Classification of triple negative breast cancer (TNBC) by DNA damage immune response (DDIR) signature and homologous recombination deficiency (HRD) status: Analysis of SWOG S9313 adjuvant trial

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Introduction

• Triple negative breast cancer (TNBC) is heterogeneous.
• Systemic chemotherapy is recommended for most patients with early-stage TNBC.
• Chemotherapy response biomarkers:
  • Number of stromal tumor infiltrating lymphocytes (sTILs)\textsuperscript{1,2,5}
  • Homologous recombination deficiency (HRD)\textsuperscript{3,7}
  • Gene expression signatures
    • TNBC molecular subtype\textsuperscript{4}
    • Proliferation associated gene expression signatures\textsuperscript{5}
    • Immune signatures, including DDIR\textsuperscript{6}
  • PD-L1 expression\textsuperscript{8}
• The overlap and prognostic interaction between HRD, sTILs, immune signatures, and molecular subtype in early stage TNBC has not been examined.

\textsuperscript{1}Denkert et al., Lancet Oncology, 2018; \textsuperscript{2}Loi et al., JCO, 2019; \textsuperscript{3}Telli et al., ASCO (2018); \textsuperscript{4}Masuda et al., Clin Cancer Res, 2013; \textsuperscript{5}Stover et al., Clin Cancer Res, 2016; \textsuperscript{6}Sharma et al., JCO, 2019; \textsuperscript{7}Sharma et al., Ann Oncol, 2018; \textsuperscript{8}Schmid, NEJM, 2020

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SWOG S9313

Design

Arm I (Concurrent)
High-Risk* Stage I-II Breast Cancer
+1 cm and ER/PR-negative, ≥2 cm (regardless of ER/PR expression), or 1-3 positive solitary nodes

Arm II (Sequential)

Doxorubicin: 54 mg/m² + Cyclophosphamide: 1.2 g/m² D1 Q3weeks x 6 cycles

Linden et al., JCO, 2007

Sharma et al., JCO, 2019

Median Age: 45 years
pN+: 33%
5-Year DFS: 74%
5-Year OS: 82%

13.6% of S9313 cohort
Defined as Allred = 0
ASCO-CAP Criteria

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Assessing impact of BRCAneus, Immune Markers, and Subtype on outcomes in TNBC Patients Treated with Adjuvant AC on S9313

- DNA Damage Immune Response (DDIR) signature (ALMAC Diagnostic Services)
  - FFPE samples analyzed by Xcel™ Array
  - 44 gene signature
  - DDIR+ = Score of ≥ 0.3681

- myChoice® HRD (Myriad Genetics)
  - Measurement of LOH, TAI, LST
  - Tumor BRCA1/2 mutation
  - HRD+ = HRD score of ≥ 42 or mutation in BRCA1 or BRCA2 detected in tumor

- sTIL quantification
  - Scored blindly by two breast pathologists

- TNBC Subtype
  - Microarray gene expression analysis
  - gDNA NGS (pending)

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Assay | Available (%)
--- | ---
DDIR | 381/425 (89.6)
HRD | 363/425 (85.4)
sTIL | 423/425 (99.5)

All three markers available for 328/425 (77%)
HRD, DDIR, sTIL, and Subtype are Prognostic in TNBC

**HRD**
- **tBRCA1/2 Mutation**
  - 67.3% Positive
  - 25.5% Negative
- 97% of tBRCA1/2 mutation with HRD score > 42

**DDIR Signature**
- 62.5% Positive

**sTIL ≥ 20%**
- 43.2% Positive

**Subtype**

DFS defined as time from registration to first invasive recurrence (local/regional/distant), new primary contralateral invasive cancer, or death from any cause.

