Supplemental Online Content


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This supplemental material has been provided by the authors to give readers additional information about their work.
RNA-seq data acquisition, quality control, and processing

Total RNA was extracted and underwent whole transcriptome RNA sequencing (RNAseq) on an Illumina HiSeq 3000 with single end 50bp reads using RiboZero Gold rRNA depletion. Samples with >10 million unique reads were included for further analyses as Reads per Kilobase per Million Reads (RPKM). RNA-seq reads were aligned to the Ensembl release 76 top-level assembly with STAR version 2.0.4b. Gene counts were derived from the number of uniquely aligned unambiguous reads by Subread:featureCount version 1.4.5. Transcript counts were produced by Sailfish version 0.6.3. Sequencing performance was assessed for total number of aligned reads, total number of uniquely aligned reads, genes and transcripts detected, ribosomal fraction known junction saturation and read distribution over known gene models with RSeQC version 2.3.

All gene-level and transcript counts were then imported into the R/Bioconductor package EdgeR and TMM normalization size factors were calculated to adjust samples for differences in library size, resulting in RPKM which were used in downstream analyses. Genes or transcripts not expressed in any sample or less than one count-per-million in the minimum group size minus one were excluded from further analysis. The TMM size factors and the matrix of counts were then imported into R/Bioconductor package Limma and weighted likelihoods based on the observed mean-variance relationship of every gene/transcript and sample were then calculated for all samples with the voomWithQualityWeights function. Performance of the samples was assessed with a spearman correlation matrix and multi-dimensional scaling plots. Gene/transcript performance was assessed with plots of residual standard deviation of every gene to their average log-count with a robustly fitted trend line of the residuals.

PAM50 intrinsic breast cancer subtype and PAM50 ‘proliferation signature’ were determined using the ‘Bioclassifier’ package.1 TNBC subtype was determined using the TNBCtype tool after normalization to fixed upper quantile.2,3 The GeparSixto ‘immune signature’ was calculated as the median expression/RPKM of seven genes (CXCL9, CD8A, CCL5, CXCL13, CD21, FOXP3, CD80) associated with TILs in the GeparSixto
clinical trial. The proportion of infiltrating immune cell subsets was calculated using the CIBERSORT and TIMER algorithms. Data visualization was performed using ggplot2.
**eTable.** Population characteristics overall and by planned therapy arm

<table>
<thead>
<tr>
<th>Planned administration schedule</th>
<th>Overall</th>
<th>Arm A</th>
<th>Arm B</th>
<th>Arm C</th>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
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<tr>
<td>Every 2 weeks</td>
<td>271</td>
<td>56.2</td>
<td>130</td>
<td>54.9</td>
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<tr>
<td>Every 3 weeks</td>
<td>211</td>
<td>43.8</td>
<td>107</td>
<td>45.2</td>
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<table>
<thead>
<tr>
<th>BRCA status</th>
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<tr>
<td>BRCA1 and/or BRCA2 mutation</td>
<td>75</td>
<td>15.6</td>
<td>39</td>
<td>16.5</td>
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<td>No mutation</td>
<td>407</td>
<td>84.4</td>
<td>198</td>
<td>83.5</td>
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<table>
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<th>Lymph node stage</th>
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<tr>
<td>N0</td>
<td>274</td>
<td>56.9</td>
<td>133</td>
<td>56.1</td>
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<td>N1-2</td>
<td>208</td>
<td>43.2</td>
<td>104</td>
<td>43.9</td>
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<tr>
<td>0</td>
<td>427</td>
<td>88.6</td>
<td>205</td>
<td>86.5</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>11.4</td>
<td>32</td>
<td>13.5</td>
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<table>
<thead>
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<th>Pathologic response status</th>
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<tbody>
<tr>
<td>Residual disease</td>
<td>246</td>
<td>51.0</td>
<td>110</td>
<td>46.4</td>
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<tr>
<td>Complete response</td>
<td>236</td>
<td>49.0</td>
<td>127</td>
<td>53.6</td>
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<table>
<thead>
<tr>
<th>PAM50 subtype</th>
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<tbody>
<tr>
<td>Basal</td>
<td>386</td>
<td>80.1</td>
<td>191</td>
<td>80.6</td>
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<tr>
<td>Nonbasal</td>
<td>96</td>
<td>19.9</td>
<td>46</td>
<td>19.4</td>
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<table>
<thead>
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<th>TNBC subtype</th>
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<tbody>
<tr>
<td>BL1</td>
<td>79</td>
<td>16.4</td>
<td>42</td>
<td>17.7</td>
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<tr>
<td>BL2</td>
<td>33</td>
<td>6.9</td>
<td>21</td>
<td>8.9</td>
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<tr>
<td>IM</td>
<td>108</td>
<td>22.4</td>
<td>53</td>
<td>22.4</td>
</tr>
<tr>
<td>LAR</td>
<td>29</td>
<td>6.0</td>
<td>12</td>
<td>5.1</td>
</tr>
<tr>
<td>M</td>
<td>102</td>
<td>21.2</td>
<td>48</td>
<td>20.3</td>
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<tr>
<td>MSL</td>
<td>51</td>
<td>10.6</td>
<td>26</td>
<td>11.0</td>
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<tr>
<td>Unselected</td>
<td>80</td>
<td>16.6</td>
<td>35</td>
<td>14.8</td>
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Abbreviations: BL1, basal-like 1; BL2, basal-like 2; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IM, immunomodulatory; LAR, luminal androgen receptor; M, mesenchymal; MSL, mesenchymal stem-like; TNBC, triple-negative breast cancer
eFigure 1. Triple-Negative Breast Cancer Expression-Based Subtypes and Response to Neoadjuvant Chemotherapy. **A.** Percent pathologic complete response across all arms by PM50 intrinsic subtype;\(^1,8\) error bars denote 95% confidence intervals based on normal approximation. **B.** Percent pathologic complete response across all arms by TNBC subtype;\(^2,9\) error bars denote 95% confidence intervals based on normal approximation.

