

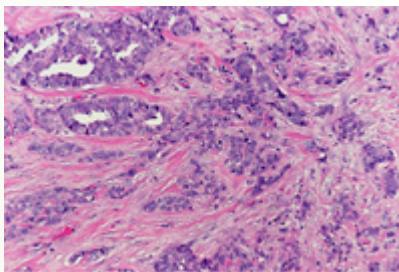


# Molecular Subtypes of Invasive Lobular Cancer in Locally Advanced Patients

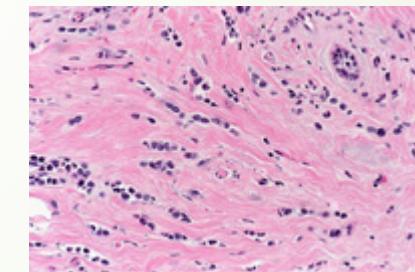
By Zelos Zhu

# Background

- ▶ Invasive lobular carcinoma (ILC) of the breast differs from invasive ductal carcinoma (IDC) by its non-cohesive, diffuse growth pattern.
- ▶ This is characterized by the unique lack of the protein E-cadherin present in the tumor



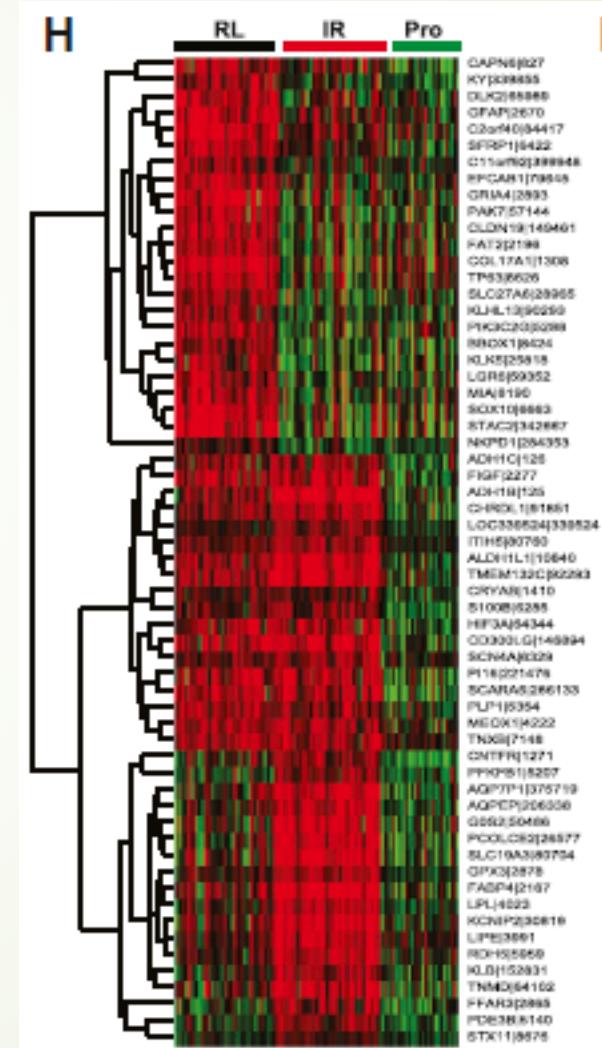
IDC



ILC

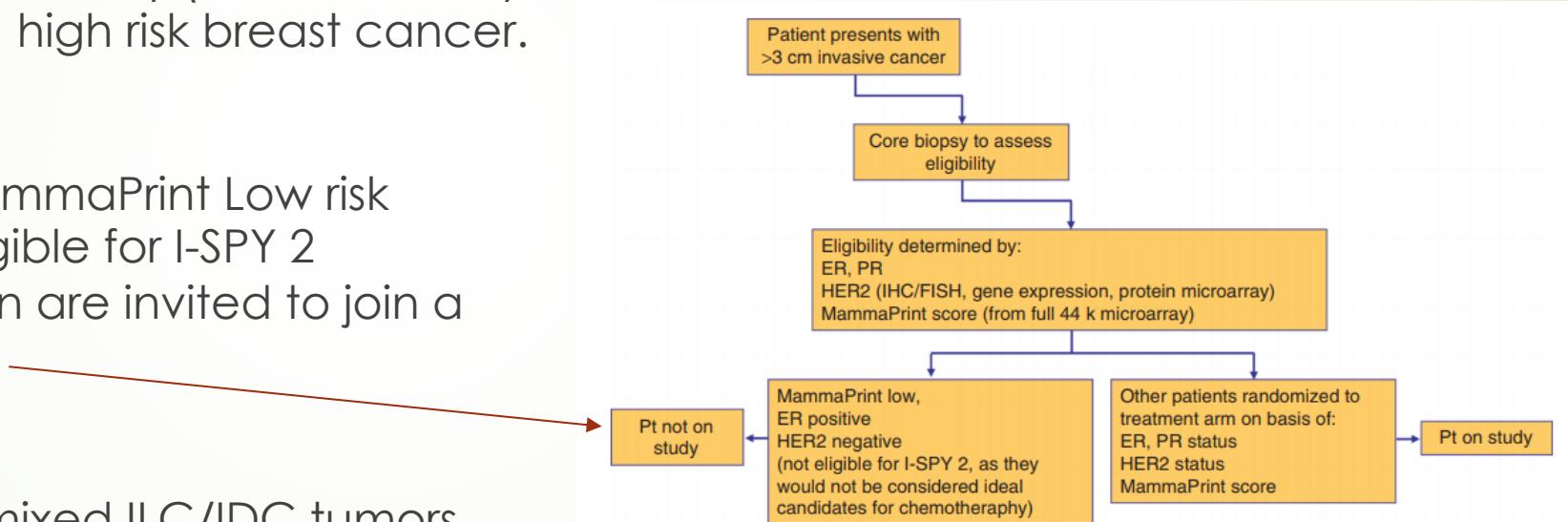
# Background

- ▶ In 2015, the TCGA published the “Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer” which created an ILC molecular subtype classifier
  - ▶ Created 3 subtypes: Reactive-Like, Immune-Related, Proliferative
