Molecular subtypes of invasive lobular breast cancer in the I-SPY2 Trial

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BACKGROUND

- Invasive lobular carcinoma (ILC) of the breast has distinct histological and molecular features compared to invasive ductal carcinoma (IDC), including absence of the adhesion protein E-cadherin.
- A recent analysis from The Cancer Genome Atlas (Ciriello *et al*) identified three distinct molecular subtypes within ILC based on gene expression:
  - **Reactive-like, Immune-related, and Proliferative**
- In this study, we applied this 60-gene classifier to a locally advanced cohort of ILC and mixed ILC/IDC cases screened for the I-SPY 2 TRIAL and evaluated associations with response to treatment.
- We evaluated concordance with signatures derived from

I-SPY 2 TRIAL ELIGIBILITY

Clinical Eligibility Criteria: Stage II or III, or T4, any N, M0, including clinical or pathologic inflammatory cancer or Regional Stage IV, where supraclavicular lymph nodes are the only sites metastasis

Molecular Eligibility Criteria: Triple Negative, or HER2+, or MammaPrint High risk HR+HER2-

HR+HER2- MammaPrint Low risk patients ineligible for I-SPY 2 randomization are invited to join a Low risk registry.

I-SPY 2's ADAPTIVE TRIAL DESIGN

I-SPY 2 is a multicenter, phase 2 trial using response-adaptive randomization within biomarker subtypes to evaluate a series of novel agents when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer (FIG.1). Within each patient subtype, participants are assigned to one of several investigational therapies or the control regimen (4:1). Randomization probabilities are weighed by the probability of achieving a pCR within each subtype for each agent and adapts over the course of the trial. *The primary endpoint is pathologic complete response (pCR, no residual disease in breast or nodes) at surgery*.

The goal is to identify/graduate regimens that have ≥85% Bayesian predictive probability of success (statistical significance) in a 300-patient phase 3 neoadjuvant trial, defined by hormone-receptor (HR) & HER2 status & MammaPrint (MP). To date, 10 experimental regimens have been evaluated.

Regimens may leave the trial for one of four reasons: Graduate, Drop for futility (< 10% probability of success), Drop for safety issues, or accruing maximum sample size (10%< probability of success <85%).

Figure 1: I-SPY2 study schema and adaptive randomization based on probabilities of agents of achieving pCR within a given subtype

METHODS

- 132 ILC and mixed ILC/IDC tumors from I-SPY 2 and Low Risk Registry with pre-treatment Agilent microarrays were available for analysis.
- We used the Classification to Nearest Centroid technique to assign TCGA subtype to our cohort.
- We assessed association between TCGA subtype, clinical covariates and response to therapy using a chi-square test.

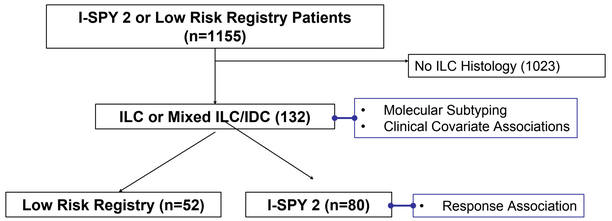
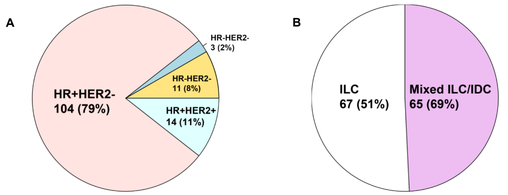


Figure 2. Consort Diagram

- We also evaluated the Euclidean distance between each sample and the three subtype centroids.
- In an exploratory analysis, we used consensus clustering based on the 1000 most varying genes within the HR+HER2- I-SPY ILC cases to generate new unsupervised groupings, and assessed the concordance with the TCGA reactive-like, immune-related and proliferative subtype assignments.

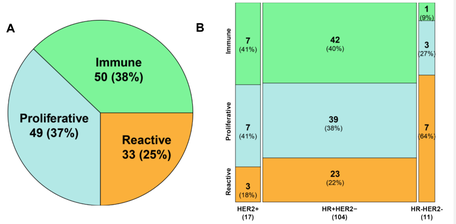
RESULTS

Figure 3. Characteristics of I-SPY 2 and Low Risk Registry Lobular Cohort . (A) HR/HER2 Distribution showing most cases were HR+HER2- (B) Distribution of histology showing 51% were pure ILC.



- Upon applying the TCGA 60-gene classifier, the distribution of ILC subtypes was as follows: 33 (25%) were classified as reactive-like, 50 (38%) were immune-related, and 48 (37%) were proliferative.

Figure 4. Distribution of ILC Molecular Subtype within the I-SPY 2 and Low Risk Registry. (A) Pie chart showing proportion of ILC subtype; (B) Mosaic plot showing distribution of ILC molecular subtype within each HR/HER2 subtype



- **64% of triple negative cases were reactive-like; while the HR+HER2- and HER2+ cases were more likely to be in the proliferative or immune-related subtypes (p=0.037).**
- **Among the 80 I-SPY 2 cases, the overall pathologic complete response rate was low (16.3%) but equivalent to the overall HR+HER2 - I-SPY2 population (16.5%). This did not differ across groups defined by the TCGA ILC subtypes (p=0.79).**

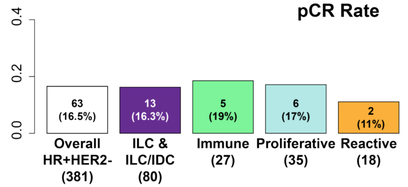


Figure 5. Barplots of pCR rates in the overall HR+HER2- I-SPY population, the lobular cohort, and within TCGA ILC subtypes

A subset of reactive-like and immune-related cases were of similar distance to the proliferative subtype centroid as the proliferative cases.

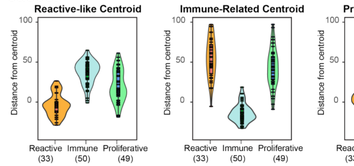


Figure 6. Violin plots showing distance to subtype centroids of samples within each ILC subtype

When we used consensus clustering to identify new subsets within our locally advanced ILC cohort, our unsupervised groupings had only 34% concordance with the TCGA ILC subtype assignments.

	Immune	Proliferative	Reactive
CCP1	8	**18**	4
CCP2	**24**	14	16
CCP3	10	7	**3**

Table 1. Low concordance between consensus cluster and TCGA Subtypes

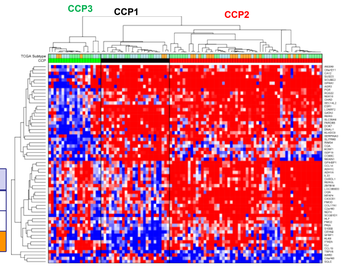


Figure 7. Heatmap of 60 most discriminating genes between consensus clusters

CONCLUSIONS

- We found associations between TCGA molecular subtypes and HR/HER2 status in ILC patients from the ISPY2 Trial.
- There was no association between TCGA subtype and pCR.
- The TCGA subtypes were not the best classifiers for ILC cases in ISPY, possibly reflecting underlying differences within a locally advanced/high risk cohort compared to the overall lower stage TCGA cohort.
- These findings suggest that considerable molecular heterogeneity exists in lobular cancers, which merits further investigation.
- Future work will include pathway analysis of CC subtypes and comparison to signatures derived other groups (Michaut *et al*)

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