Elucidating the immune active state of HR+HER2- MammaPrint High 2 early breast cancer

Erin Frances Cobain[1], Lajos Pusztai[2], Cathy Lynne Graham[3], Pat W. Whitworth[4], Peter D. Beitsch[5], Cynthia R. C. Osborne[6], Rakhshanda Layeequr Rahman[7], Nathalie M. Johnson[8], Adam Brufsky[9], Reshma L. Mahtani[10], VK Gadi[11], Kent Hoskins[11], Hannah M. Linden[12], Rita A. Mukhtar[13], Laura Esserman[14], Josien Haan[15], Katie Quinn[16], Andrea Menicucci[16], William Audeh[16], Joyce O'Shaughnessy[17], **FLEX Investigators Group**

[1]University of Michigan, Ann Arbor, MI; [2]Yale Cancer Center, Yale School of Medicine, New Haven, CT; [3]Emory University School of Medicine, Atlanta, GA; [4]Nashville Breast Center, Nashville, TN; [5]Dallas Surgical Group, Dallas, TX; [6]Texas Oncology-Baylor Charles A. Sammons Cancer Center, The US Oncology Network, Dallas, TX; [7]Texas Tech University Health Sciences Center School of Medicine, Lubbock, TX; [8]Legacy Health System, Portland, OR; [9]University of Pittsburgh, Pittsburgh, PA; [10]Miami Cancer Institute, Baptist Health South Florida, Miami, FL; [11]University of Illinois Chicago, Chicago, IL; [12]University of Washington, Fred Hutchison Cancer Research Center, Seattle, WA; [13]University of California, San Francisco, San Francisco, CA; [14]Department of Surgery, University of California, San Francisco, San Francisco, CA; [15]Agendia, Amsterdam, Netherlands; [16]Medical Affairs, Agendia Inc., Irvine, CA; [17]Baylor University Medical Center, Texas Oncology, The US Oncology Network, Dallas, TX

PRESENTED BY: Erin Frances Cobain, MD, University of Michigan, Ann Arbor, MI

Key Takeaway Points/Conclusions:

- MammaPrint H2 tumors exhibit a heightened immune active state compared to MammaPrint H1 tumors among hormone receptor positive (HR+), HER2-negative (HER2-) early-stage breast cancers
- The presence of immune cells and upregulation of genes involved in antigen presentation, which are critical in eliciting T- and B-cell activation, may explain improved response rates to immunotherapy observed in MammaPrint H2 tumors
- Selecting patients with HR+ HER2- early-stage breast cancer for immunotherapy treatment based on grade or percent estrogen receptor positivity (ER%) alone may exclude patients likely to benefit. Conversely, patients with high grade tumors that are not MammaPrint H2 may be at risk of toxicities associated with overtreatment
- These data support the ongoing SWOG2206 clinical trial in evaluating the impact of neoadjuvant chemo-immunotherapy on rates of pathologic complete response and invasive disease-free survival in HR+ HER2- early-stage breast cancer with MammaPrint H2 tumors

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Neoadjuvant immunotherapy in HR+HER2- breast cancer

- Patients with clinically and genomically high-risk HR+HER2- breast cancer continue to have 20% recurrence risk despite current adjuvant therapies (i.e., chemotherapy [CT], CDK4/6i)
- The addition of immune checkpoint inhibitors to neoadjuvant CT in HR+HER2- breast cancer has been evaluated in Phase III clinical trials (CheckMate-7FL and KEYNOTE-756)
  - These trials used clinical stage, tumor grade (grade 3), or ER% (≤ 10%) to select patients with high risk for recurrence for treatment on study
- Genomic classifiers predicting recurrence risk, such as MammaPrint® 70-gene signature, may offer enhanced precision in identifying HR+HER2- breast cancer patients who benefit from chemotherapy and immune checkpoint therapy

References: Loi et al. Ann Oncol 2023; Cardoso et al. Ann Oncol 2023

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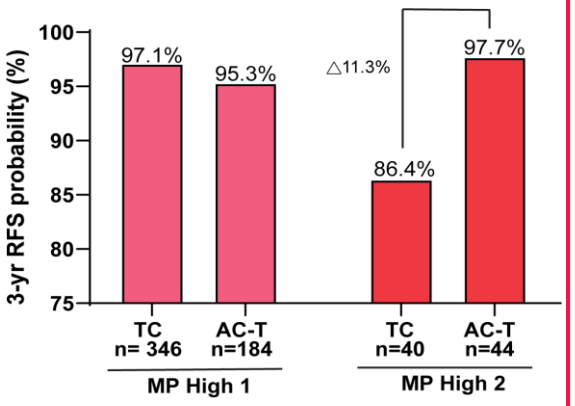
70-gene MammaPrint test: Implications for ET and CT Decisions

