Genomic signatures predict the immunogenicity of BRCA-deficient breast cancer

Adam A Kraya et al. 2019/03, AACR

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Introduction

Mutations in BRCA1 and BRCA2 are the most common causes of hereditary breast cancer.

- BRCA1 and 2 play essential functions in maintaining genome integrity
  - primarily through their roles in homologous recombination (HR)
  - And contribution to double strand DNA break repair.
- Breast cancers associated with germline BRCA1 and BRCA2 mutations have higher sensitivity to DNA damaging agents
- However, outcomes can vary widely across patients with germline BRCA1 and BRCA2 mutations receiving DNA damaging agents,
  - may be due to the varying degree of HR deficiency in these tumors.

Purpose

- Breast cancers with BRCA1/2 alterations have a relatively **high mutational load**
- Suggesting **immune checkpoint blockade** may be a potential treatment option.
- However, the degree of immune cell infiltration varies widely and molecular features contributing to this variability **remain unknown**.

Hypothesis

- Tumors with somatic or germline defects in BRCA1/2 are hypothesized to be **more immunogenic** than tumors without genetic defects in the HR pathway,
    - potential candidates for immune checkpoint blockade.

- BRCA1/2 mutation-associated breast cancers are more genomically unstable than tumors without such genetic alterations
    - with increased numbers of non-synonymous single nucleotide variants likely driving the heightened immunogenicity observed in these tumors

Experimental Design

Used the Cancer Genome Atlas (TCGA) genomic data,

- Compared breast cancers with (89) and without (770) either germline or somatic BRCA1/2 alterations.

- Also studied 35 breast cancers with germline BRCA1/2 mutations from U of Penn using WES and immunohistochemistry.

Analysis

- **Matching:**
  - All WES data from TCGA tumors and matched germline were aligned to the hg38 assembly of the human genome.
  - All WES data from Penn tumors and matched germline were aligned to the hg19 assembly of the human genome.

Analysis

In total, identified 35 breast tumors from the TCGA associated with germline BRCA1 (n=18) or BRCA2 (n=17) mutations with RNAseq data.

- **Threshold:**
  - If the germline allelic fraction (AF) >0.30 for the mutation, and the total depth was >30 in germline and tumor at the mutation locus.
    - Considered tumors were associated with germline BRCA1 and BRCA2 mutations

Statistical Methods

- **Dichotomization using Median:**
  - Median HRD-total scores were determined for BRCA1/2 (median=50.65)
  - HR mutant (median=12.08)
  - HR wild type (median=-4.58)

- **Student's T-test:**
  - Log-normalize the data first
  - P-values for correlation between gene expression and sample traits (HRD and allele-specific LOH status)

Statistical Methods

Else...

- Normalized enrichment scores from GSVA were computed by assuming unimodal and approximately Gaussian distribution of enrichment scores.

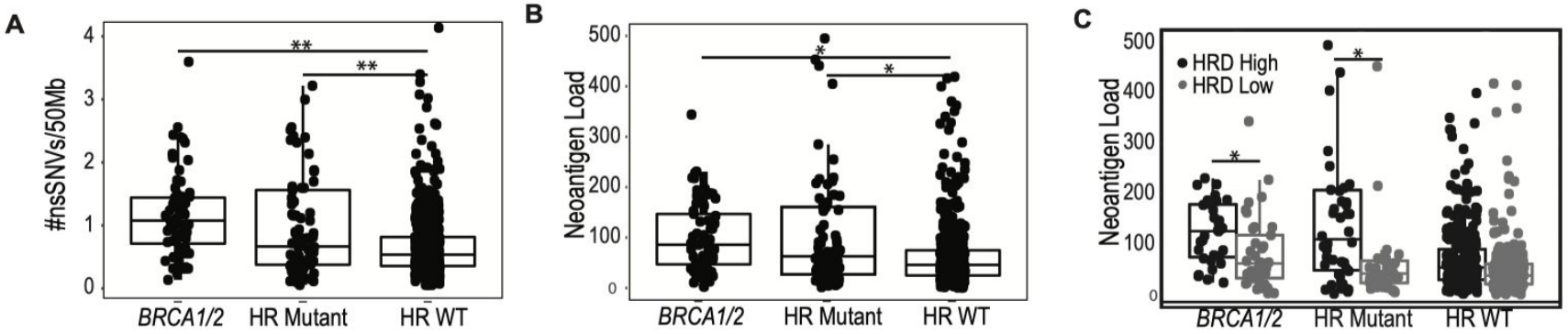
- T-statistics were adjusted using an empirical Bayesian model, and p-values were adjusted using the Benjamini-Hochberg method.

