed in organoids
- Breast cancer subtype changed post-treatment
- Other markers changed pre- vs. post-treatment
- Markers or signatures can be identified in a subset of pre-treatment cells

- Organoid culturing of residual disease after neoadjuvant endocrine therapy is feasible.
- Neoadjuvant treatment with a SERD can render ER and PR low or absent at the time of surgical resection, which does not necessarily imply the presence of endocrine therapy resistant disease.
- The use of organoids and additional IHC markers demonstrate that receptor negativity may be an indicator of the drug hitting its target with ER signaling still intact.
- Tumor organoids modeling residual disease states can be a useful adjunct to existing methods used to monitor the effects of neoadjuvant endocrine therapy and is being explored in the I-SPY EOP trial.

Advocate statement

- *"ER expression or suppression in organoids created with post-surgical residual tissue treated preoperatively with a SERD may be a useful tool in determining when and how to use endocrine or other post-surgery therapies. This important preliminary research, which will be further explored in the I-SPY trial, could potentially lead to more precise and targeted post-surgical treatments and is therefore of great interest to patients."*

- Carol Simmons, Advocate, UCSF Breast Cancer Advocacy Core

I-SPY2 TRIAL Consortium Acknowledgements

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