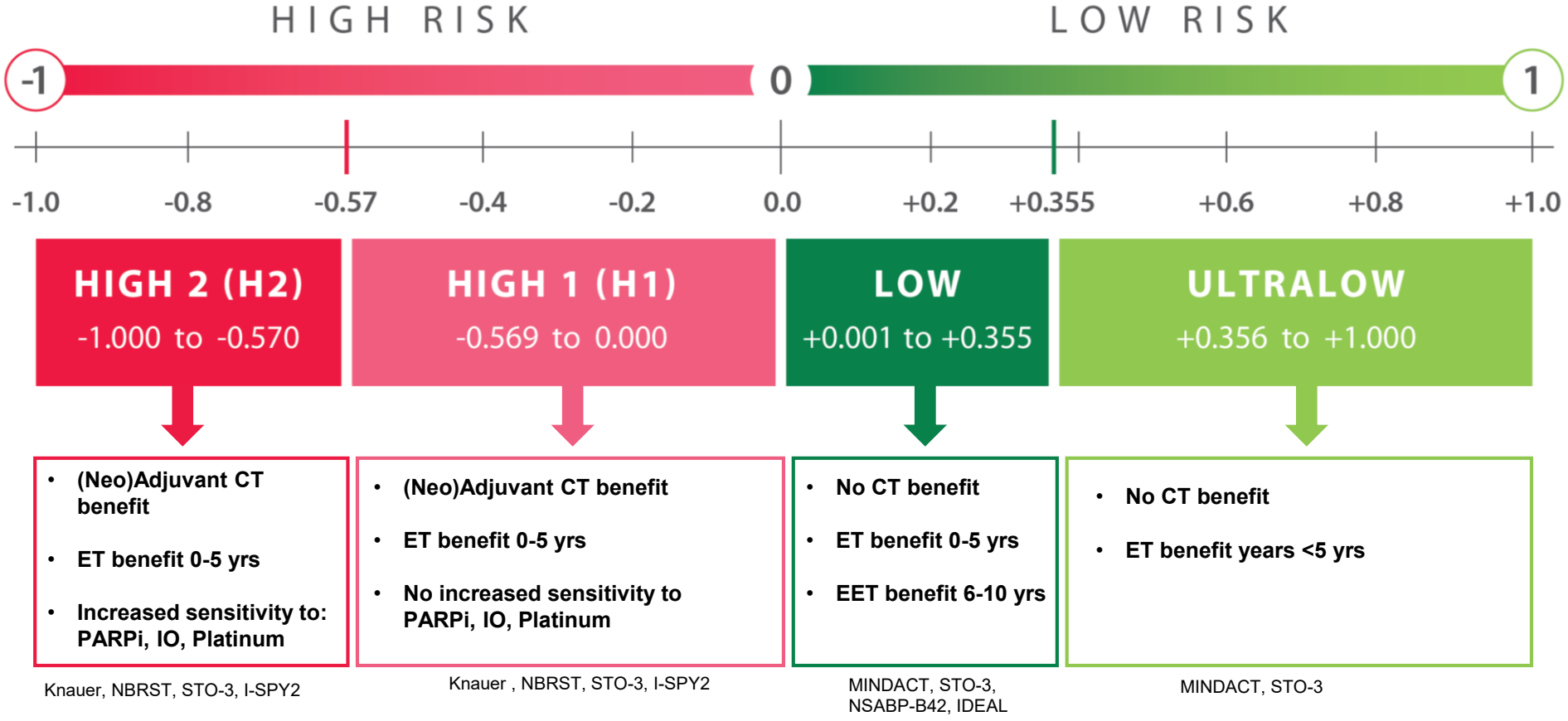
- MammaPrint (MP) classifies patients with HR+HER2- early breast cancer as having an Ultra Low, Low, High 1, or High 2 Risk of distant recurrence

Additional Recent High 2 Findings

Abstract Title: Association of MammaPrint index and 3-year outcome of patients with HR+HER2- early-stage breast cancer treated with chemotherapy with or without anthracycline