*Continuous comparison
**Categorical comparison (by threshold)

HRD and tBRCA1/2 Mutation are Associated with Induction of DDIR but not with sTIL Infiltration

**HRD**
- tBRCA1/2 Mutation
  - P=0.0062**
P=0.0027*

**tBRCA1/2 Mutation**
- HRD Status
  - P=0.0092**
P=0.0196*

What is the prognostic utility of dual classification of TNBC by DDIR and HRD status?
Classification by DDIR and HRD status

DDIR+/HRD+

DDIR+/HRD-

DDIR-/HRD+

DDIR-/HRD-

Molecular subtypes distribution within DDIR-HRD classes

Immunomodulatory (IM+)

Combined DDIR-HRD Classes are Prognostic
DDIR-HRD Class and Immune Cell Infiltration

<table>
<thead>
<tr>
<th>DDIR/HRD Class</th>
<th>Median sTIL</th>
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</thead>
<tbody>
<tr>
<td>DDIR+/HRD+</td>
<td>20%</td>
</tr>
<tr>
<td>DDIR+/HRD-</td>
<td>20%</td>
</tr>
<tr>
<td>DDIR-/HRD+</td>
<td>5%</td>
</tr>
<tr>
<td>DDIR-/HRD-</td>
<td>5%</td>
</tr>
</tbody>
</table>

DDIR+/HRD+ vs. DDIR-/HRD-:
P < 0.05 (Enriched in DDIR+)

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Gene Expression Signature Cluster Analysis Based on DDIR and HRD Status
IO Signatures Dominate DDIR+ TNBC and EMT Signatures Dominate DDIR- TNBC

Signature scores generated by clara© analysis (ALMAC)

Mariathasan et al., Nature, 2018; Tescchendorff et al., BMC Cancer, 2010; Yuan et al., Scientific Reports, 2015

Genomic Instability and Metabolism Signatures Distinguish DDIR-/HRD+ and DDIR-/HRD- TNBC

Signature scores generated by clara© analysis (ALMAC)

Severson et al., Breast Cancer Res, 2017; Knijnenburg et al., Cell Reports, 2018

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Conclusions

- Immune activation and DNA repair deficiency have incomplete overlap in TNBC
- Classification of TNBC by DDIR and HRD status identifies biology-driven prognostic categories
- Immunologic and DNA repair-mediated therapeutic vulnerabilities may be independent
  - Both contribute to favorable outcomes noted in DDIR+/HRD+ subgroup
- Immunologically “cold” DDIR-/HRD+ subgroup does not have poor outcome
  - Rescued by sensitivity to DNA damaging chemotherapy
- These findings should be validated in other cohorts
Conclusions

Future questions:

• What mechanisms suppress DNA damage-induced anti-tumor immunity in DDIR-/HRD+ tumors?
• Can DNA repair deficiency be induced in immune activated DDIR+/HRD- tumors?
• Classification of TNBC by DDIR and HRD has potential therapeutic implications

<table>
<thead>
<tr>
<th>DDIR/HRD Category</th>
<th>Prognosis</th>
<th>Features</th>
<th>Therapeutic Opportunities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>sTIL Infiltration</td>
<td>BRCAness &amp; DNA Repair Deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune Activation (ICI) Target Expression</td>
<td></td>
</tr>
<tr>
<td>DDIR+/HRD+</td>
<td>Favorable</td>
<td>✓</td>
<td>(7) De-intensified chemotherapy</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>DNA damaging agents</td>
</tr>
<tr>
<td>DDIR+/HRD-</td>
<td>Favorable</td>
<td>✓</td>
<td>Immunotherapy</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td>(7) Intensified chemotherapy (ADC)</td>
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<tr>
<td>DDIR-/HRD+</td>
<td>Favorable</td>
<td>X</td>
<td>DNA damaging agents and PARPi</td>
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<td>Intermediate</td>
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<tr>
<td>DDIR-/HRD-</td>
<td>Unfavorable</td>
<td>X</td>
<td>Novel agents</td>
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• Patients and their families
• Participating sites
  • Physicians, nurses, and research coordinators
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