- ▶ Each of these subtypes are affiliated with higher expression of certain genes:
  - ▶ Reactive like – EGFR, MET, PDGFRA, KIT, TP53, TP63, TP73
  - ▶ Immune Related – IDO1, IFNG
  - ▶ Proliferative – CCNE1, FoxM1, PCNA, RAD50, RAD51, XRCC1, BRCA2, IL23R, JAK2, TYK2MAPK3, RB1, ERK1



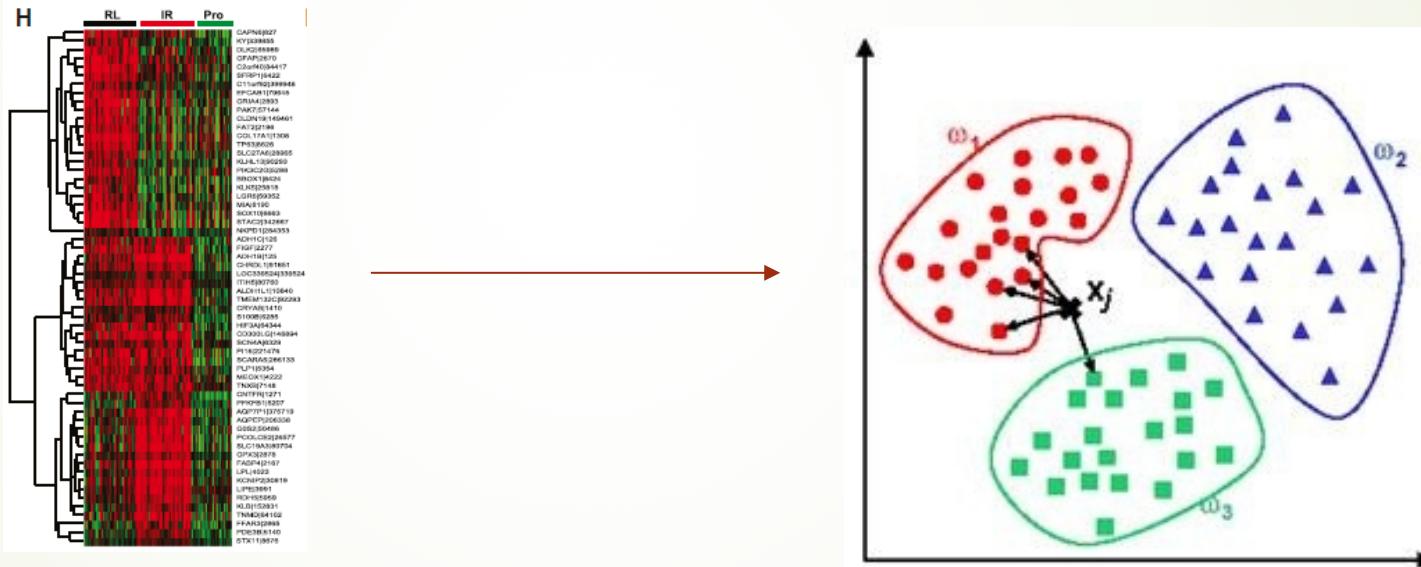
# Methods – Patients Involved

- The I-SPY 2 TRIAL is open to women with locally advanced, clinically/molecularly (as assessed by MammaPrint) high risk breast cancer.
- HR+HER2- MammaPrint Low risk patients ineligible for I-SPY 2 randomization are invited to join a registry.
- 131 ILC and mixed ILC/IDC tumors from these cohorts (I-SPY 2: n=80; low risk registry: n=51) with pre-treatment Agilent microarrays were available for analysis.