O'Shaughnessy et al., Rapid Oral # 511, 5/31 2:45-4:15pm



References: **Knauer (**Breast Cancer Res Treat 2010**), NBRST** (Whitworth, Ann Surg Oncol 2022), **STO-3** (van't Veer, Breast Cancer Res Treat, 2017, Esserman, JAMA Onc 2017), **I-SPY2** (https://www.ispytrials.org/i-spy-platform/i-spy2; Pusztai, Cancer Cell 2021), **MINDACT** (Piccart, Lancet Oncol, 2021; Lopes Cardozo, JCO, 2022), **NSABP-B42** (Rastogi, ASCO 2021), **IDEAL** (Liefers, SABCS 2022)

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MammaPrint High 2 and neoadjuvant immunotherapy

- I-SPY2 trials demonstrated improved pCR when immune checkpoint inhibitors were included with neoadjuvant chemotherapy (nCT) in MP High 2, HR+HER2- tumors

Immunotherapy (IO) arm	pCR% in control (nCT) and IO					
	High 1			High 2		
	Control	IO	**Δ**	Control	IO	**Δ**
Pembrolizumab	13%	18%	**5%**	21%	61%	**40%**
Pembrolizumab + SD-101	10%	17%	**7%**	21%	45%	**24%**
Durvalumab + Olaparib	9%	10%	**1%**	22%	66%	**44%**

- The currently enrolling phase III randomized trial SWOG S2206 (NCT06058377) assigns patients with ER+HER2-, MP High 2 breast cancer to durvalumab + nCT vs nCT alone

Reference: Nanda et al., JAMA Oncol, 2020; Wolf et al., Cancer Res, 2022; Pusztai et al., Cancer Cell 2021

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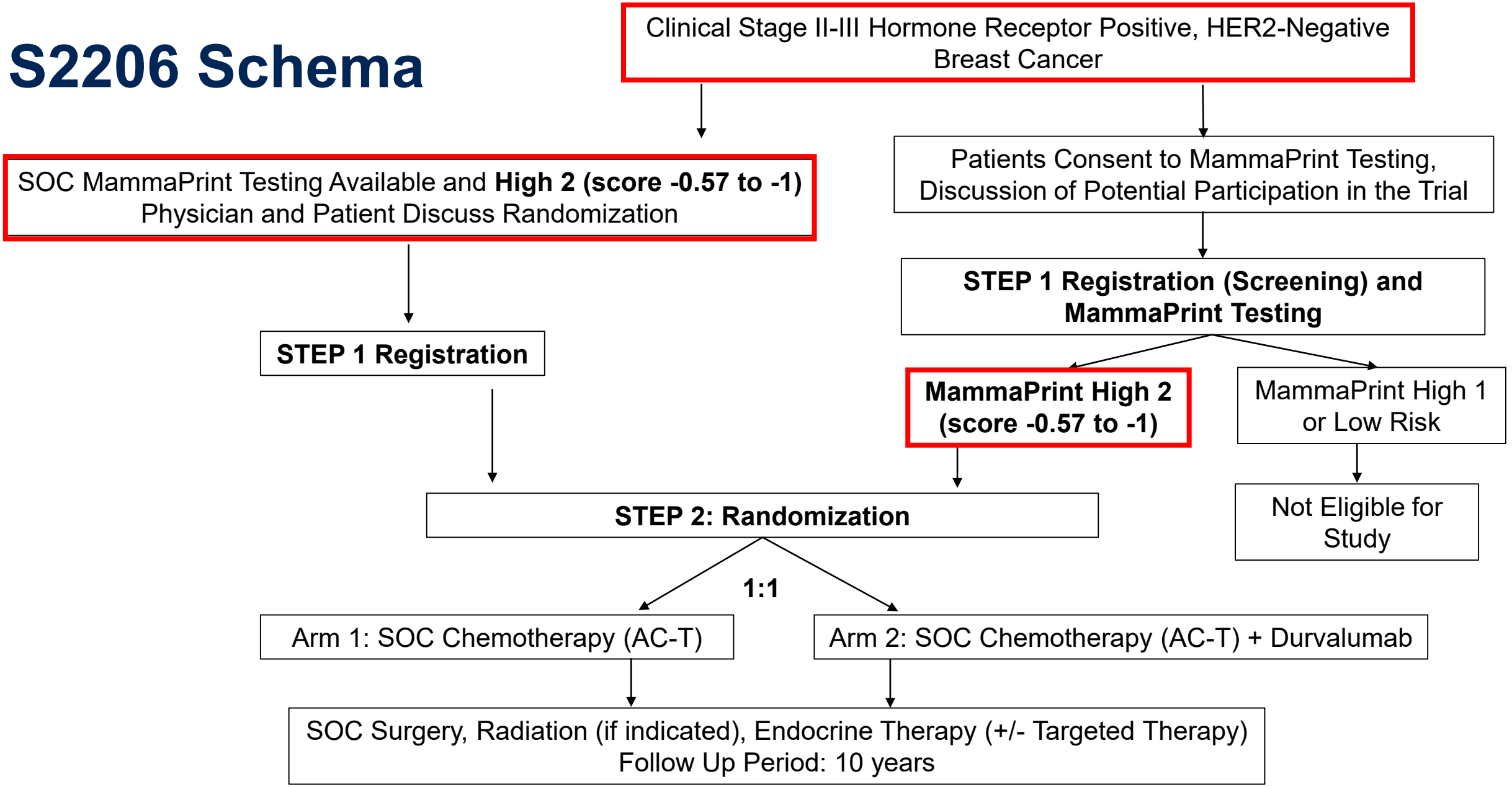
S2206 Schema