Results

Association of homologous recombination deficiency (HRD) with mutational and neoantigen burden in TCGA breast cancers

- Found homologous recombination deficiency (HRD) scores were negatively associated with:
  - expression-based immune indices
    - cytolytic index (p=0.04)
    - immune ESTIMATE (p=0.002)
    - type II IFN signaling (p=0.002)
  - although being associated with a higher mutational/neo-antigen burden, in BRCA1/2 mutant breast cancers.

Figure1. HRD and immunogenicity in TCGA breast cancers



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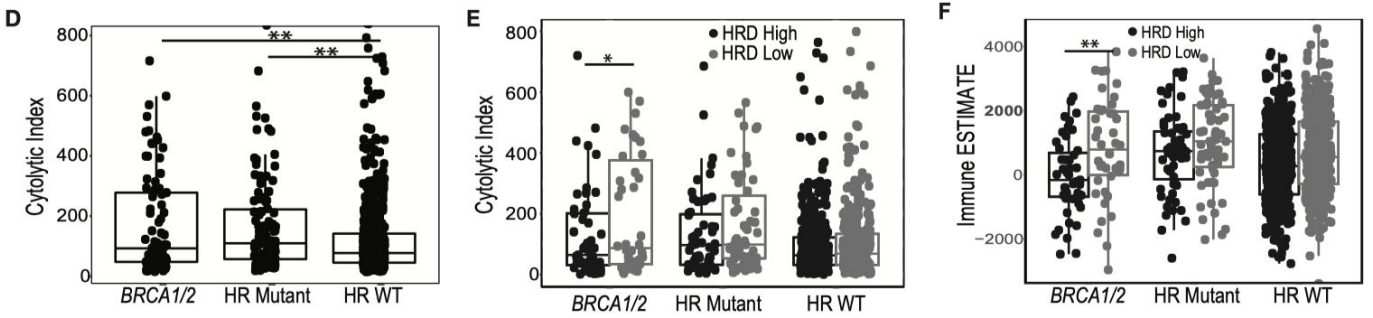
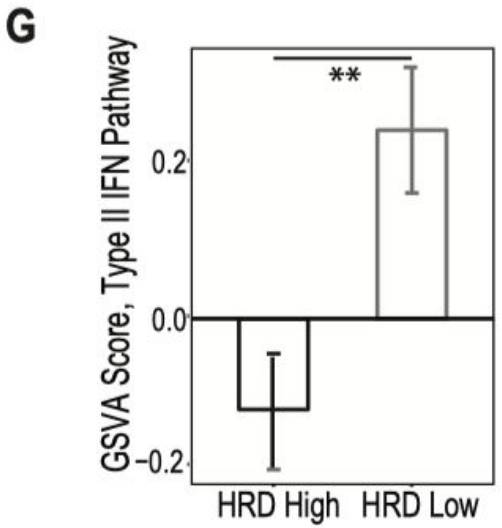


- HRD high BRCA1/2 cancers more closely resembled HR wild type cancers
- **(1D)** Cytolytic index was higher in BRCA1/2 mutation-associated (p=0.0016) and HR gene mutation-associated (p=0.0019) than HR wild type breast cancers
- **(1E, 1F)** higher HRD scores were associated with lower cytolytic index (p=0.043) and immune ESTIMATE score (p=0.002), despite correlating with a higher predicted neoantigen load