# Methods – Analysis Plan

- We used a 60 gene classifier developed by the TCGA based on Classification to Nearest Centroid technique to assign TCGA subtype to our cohort.



- Associations between TCGA subtype, clinical covariates and response to therapy were conducted using a chi-square test.

# Results

Variable Result	Total (N=131)
<b>Type of Study</b>	
Low-Risk Registry	51 (39%)
I-SPY 2	80 (61%)
<b>Histology</b>	
Pure ILC	66 (50%)
Mixed ILC/IDC	65 (50%)
<b>Receptor Status</b>	
HER2+	17 (13%)
HR+HER2-	103 (79%)
TN	11 (8%)
<b>Risk Status</b>	
High 1	66 (50%)
High 2	14 (11%)
Low Risk	51 (39%)
<b>PCR</b>	
PCR	13/80 (16%)
<b>Molecular Subtype</b>	
Reactive Like	33 (25%)
Immune-Related	50 (38%)
Proliferative	48 (36%)

Variable Result	Reactive Like (N=33)	Immune Related (N=50)	Proliferative (N=48)	P-Value
<b>Type of Study</b>				
Low-Risk Registry	15 (29%)	23 (45%)	13 (26%)	0.107
I-SPY 2	18 (23%)	27 (34%)	35 (44%)	
<b>Histology</b>				
Pure ILC	16 (24%)	32 (48%)	18 (27%)	0.031
Mixed ILC/IDC	17 (26%)	18 (28%)	30 (46%)	
<b>Receptor Status</b>				
HER2+	3 (18%)	7 (41%)	7 (41%)	0.037
HR+HER2-	23 (22%)	42 (40%)	38 (38%)	
TN	7 (64%)	1 (9%)	3 (27%)	
<b>Risk Status</b>				
MP1	10 (15%)	25 (38%)	31 (47%)	0.004
MP2	8 (57%)	2 (14%)	4 (29%)	
MPO	15 (29%)	23 (45%)	13 (26%)	
<b>PCR</b>				
PCR	2/18 (11%)	5/27 (19%)	6/35 (17%)	0.790

## Summary of previous results

- ▶ As expected, the I-SPY 2 patients seemed to be much more proliferative, considering our higher risk population
- ▶ Most triple negative patients seemed to be grouped into the reactive-like subtype
- ▶ There does NOT seem to be any significant difference in pathological complete response (PCR) between the three subtypes

## Reminders for ourselves

- ▶ Our population has more locally advanced patients, skewing it toward the high risk
- ▶ The I-SPY group used different ways to measure the gene expression data: Agilent Microarrays vs RNAseq
- ▶ Nearest centroid techniques rely heavily on baseline population assumptions and ours does not necessarily match that of the TCGA