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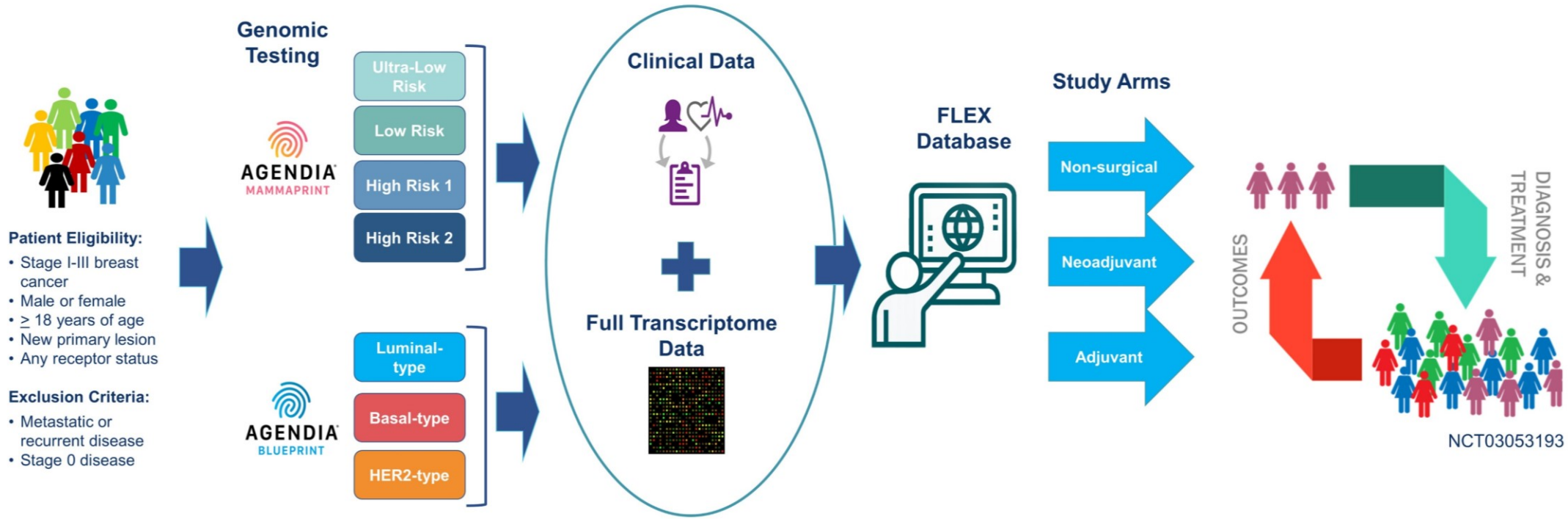
Study Objective

Characterize the underlying biology that mediates immune therapy response in HR+HER2- early breast cancers classified as MammaPrint High 2, an immunotherapy biomarker in SWOG 2206, using the FLEX Study

