Genomic Profiling of PAM50 Based Intrinsic Subtypes in HR+, HER2– Advanced Breast Cancer Across the MONALEESA Studies

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Introduction

• The phase 3 MONALEESA (ML) trials (ML-2, -3, and -7) reported statistically significant progression free survival (PFS) and overall survival (OS) benefit with ribociclib plus endocrine therapy (ET) compared with ET alone in patients with hormone receptor positive, human epidermal growth factor receptor 2 negative (HR+, HER2–) advanced breast cancer (ABC)\textsuperscript{1-4}

• A retrospective exploratory analysis of primary and metastatic tumor samples from these trials found that PAM50 based intrinsic subtypes, including, luminal A (LumA), luminal B (LumB), HER2-enriched (HER2E), and Basal-like, were prognostic and predictive of ribociclib PFS and OS benefit\textsuperscript{5,6}

• A consistent PFS and OS benefit with ribociclib was observed across subtypes (except for the Basal-like subtype), including the HER2E subtype, which is typically associated with ET resistance and poor outcomes\textsuperscript{5-9}

• Here, we report the results of genomic profiling of baseline circulating tumor DNA (ctDNA) by PAM50 based intrinsic subtype across the ML studies
A total of 883 of 2066 patients recruited in the ML-2, -3, and -7 phase 3 trials had both tumor (primary, 70%; metastatic, 30%) intrinsic subtype defined by PAM50 and plasma ctDNA next-generation sequencing (NGS)-based data obtained at baseline (ie, before starting treatment) - Patients in both first- and second-line settings of ML-3 were included in the analysis

The NGS-based panel targeted exonic regions in approximately 550 genes sequenced on an Illumina HiSeq instrument

A total of 130 patients had the normal-like subtype and were excluded from this analysis

We assessed the differences in frequency across intrinsic subtypes for genes that were altered in > 5% of patients - Genetic alterations included mutations, indels, and copy number alterations
For each gene, a Fisher exact test was used to test for differences in frequency across the subtypes. A false discovery rate (FDR) correction was used to adjust for multiple testing; for genes with FDR < 0.10, a logistic regression model was used to quantify the relationship between subtypes and alteration status. We evaluated differences across subtypes for tumor mutational burden (TMB) using analysis of variance. The Kruskal-Wallis test was used to measure the differences across subtypes for ctDNA fraction (an estimate of the amount of tumor DNA in the circulation and indicative of prognosis).
Results (1 of 9)

Differences in baseline genomic profiles were observed across intrinsic subtypes

- A total of 396 patients (44.8%) had LumA subtype, 210 (23.8%) had LumB subtype, 123 (13.9%) had HER2E subtype, and 24 (2.7%) had Basal-like subtype

- Gene amplifications were more frequent in the LumB, HER2E, and Basal-like subtypes than in the LumA subtype (Figure 1)

Figure 1A. Oncoprints of Genes Altered in > 5% of patients by Intrinsic Subtype (1 of 3)

BOR, best overall response; HER2E, HER2-enriched; LumA, luminal A; LumB, luminal B.
Results (2 of 9)

Figure 1B. Oncoprints of Genes Altered in > 5% of patients by Intrinsic Subtype

BOR, best overall response; CR, complete response; LumB, luminal B; NCRNPD, neither complete response nor progressive disease; PD, progressive disease; PR, partial response; SD, stable disease; UNK, unknown.
**Results (3 of 9)**

Figure 1C&D. Oncoprints of Genes Altered in > 5% of patients by Intrinsic Subtype (3 of 3)

BOR, best overall response; HER2E, HER2-enriched.
Results (4 of 9)

Several genes showed differences in baseline alteration frequency across intrinsic subtypes

- **CCND1** region (FGF3/4/19, ANO1), **FGFR1** region (WHSC1L1, ZNF703, ADGRA2, TACC1), **MYC** region (ATAD2, RAD21), **PIK3CA**, **TP53**, and **SPEN**, showed differences in alteration frequency across subtypes (Table 1, Figure 2)

- **CCND1** alterations were more frequent in HER2E (14.6%) and LumB (14.3%) subtypes than in the LumA (4.8%) subtype (Table 1, Figure 2)

- **FGFR1** and **MYC** alterations were more frequent in HER2E (13% and 9.8%), Basal-like (12.5% and 12.5%), and LumB (8.6% and 10%) subtypes than in the LumA (3.3% and 2.3%) subtype (Table 1, Figure 2)

- **PIK3CA** alterations, including hotspot somatic mutations, were less frequent in the Basal-like (12.5%) than in the LumA subtype (33.8%) (Table 1, Figure 2)

- **TP53** was more frequently altered in Basal-like (66.7%) and HER2E (29.3%) subtypes than LumA (12.4%) subtype (Table 1, Figure 2)

- **SPEN** alterations were more frequent in Basal-like (16.7%) than in LumA (5.1%) subtype (Table 1, Figure 2)
Results (5 of 9)