Figure1. HRD and immunogenicity in TCGA breast cancers

Lower immune effector activity in the tumor subset



- Interrogated immune metagenes by gene set variation analysis (GSVA)
- Found lower enrichment of the type II interferon (IFN) metagene (p=0.002) amongst HRD high BRCA1/2 mutation-associated breast cancers

Figure2. Effects of complete loss of wild type BRCA1/2 on breast cancer immunogenicity

- **(2A)** Found homologous recombination deficiency (HRD) scores were negatively associated with:
    - expression-based immune indices
        - cytolytic index (p=0.04)
        - immune ESTIMATE (p=0.002)
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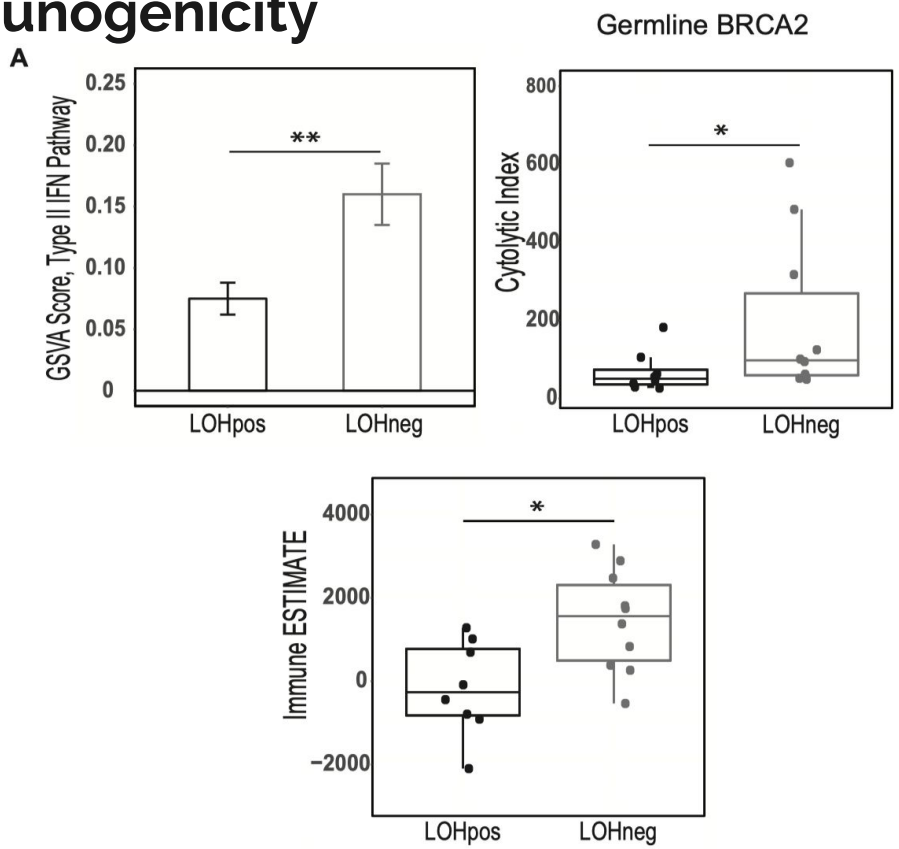
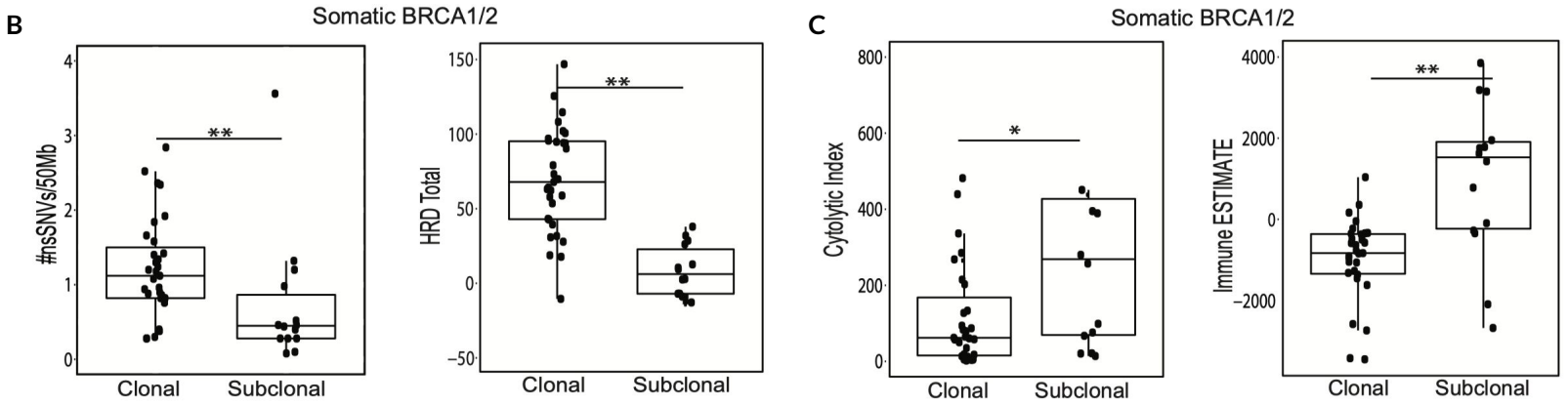