**A**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Basal</th>
<th>Her2Like</th>
<th>LumA</th>
<th>LumB</th>
<th>Normal</th>
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<tbody>
<tr>
<td>pCR n/Total n</td>
<td>202/386</td>
<td>8/23</td>
<td>2/18</td>
<td>1/3</td>
<td>23/52</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>BL1</th>
<th>BL2</th>
<th>IM</th>
<th>LAR</th>
<th>M</th>
<th>MSL</th>
<th>UNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR n/Total n</td>
<td>40/79</td>
<td>15/33</td>
<td>70/109</td>
<td>8/29</td>
<td>38/102</td>
<td>28/51</td>
<td>38/80</td>
</tr>
</tbody>
</table>

\(52.3\%\)  \(34.8\%\)  \(11.1\%\)  \(33.3\%\)  \(44.2\%\)  \(50.6\%\)  \(45.5\%\)  \(64.2\%\)  \(27.6\%\)  \(37.3\%\)  \(54.9\%\)  \(47.5\%\)
eFigure 2. Proliferation and immune signatures by expression-based subtypes. (A-B): PAM50 proliferation signature was determined as part of the BioClassifier package and visualized by PAM50 subtype (A) or Lehmann/Pietenpol TNBC subtype (B). (C-D) The GeparSixto ‘immune signature’ was calculated as the median expression of seven genes (CXCL9, CD8A, CCL5, CXCL13, CD21, FOXP3, CD80) positively associated with TILs in the GeparSixto clinical trial for all available samples and visualized by PAM50 subtype (C) or Lehmann/Pietenpol TNBC subtype (D).
eFigure 3. Distribution of Proliferation and Immune Signatures and Response to Neoadjuvant Chemotherapy for All Patients. The ‘proliferation signature’ was calculated from a subset of PAM50-related genes as part of PAM50 classification. The GeparSixto ‘immune signature’ was calculated as the median expression of seven genes (CXCL9, CD8A, CCL5, CXCL13, CD21, FOXP3, CD80) positively associated with TILs in the GeparSixto clinical trial. The distribution of tumors by proliferation signature score (y-axis) and immune signature score (x-axis), with pathologic complete response (black dots) and residual disease (grey dots) indicated for all patients in the study. Proportion of pathologic complete response (pCR) for each quartile defined as above/below median for each score are indicated in each corner.
eFigure 4. Immune Signatures and Response to Neoadjuvant Chemotherapy. A. Distribution of TIMER subsets (z-score) by GeparSixto immune signature score. Line of best fit indicated. B-E. Forest plots of likelihood of pathologic complete response by treatment arm (A+B: AC-T plus carboplatin with or without veliparib) versus (C: AC-T only) by B. GeparSixto immune signature score and TIMER subsets; C. CIBERSORT subsets, determined as sum of relative proportion of each cell type; D. CIBERSORT macrophage subsets. AC-T: doxorubicin, cyclophosphamide, and paclitaxel.
eFigure 5. Exploratory Immunophenotype Analyses of Immune High versus Immune Low Triple-Negative Breast Cancers. A. Schematic figure of Gene Set Enrichment Analyses comparing GeparSixto immune signature 'high' (above median) versus 'low' (below median) in Arms A/B and, independently, Arm C. Immune GSEA focused on 96 signatures from Immune Response in Silico/ImmuneSigDB. Higher NES demonstrates enrichment in ‘immune high’ tumors. Negative log10 FDR normalized enrichment score (NES) for each of the 48 ‘up’ signatures in Immune Response in Silico/ImmuneSigDB. Higher NES demonstrates enrichment in ‘immune high’ tumors. Negative log10 FDR q-value indicated in bar graph, right panel. C. Two signatures with the highest NES in Arms A/B (left panels) and Arm C (right panels). D. Top 50 individual genes most highly enriched in ‘immune high’ tumors. Venn diagram indicates overlap between Arms A/B and Arm C.
eFigure 5. (continued)

C

Arms A/B
AC-T + Carbo +/- Veliparib

Arm C
AC-T only

NES = 2.47
FDR q = 0

NES = 2.59
FDR q = 0

NES = 2.59
FDR q = 0

NES = 2.45
FDR q = 0

D

Arm AB

Arm C

CTLA4
LINC00426
SNX20
D2Z
FASLG
ZC3H12D
CD48
TBX21
TRIT1
APOBEC3H
ZC3H12L
CD3E
CD3D
CD8A
CXCR3
SP140
IL12RB1
BSIPG
ST11
HLA1
SLAMF6
LZRB2
ZC3H12L
CD96
GZMB
TAC1
QRPR174
SLAMF1
GZMB
PTPN7
UBASH3A
ZBED2
UBASH3A
ZAP70
LCK
SLAMF7
P2RY10
CD38
PYHIN1
CD274

CTLA4
LINC00426
SNX20
D2Z
FASLG
ZC3H12D
CD48
TBX21
TRIT1
APOBEC3H
ZC3H12L
CD3E
CD3D
CD8A
CXCR3
SP140
IL12RB1
BSIPG
ST11
HLA1
SLAMF6
LZRB2
ZC3H12L
CD96
GZMB
TAC1
QRPR174
SLAMF1
GZMB
PTPN7
UBASH3A
ZBED2
UBASH3A
ZAP70
LCK
SLAMF7
P2RY10
CD38
PYHIN1
CD274
eReferences