Table 1. Genes with differences (FDR < 0.10) in alteration frequency in ctDNA across the intrinsic subtypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Subtype</th>
<th>% Altered</th>
<th>OR (95%CI)</th>
<th>P Valuea</th>
<th>Fisher P valueb</th>
<th>FDR-Adjusted Fisher P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1</td>
<td>HER2E</td>
<td>14.6</td>
<td>3.25 (1.63-6.48)</td>
<td>.00076</td>
<td>&lt;.0001</td>
<td>.00023</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>14.3</td>
<td>3.25 (1.79-6.03)</td>
<td>.00013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>8.3</td>
<td>1.65 (0.25-6.26)</td>
<td>.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR1</td>
<td>HER2E</td>
<td>13.0</td>
<td>4.41 (2.04-9.68)</td>
<td>.0016</td>
<td>.00028</td>
<td>.00062</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>8.6</td>
<td>2.71 (1.31-5.78)</td>
<td>.0079</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>12.5</td>
<td>4.00 (0.86-13.77)</td>
<td>.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>HER2E</td>
<td>9.8</td>
<td>4.18 (1.71-10.58)</td>
<td>.0018</td>
<td>&lt;.0001</td>
<td>.00012</td>
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<tr>
<td></td>
<td>LumB</td>
<td>10.0</td>
<td>4.58 (2.11-10.76)</td>
<td>.0021</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>12.5</td>
<td>5.03 (1.04-18.71)</td>
<td>.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>HER2E</td>
<td>37.4</td>
<td>1.21 (0.79-1.84)</td>
<td>.38</td>
<td>.035</td>
<td>.059</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>27.6</td>
<td>0.78 (0.52-1.09)</td>
<td>.14</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Basal-like</td>
<td>12.5</td>
<td>0.30 (0.07-0.89)</td>
<td>.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>33.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>HER2E</td>
<td>29.3</td>
<td>2.92 (1.77-4.79)</td>
<td>.00022</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>16.2</td>
<td>1.38 (0.85-2.22)</td>
<td>.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>66.7</td>
<td>14.55 (6.02-37.88)</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>12.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPEN</td>
<td>HER2E</td>
<td>8.9</td>
<td>1.87 (0.84-3.99)</td>
<td>.11</td>
<td>.018</td>
<td>.032</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>3.3</td>
<td>0.66 (0.26-1.53)</td>
<td>.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>16.7</td>
<td>4.03 (1.08-12.18)</td>
<td>.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HER2E, HER2-enriched; LumA, luminal A; LumB, luminal B.

aOdds ratio and P value from a logistic regression model of alteration status and subtype (LumA as reference group).

bP value from Fisher exact test evaluating differences in frequency across the subtypes (unadjusted).

cFalse discovery rate-adjusted P value from Fisher exact test.

dSimilar results were obtained for FGFR3/4/19 and ANO1.

eSimilar results were obtained for WHSC1L1, ZNF703, ADGRA2, and TACC1.

fSimilar results were obtained for ATAD2 and RAD21.
Results (6 of 9)

Several genes showed differences in baseline alteration frequency across intrinsic subtypes

- ESR1 did not show any significant difference across subtypes (Figure 2)

Figure 2. Differences in Gene Alteration Frequencies in ctDNA Across Intrinsic Subtypes
Results (7 of 9)

**ERBB2** alteration frequency was similar across LumA, LumB, and HER2E subtypes

- ERBB2 alterations (n = 25), which were mainly missense mutations (n = 22), were observed at similar frequencies (3%–4%) across the LumA, LumB, and HER2E subtypes (Table 2).

- No ERBB2 alterations were detected in the Basal-like subtype (Table 2).

### Table 2. ERBB2 Alteration Frequency Across Intrinsic Subtypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Subtype</th>
<th>n</th>
<th>Altered, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2</td>
<td>HER2E</td>
<td>123</td>
<td>5 (4.1)</td>
</tr>
<tr>
<td></td>
<td>LumB</td>
<td>210</td>
<td>8 (3.8)</td>
</tr>
<tr>
<td></td>
<td>Basal-like</td>
<td>24</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>LumA</td>
<td>396</td>
<td>12 (3.0)</td>
</tr>
</tbody>
</table>

HER2E, HER2-enriched; LumA, luminal A; LumB, luminal B.
Results (8 of 9)

TMB was similar across intrinsic subtypes

- No significant difference in TMB was observed across subtypes ($P = .20$), including when a cutoff of $\geq 10$ was used ($P = .23$) (Figure 3)
ctDNA fraction differed across intrinsic subtypes

- Differences in ctDNA fraction were observed across subtypes ($P < .001$) (Figure 4)
  - Compared with LumA, ctDNA fraction was significantly higher for patients with LumB ($P < .001$) and for those with HER2E ($P < .001$)
Conclusions

These findings are the first combined report of baseline ctDNA NGS profiling and intrinsic molecular subtype in patients with ABC

- Although ctDNA samples were collected at baseline, the intrinsic subtype data may have been driven by the large proportion of primary tumor samples (70%) in this analysis

- Differences in ctDNA profiles were observed across intrinsic subtypes; there was a trend for higher copy number alterations in HER2E and LumB subtypes than in the LumA subtype

- LumA and Basal-like subtypes showed the most distinct genomic features among the subtypes

- From a clinical and biological perspective, the Basal-like subtype is known to be similar to triple-negative BC, which may explain the limited activity of ribociclib in this subgroup, as shown previously; however, these results should be interpreted with caution due to the limited sample size of this subgroup

- The consistent benefit of ribociclib observed in the luminal subtypes and the HER2E subtype, particularly in the LumB and HER2E subtypes which are enriched with somatic alterations associated with ET resistance and tend to show worse prognosis, warrants further investigation
References

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