Characterizing the HER2-/Immune-/DNA repair (DRD-) response predictive breast cancer subtype: the hunt for new protein targets in a high-needs population with low response to all I-SPY2 agents

Denise Wolf[1], Christina Yau[1], Julia Wulfkuhle[2], Isela Gallagher[2], Lamorna Brown-Swigart[1], Gillian Hirst[1], Jean-Phillipe Coppe[1], Mark Magbanua[1], Rosalyn Sayaman[1], Susan Samson[3], I-SPY2 Investigators, Laura Sit[4], Nola Hylton[1], Angela De Michele[5], Lajos Pusztai[6], Doug Yee[7], Laura Esserman[1], Chip Petricoin[2], Laura van 't Veer[1]

[1]University of California San Francisco, [2]George Mason University, [3]UCSF Breast Science Advocacy Core, [4]Quantum Leap Healthcare Collaborative; [5]University of Pennsylvania; [6]Yale University; [7]University of Minnesota

1. Background

- **Previously we leveraged the I-SPY2 trial to create treatment response predictive subtypes (RPS) incorporating tumor biology beyond clinical HR/HER2, to better predict drug responses in an expanded treatment landscape that includes platinum agents, dual HER2-targeting regimens and immunotherapy [1].**
- **Best performing schemas incorporate Immune, DRD and HER2/Luminal phenotypes, and treatment allocation based on these should increase the overall pCR rate up to 63% from 51% using HR/HER2-based treatment selection.**
- **An RPS schema has been selected for prospective evaluation in I-SPY2.2.**
- **Using this RPS, one would prioritize platinum-based therapy for HER2-/Immune-/DRD+, immunotherapy for HER2-/Immune+, and dual-anti-HER2 for HER2+ that are not luminal [Fig. 1].**
- **HER2+/Luminal patients have low response to dual-anti-HER2 therapy but may respond better to an anti-AKT therapy.**
- **However, there is still a 'biomarker-negative' group of resistant cancers (HER2-/Immune-/DRD-) with low pCR rates to all tested agents, that require a new therapeutic approach.** ***Here we characterize the protein signaling of these tumors to identify new target candidates.***

1 Wolf, Yau, …van 't Veer, et al. Redefining breast cancer subtypes to guide treatment prioritization and maximize response: Predictive biomarkers across 10 cancer therapies. Cancer Cell. 2022 Jun 13;40(6):609-623.

2. THE PATIENTS: I-SPY 2 TRIAL Standing Platform

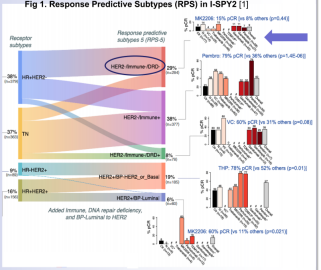
- **Phase II, adaptively-randomized neoadjuvant trial**
- **Shared control arm**
  Standard neoadjuvant chemotherapy
- **Simultaneous experimental arms**
  Up to four
- **Primary endpoint:** pathologic complete response (pCR)
  No residual invasive cancer in breast or nodes
- **Match therapies with most responsive breast cancer subtypes**
  Defined by HR, HER2, and Mammaprint High1/ultra/High2 (MP1/2) status
- **Agents/combinations "graduate" for efficacy** = reaching >85% predictive probability of success in a subsequent 300 pt phase III trial in the most responsive patient subset

- *I-SPY 2 is a biomarker rich trial: Tumor assayed for: mRNA, DNA, protein/phospho-protein, MIF; blood assayed for: ctDNA, oncRNA, cyTOF*

3. DATA/METHODS: patients and comparisons

- 987 I-SPY 2 patients from 10 arms of the trial were considered for this analysis.
  - All have gene expression, pCR and RPS class assignments;
  - 944 have distant recurrence free survival (DRFS) data;
  - 736 have reverse phase protein array (RPPA) data from laser capture micro-dissected tumor sections.
- These data – known collectively as the **I-SPY2-990 mRNA/RPPA Data Resource** - were recently made public on NCBI's Gene Expression Omnibus [GEO: GSE196096].

Fig 1. Response Predictive Subtypes (RPS) in I-SPY2 [1]



- We focus on **HER2-/Immune-/DRD- tumors** (top right, circled)
- We apply Wilcoxon and t-tests to identify:
  1. Proteins/phosphoproteins that differ between HR+HER2-/Immune-/DRD- and other HR+HER2- tumors;
  2. Proteins/phosphoproteins that differ between TN/Immune-/DRD- and other TNs.
- The Benjamini-Hochberg (BH) method is used to adjust p-values for multiple hypothesis testing.
- In addition, the Kaplan-Meier method is used to estimate DRFS.