Figure2. Effects of complete loss of wild type BRCA1/2 on breast cancer immunogenicity



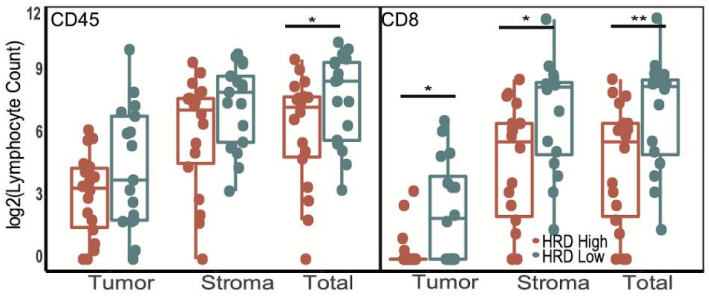
- **(2B)** Found that cancers with clonal BRCA1/2 mutations had higher mutational burden (p=0.05) and HRD scores (p=2.37E-07),

- **(2C)** but lower cytolytic index (p=0.0033) and immune ESTIMATE scores (p=4.98E-05) than cancers with subclonal BRCA1/2 mutations.

Figure3. Immune infiltrates and T-cell effector activity in Penn BRCA1/2 breast cancers

T-cells were lower in BRCA1/2 breast cancers with elevated levels of HRD

A



B

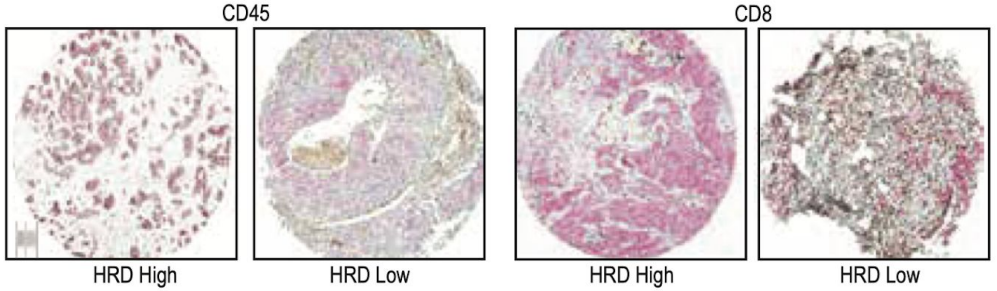
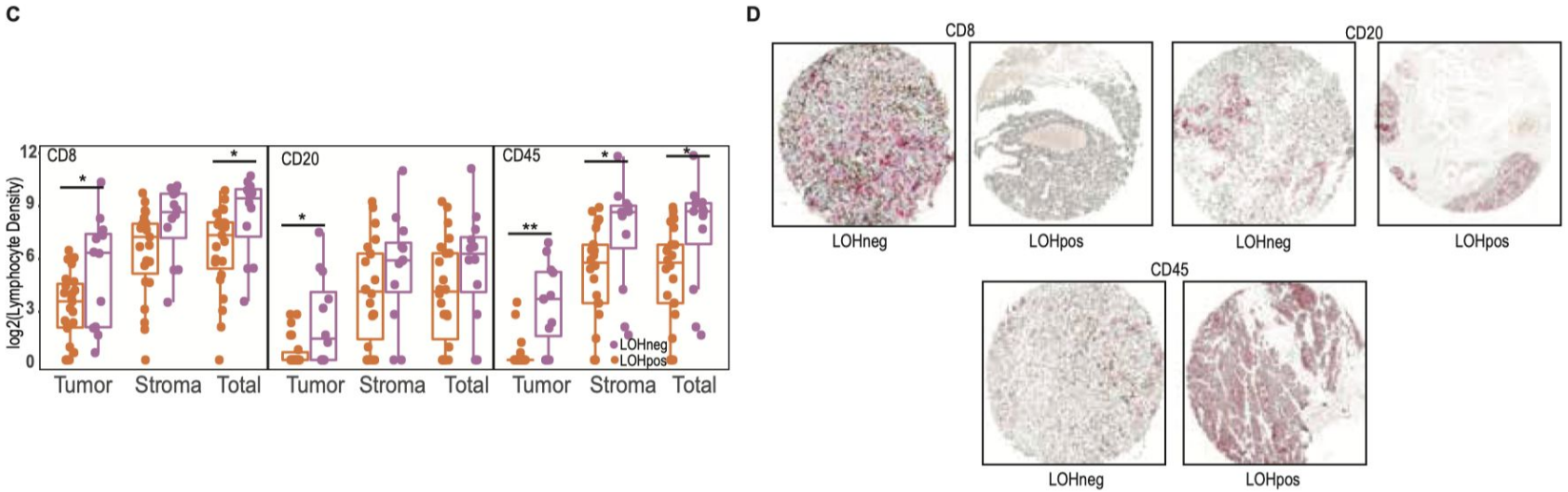


Figure3. Immune infiltrates and T-cell effector activity in Penn BRCA1/2 breast cancers