- Characterize clinicopathologic features associated with MammaPrint High 2
- Evaluate gene expression levels to define immune biology in High 2 (BluePrint Luminal and Basal subtypes) vs High 1 tumors
  - Innate and adaptive immunity cell types
  - Antigen processing and presentation pathway
  - Immune checkpoint PD-1 and PD-L1 expression

FLEX Study Overview

- FLEX (NCT03053193) is a prospective real world evidence study designed to support investigator-initiated breast cancer research through the curation of paired full transcriptome and clinical data.
- Enrolls stage I-III breast cancer patients who receive standard of care MammaPrint



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Methods

- **Patients**: 2916 patients from FLEX with MammaPrint High Risk, HR+HER2- early breast cancer
- **Molecular Classification:**
  - MammaPrint High Risk tumors stratified into High 1 (Index 0.00 to -0.569) or High 2 (Index -0.570 to -1.00)
  - 80-gene molecular subtyping signature, BluePrint, further classified High Risk tumors as Luminal-Type or Basal-Type
- **Immune characterization of High 2 relative to High 1**
  - Immune Cell abundances were defined by the gene-signature based method, xCell, and differences were determined by t-test (adjusted p-value <0.05)
  - Gene expression of the KEGG pathway genes “antigen processing and presentation” and the expression of PD-1 and PD-L1 between High Risk groups was determined by use of the R package Limma

References: Aran et al., Genome Biology 2017; Ritchie et al., Nucleic Acids Research 2015.

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Clinical Characteristics

- 79% of tumors were MP High 1
- 21% of tumors were MP High 2
- Patients with MP High 2 tumors more likely to be younger compared to High 1
- MP High 2 more likely to be grade 3
  - 30% of MP High 2 were Grade 1-2
  - 53% of all Grade 3 were MP High 1
- MP High 2 tumors more likely to have low ER staining
  - 83% of High 2 tumors had ER% >10%

Characteristic, n (%)* *unknown excluded	MP High1 (n=2292)	MP High2 (n=624)	P-value
Age			
≤ 50 yrs	537 (23.6%)	198 (31.9%)	<0.001
> 50 yrs	1739 (76.4%)	423 (68.1%)	
Menopausal Status			
Pre-/Peri-	475 (22.0%)	170 (29.2%)	<0.001
Post-	1689 (78.1%)	412 (70.8%)	
Race			
White	1778 (82.4%)	404 (69.9%)	<0.001
Black	229 (10.6%)	112 (19.4%)	
Latin American	72 (3.3%)	34 (5.9%)	
AAPI	67 (3.1%)	25 (4.3%)	
Other	12 (0.6%)	3 (0.5%)	
T Stage			
T1	943 (62.3%)	199 (45.2%)	<0.001
T2	493 (32.6%)	199 (45.2%)	
T3	55 (3.6%)	27 (6.1%)	
T4	22 (1.5%)	15 (3.4%)	
N Stage			
Node Negative	1156 (80.4%)	313 (74.2%)	0.021
Node Positive	282 (19.6%)	109 (25.8%)	
Grade			
G1	361 (16.4%)	10 (1.6%)	<0.001
G2	1346 (61.1%)	163 (26.7%)	
G3	495 (22.5%)	437 (71.6%)	
ER% Staining			
1-10%	25 (1.1%)	95 (16.8%)	<0.001
>10%	2213 (98.9%)	470 (83.2%)	
BP Molecular Subtype			
Luminal B	2269 (99.0%)	348 (55.8%)	<0.001
Basal	23 (1.0%)	276 (44.2%)	