4. RESULTS: Characterization of HER2-/Immune-/DRD- breast cancers

4.1 HER2-/Immune-/DRD- subset: prevalence, response and survival

- 201/736 I-SPY 2 patients with RPPA data are classified HER2-/Immune-/DRD- (HR+/HER2-: n=138; TN: n=63).

- Of these, 8.5% (17/201) achieved pCR across arms.

- Non-responding HER2-/Immune-/DRD- had worse outcomes than responders (~75% vs. ~95% DRFS at 5 years).

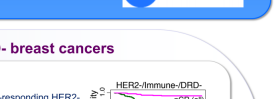
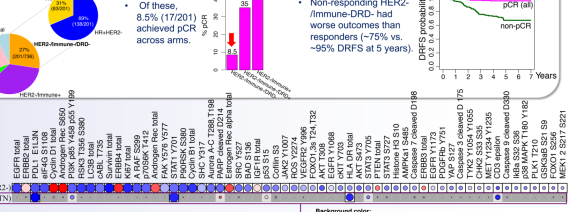
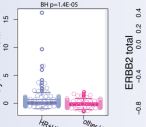


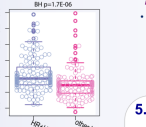
Fig 2. characteristic phosphoprotein differences: Immune-/DRD- vs other tumors, by HR status



4.2 Proteins characterizing HR+HER2-/Immune-/DRD- tumors

- 60/139 proteins and phosphoproteins differ significantly between HR+HER2-/Immune-/DRD- and other HR+HER2- tumors [Fig2. top row]
- 90% (54/60) of these are at lower levels; including immune and proliferation [Blue dots].
- Proteins at higher levels include, Cyclin D1, ERBB2, and ER.

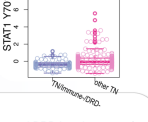




Advocate perspective: This work convincingly argues that senolytics targeting cyclin D1 and anti-AR may overcome resistance in HR+HER2-/immune- DRD- patients, whereas immune activation beyond anti-PD1/PDL1 is suggested for TN/immune-DRD-patients with low response to all ISPY2 agents. Characterizing HER2-/Immune-/DNA repair (DRD) tumors provides a golden opportunity to hunt for new protein targets that may benefit patients.

4.3 Proteins characterizing TN/Immune-/DRD- tumors

- Only 3/139 proteins and phospho-proteins differed significantly between TN/Immune-/DRD- and other TN [Fig 2. bottom row]. These were all immune, with lower expression in TN/Immune-/DRD-.



5. CONCLUSION

- HR+HER2- and TN who are Immune-Low and DRD-Low have very low pCR rates to all I-SPY2 agents including standard chemotherapy, platinum, and immunotherapy.
- Senolytics (possibly targeting Cyclin D1), anti-HER2(low?) agents, or AR modulators may overcome resistance in HR+HER2-/Immune-/DRD-
- An immune activator beyond checkpoint inhibition is suggested for TN/Immune-/DRD- patients.

ACKNOWLEDGEMENTS: With support from Quantum Leap Healthcare Collaborative, FNIH, NCI (Grant 2R01197 P-0518835, P01 CA210961), Safeway Foundation, William K. Bowes, Jr. Foundation, The Breast Cancer Research Foundation, UCSF, the Biomarkers Consortium, Salesforce, Novella Clinical, QIS Associates, Berry Consultants, Agendia, OpenClinica, Give Breast Cancer the Boot, Stand up for Cancer, Alexander Trust, CA Breast Cancer Research Program, Seagen, AstraZeneca, Daiichi-Sankyo, Merck, Dynavax, Pfizer, Puma Biotechnology, AbbVie, Madrigal Pharmaceuticals, Genentech, Amgen, Pieridion, Regeneron, G1 Therapeutics, GSK, Byondis. Initial support from IQVIA, Johnson & Johnson, San Francisco Foundation, Eli Lilly, Eisai Company, Side Out Foundation, Harlan Family, Avon Foundation for Women, Alexandria Real Estate Equities. Sincere thanks to Anna Barker, our DSMB (Harold Burstein, Elizabeth Frank, Steven Goodman, Robert Mass, Janet Witte, Tiffany Traina and Deborah Lavaguai, Ken Buetow and CellMD, our patients, advocates and investigators.