- BRCA1/2 LOH positive (LOHpos) had a lower number of CD8+ T cells
    - these changes were more strongly associated with BRCA1 mutation-associated tumors (according to supplmental data)

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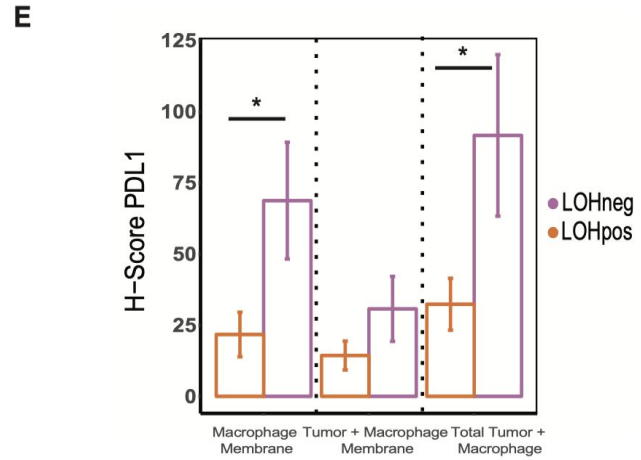

- Found lower levels of macrophage membrane (p=0.048) and macrophage + tumor PDL1 (p=0.012) in cancers with allele-specific LOH relative to cancers without allele-specific LOH
    - Indicating lower tumor inflammation
    - Driven by BRCA1 cancers

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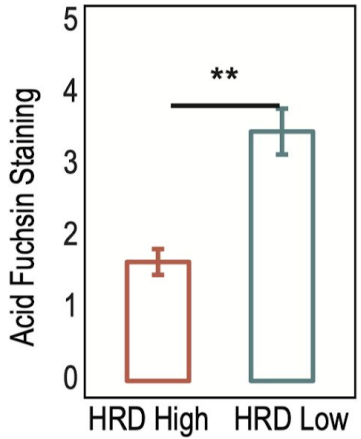
Found lower red myofibroblast staining in HRD-high versus HRD-low tumors (p=0.0071).