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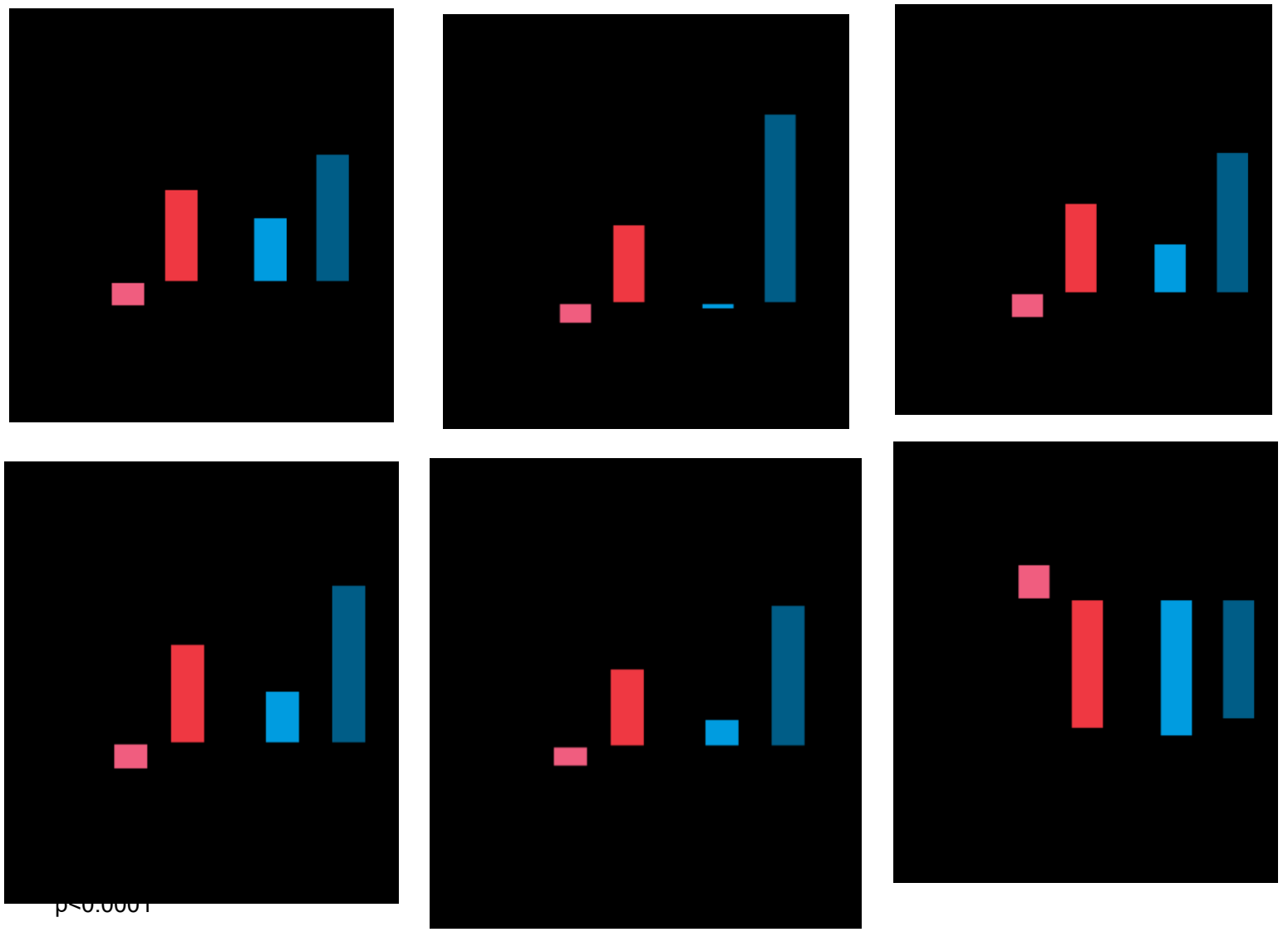
MammaPrint High 2 tumors have increased innate immunity

- MP High 2 tumors had significantly higher abundance of antigen presenting cells (APCs) compared to MP High 1 tumors:
  - M1 macrophages
  - Activated Dendritic Cells
- Compared to MP High 1, MP High 2 tumors had:
  - Higher abundance of NK cells
  - Significantly lower abundance of neutrophils