# Methods – Additional Analysis Plan

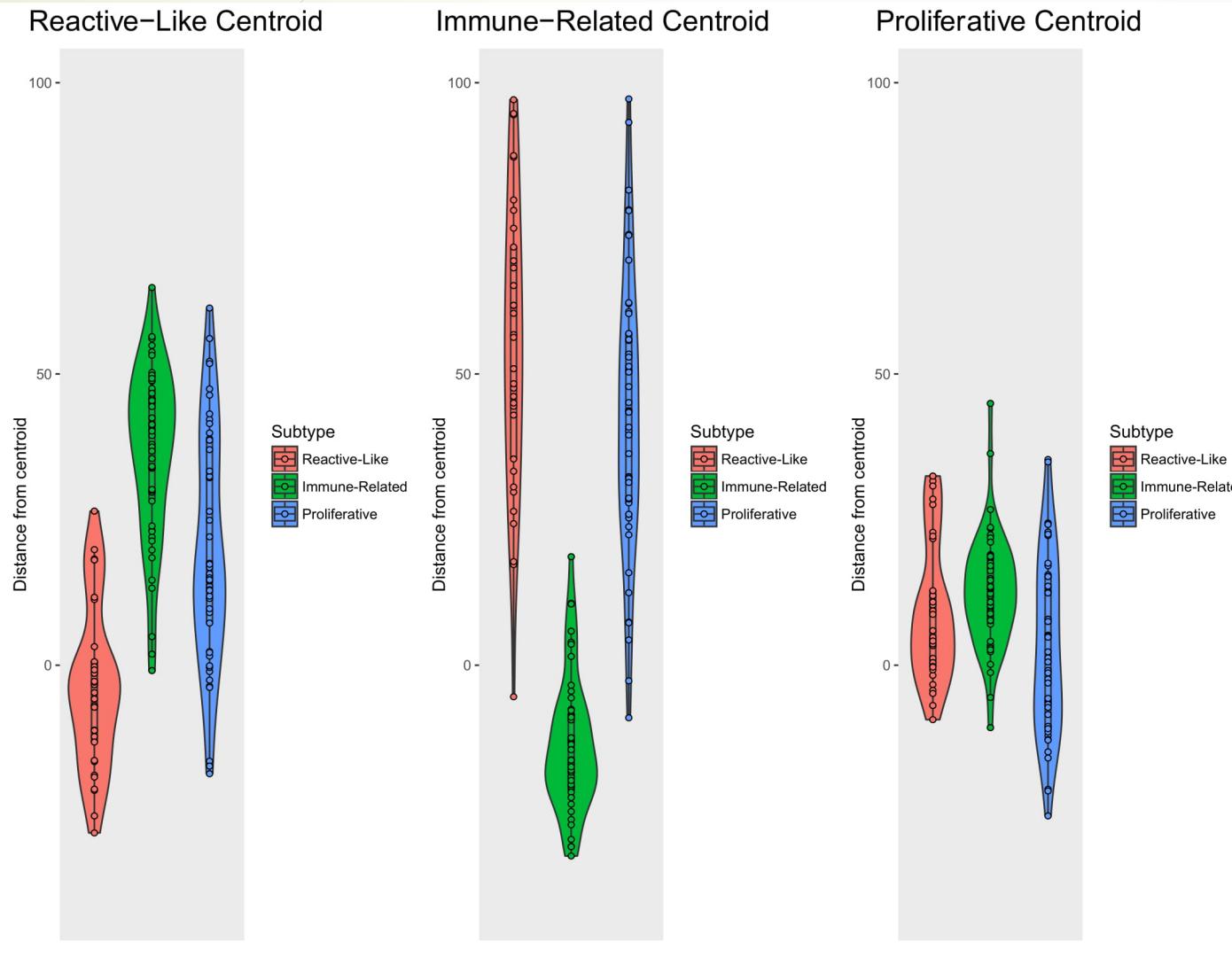
- ▶ Apply ANOVA to see if the differentially expressed genes discovered by the TCGA are different within our cohort as well
- ▶ To evaluate classifier performance in our dataset, we compared the Euclidean Distance to each molecular subtype centroid between samples assigned to each class.
- ▶ In an exploratory analysis, we used consensus clustering based on the 1000 most varying genes within the HR+HER2- I-SPY ILC cases to generate 3 new unsupervised groupings, and assessed the concordance with the TCGA reactive-like, immune-related and proliferative subtype assignments.

# Genes said to be significant by TCGA

Gene Name	Reactive Like	Immune Related	Proliferative	P-Value
EGFR	6.43	6.19	6.08	0.152
MET	7.37	7.14	7.09	0.088
PDGFRA	9.12	8.97	8.81	0.190
KIT	7.58	6.89	6.96	0.000
TP53	6.06	5.94	6.08	0.455
TP63	6.39	6.00	6.14	0.118
TP73	6.17	6.06	6.13	0.723
IDO1	8.66	8.56	8.72	0.868
IFNG	6.19	5.99	6.10	0.574
CCNE1	9.29	8.98	9.17	0.198
FOXM1	7.62	7.55	7.78	0.386
PCNA	10.99	10.96	11.02	0.899
RAD50	8.64	8.74	8.87	0.161
RAD51	8.92	8.92	9.10	0.526
XRCC1	11.17	11.23	11.20	0.881
BRCA2	6.03	5.85	6.05	0.129
IL23R	5.49	5.36	5.59	0.335
JAK2	8.27	8.06	8.00	0.078
TYK2	7.19	7.36	7.38	0.297
MAPK3	7.57	7.94	7.93	0.008
RB1	10.37	10.40	10.29	0.541

Only 2?