G



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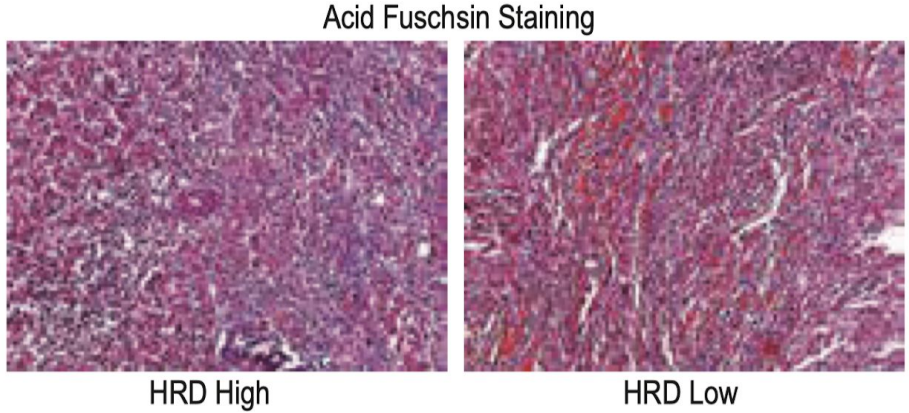
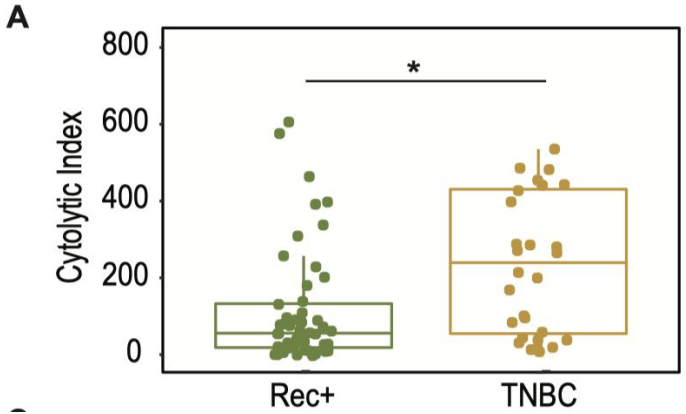


Figure4. Hormone receptor expression and HRD jointly stratify BRCA1/2 breast cancer immunogenicity



- analyzed the association between hormone receptor expression and immunogenicity in the background of BRCA1/2 alterations
- TNBCs had higher cytolytic index overall (p=0.025)

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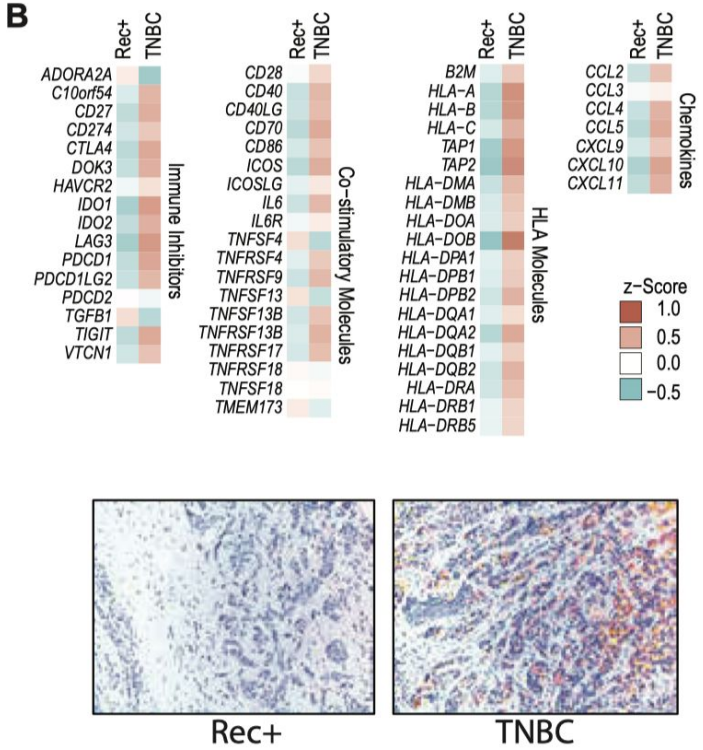