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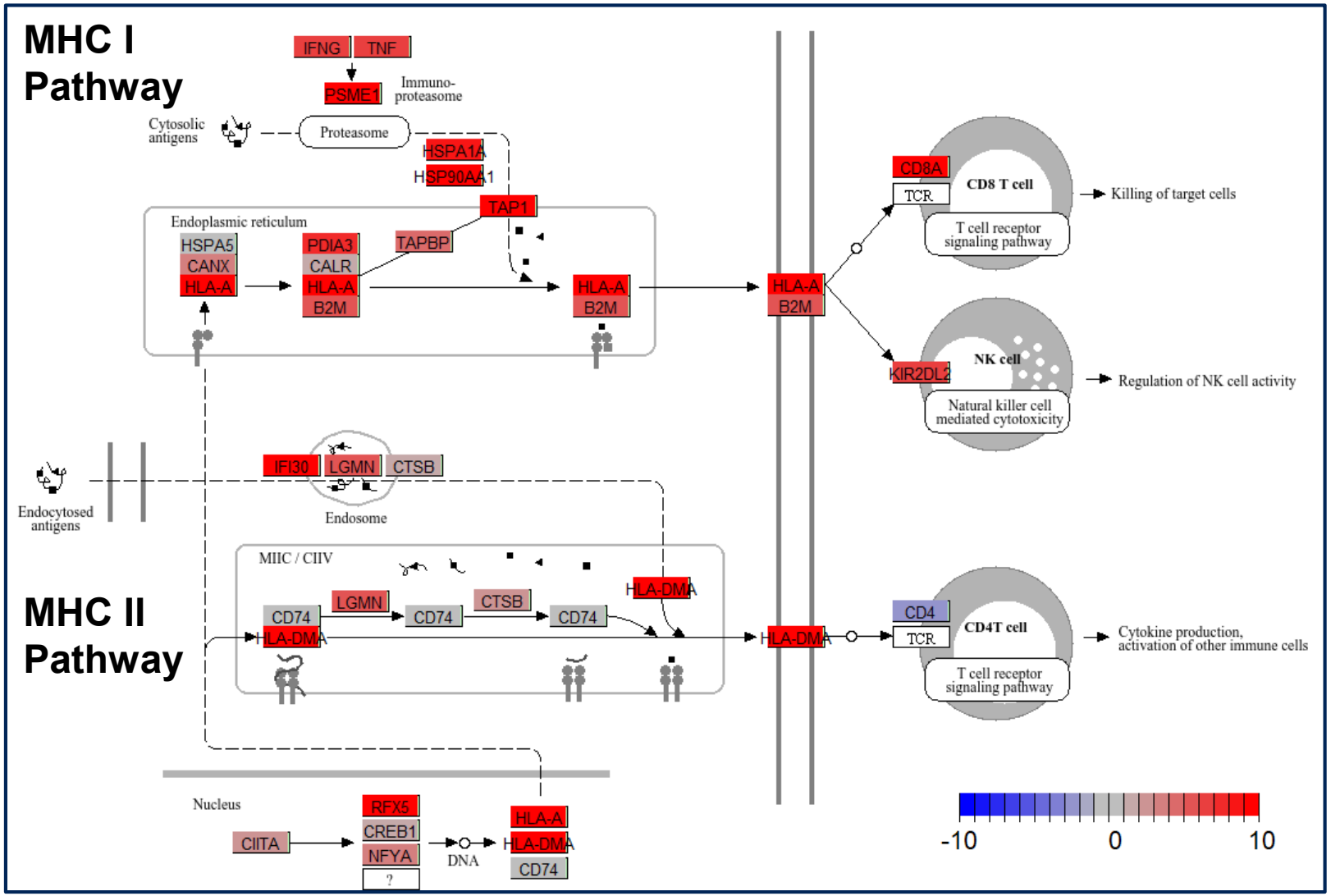
Upregulation of antigen presentation and processing in MammaPrint High 2 vs High 1

- KEGG Pathway: Antigen Presentation and Processing.
- Genes involved in MHC I pathway and MHC II pathway were upregulated (**red**) in MP High 2 tumors compared to MP High 1 tumors