# Distance from Centroid Analysis

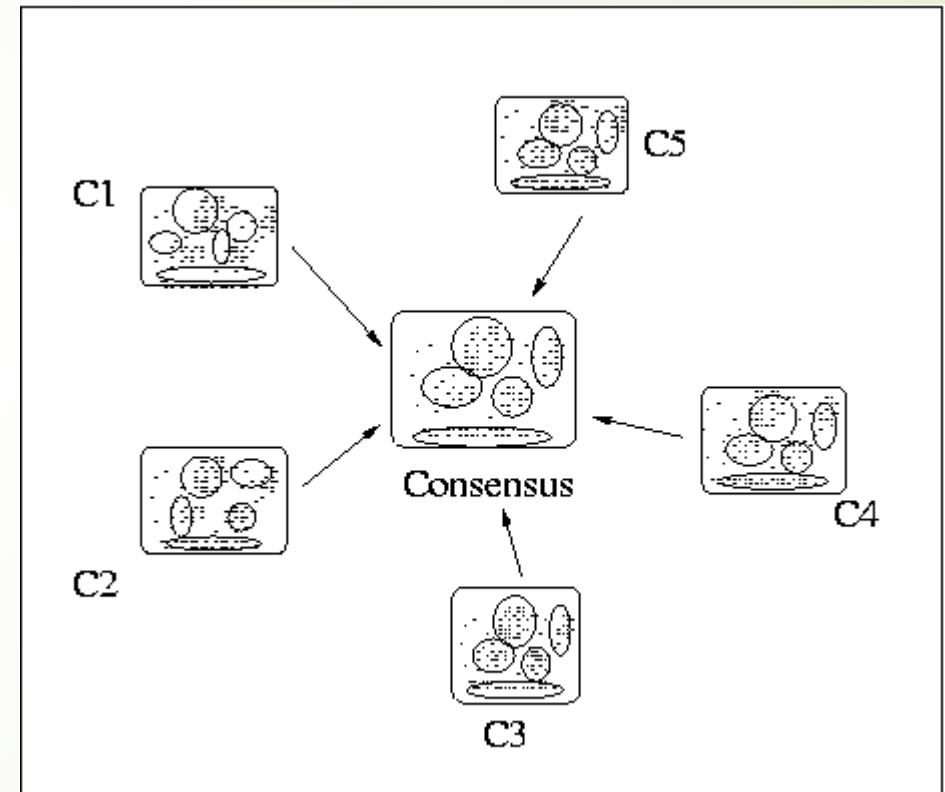


- As illustrated, a subset of cases classified as reactive-like and immune-related are of similar distance to the proliferative centroid as the proliferative cases are
- This suggests our data may not necessarily fit to be used with the TCGA classifier

# What is Consensus Clustering?



Scaled  
up



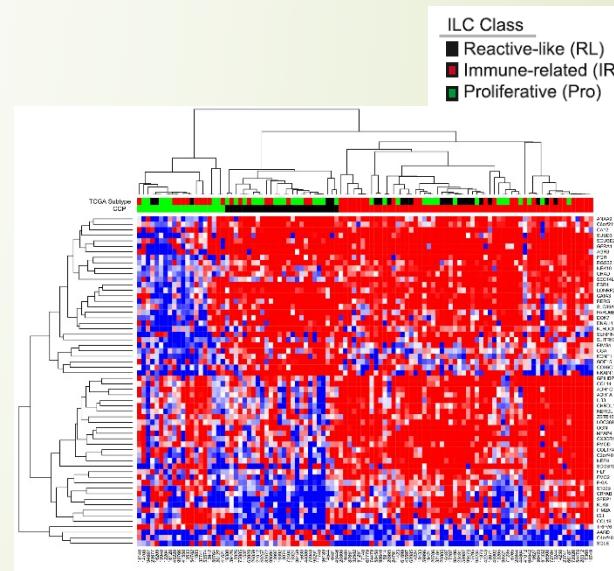
# Exploratory Analysis

## Concensus Clustering Results

	Reactive-Like	Immune Related	Proliferative
CCP 1	2	8	16
CCP 2	18	24	15
CCP 3	3	10	7

32% concordance

- We followed the TCGA protocol further to build out our own classifier
- Only 22/60 genes of the TCGA classifier are within the 1000 most varying genes
- But only 7 of the genes we discovered in our 60 classifier were shared with theirs



# Conclusion

- ▶ The low concordance between our groupings and the TCGA groupings may reflect underlying differences in our population and the population the TCGA studied
- ▶ A locally advanced cohort of ILC cases, like I-SPY, may not be captured in the 60 gene classifier developed from the overall lower stage TCGA cohort
- ▶ These findings DO suggest that a fair amount of molecular heterogeneity exists in lobular cancers, which merits further investigation
- ▶ Further work should involve exploration into pathways identified by the differentially expressed genes in the patients