- An expression level comparison of immunomodulatory genes across TNBCs and Rec+ BRCA1/2 mutant breast cancers found that:
  - TNBCs had higher expression of most immune markers than Rec+ tumors
  - suggesting **a more inflamed microenvironment**
- Inflamed tumors often express counterregulatory checkpoint proteins such as PDL1 to evade immune attack

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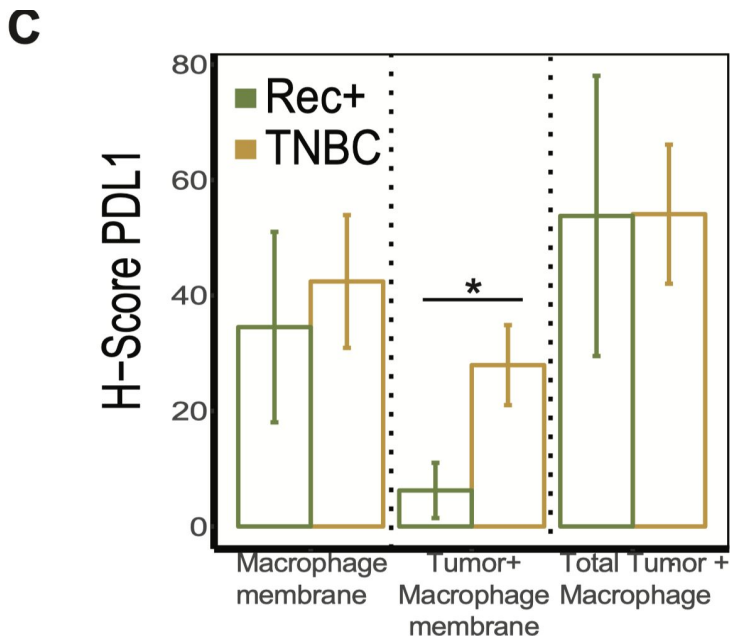
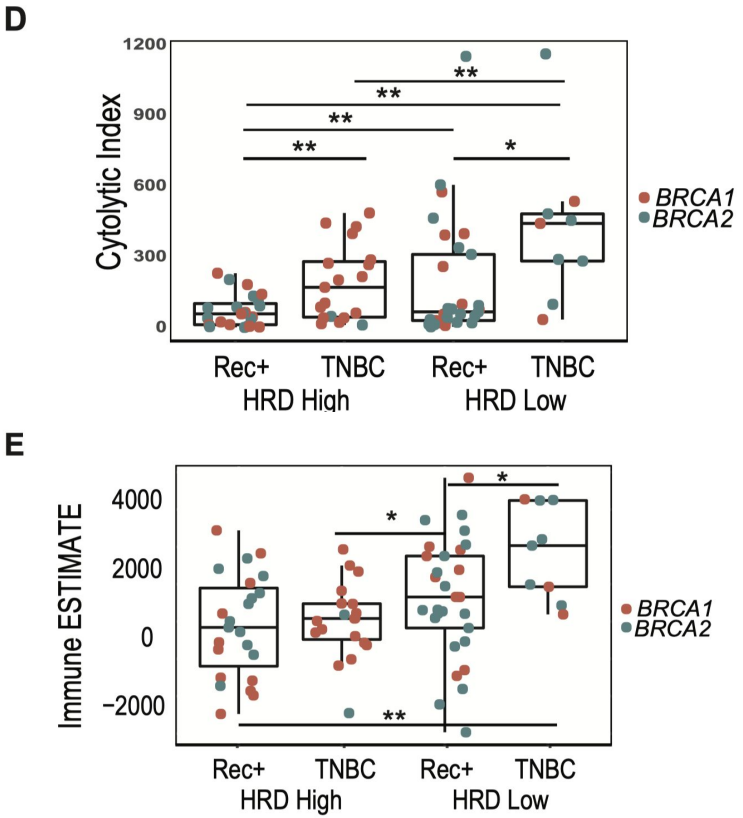