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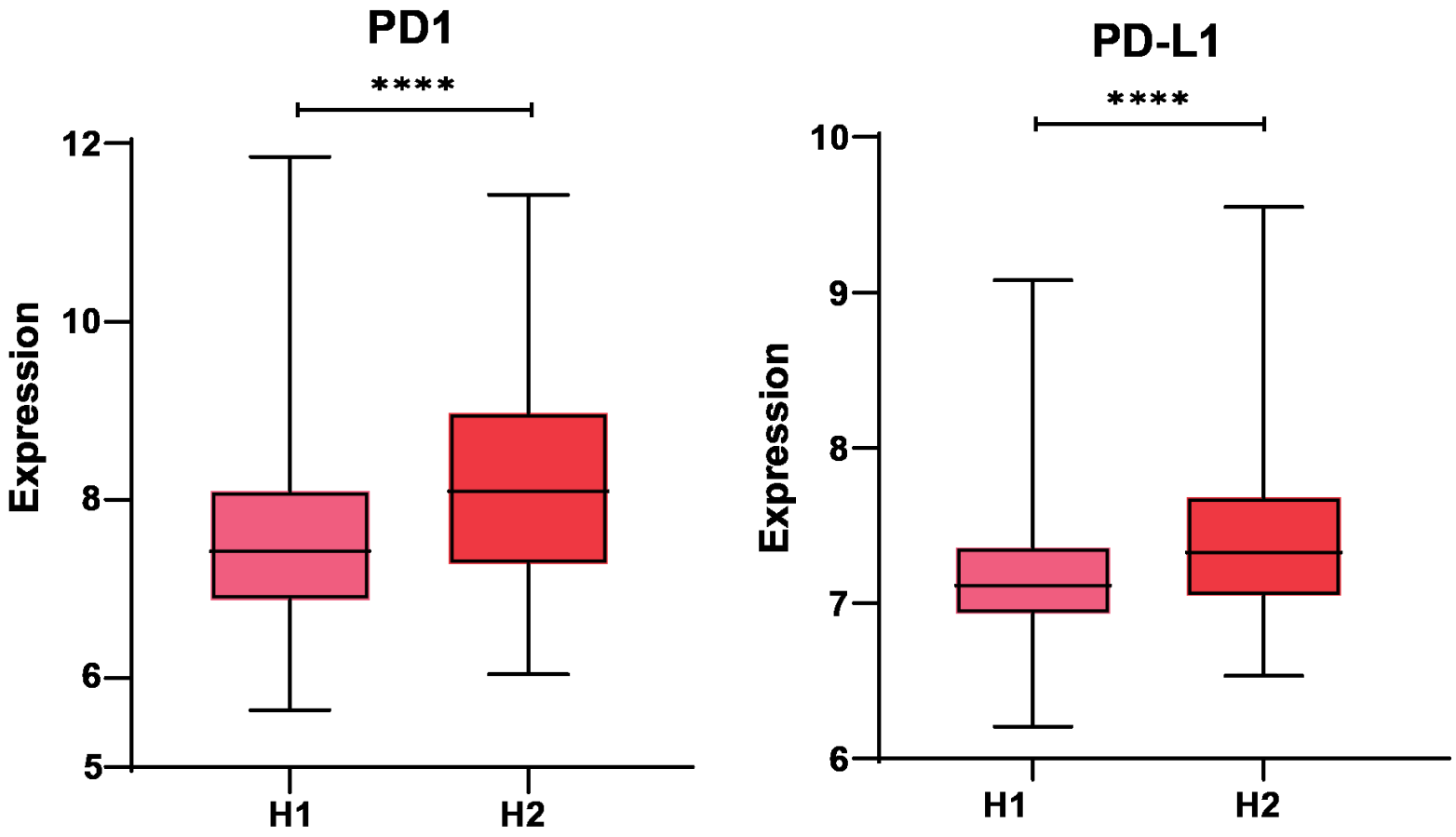
Upregulation of PD-1 and PD-L1 expression in MP High 2

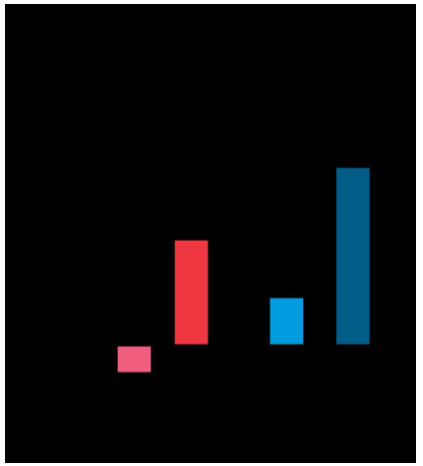


****p<0.0001

- MP High 2 tumors had significantly higher expression of *PD1* and *PD-L1* compared to MP High 1 tumors

MammaPrint High 2 tumors have increased adaptive immunity

- Compared to MP High 1, MP High 2 tumors had significantly higher frequency of:
  - CD4+ T and Memory cells
  - CD8+ T and Memory cells
  - B cells, memory B cells and antibody producing plasma cells
- Increased lymphocytes were observed in both MP High 2 Luminal and Basal tumors



****p<0.0001; **p<0.01; *p<0.05

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Conclusions:

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Acknowledgements

- We would like to thank the >16,000 patients enrolled in FLEX that make these data possible
- FLEX study site investigators, coordinators, research nurses and personnel

FLEX Trial Information

- MammaPrint, BluePrint, and Full-genome Data Linked With Clinical Data to Evaluate New Gene EXpression Profiles (FLEX) (NCT03053193)
- Agendia, Inc.