- In Penn BRCA1/2 germline mutation-associated breast cancers,
  - membrane PDL1 was higher in TNBCs when summing with tumor and macrophage membrane PDL1

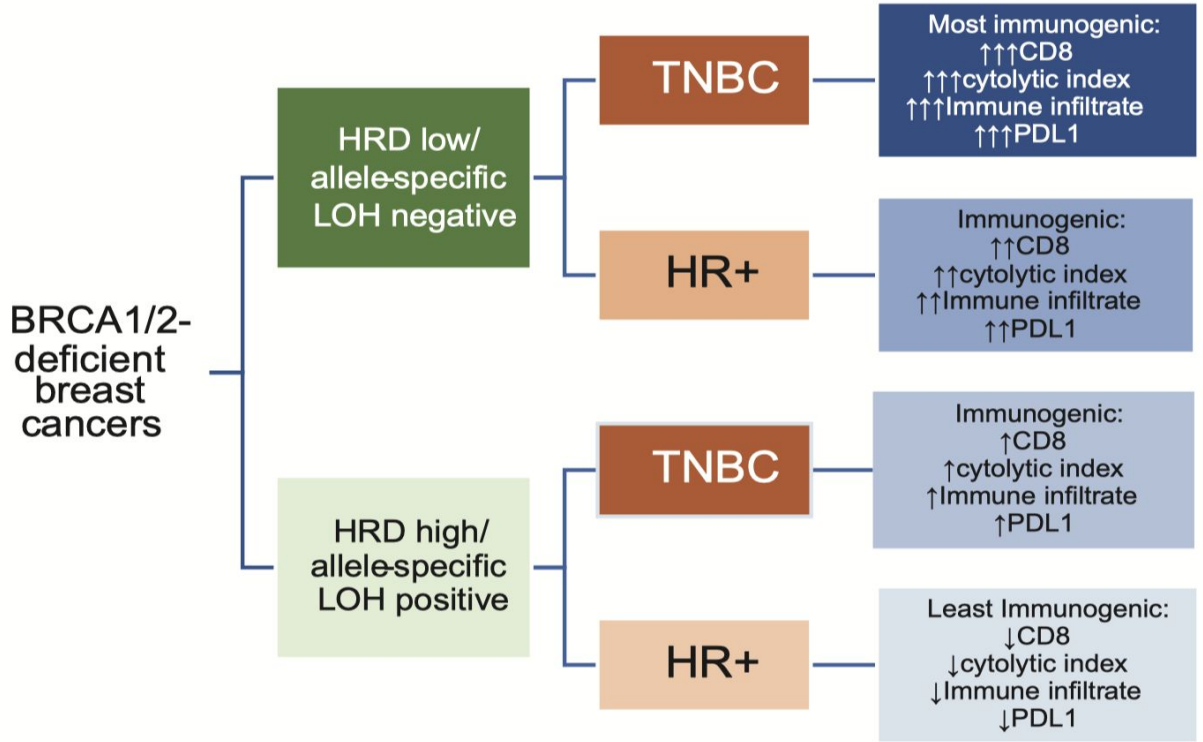
Figure4. Hormone receptor expression and HRD jointly stratify BRCA1/2 breast cancer immunogenicity

- Investigated a potential interaction effect between HRD and hormone receptor statuses, performing a stratified comparison in BRCA1/2 TCGA tumors.

- Comparing cytolytic index and immune ESTIMATE in TCGA, found the greatest difference between TNBC HRD-low (n=10) and Rec+ HRD-high BRCA1/2 breast cancers (n=22) (p=0.0013)



Executive Summary



Conclusion

- HRD scores and hormone receptor subtype are predictive of immunogenicity in BRCA1/2 breast cancers

- May inform the design of optimal immune therapeutic strategies.
    - which can potentially guide treatment strategies utilizing DNA damaging agents and checkpoint blockade alone or in combination.

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