Upregulation of Immune Response Biomarkers by Ribociclib Plus Endocrine Therapy in Paired Tumor Samples From Phase I Studies

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Introduction

- While cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors function as inhibitors of cell cycle progression, preclinical studies have revealed a role for CDK4/6 inhibitors in anticancer immunity through interferon response triggering, increased antigen presentation, and regulatory T-cell suppression.

- To explore the effect of ribociclib (RIB) on immune modulation in a clinical setting, a gene expression analysis was performed using paired biopsies from 2 phase I clinical trials (CLEE011A2115C [A2115C], CLEE011X2107 [X2107]) that studied RIB in combination with endocrine therapy (ET) in hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−) metastatic breast cancer (MBC).
Methods (1 of 3)

- A2115C enrolled Asian patients with HR+/HER2– MBC
  - Cohort A enrolled postmenopausal non-Japanese Asian patients in dose-escalation and -expansion phases. Patients were treated with RIB + letrozole (LET)
  - Cohort B enrolled pre- and postmenopausal Japanese patients in dose-escalation and -expansion phases. Patients were treated with RIB + LET in the dose-escalation phase and with RIB + ET (LET, tamoxifen + goserelin, or fulvestrant) in the dose-expansion phase
  - Prior ET or chemotherapy (CT) for advanced breast cancer (ABC) was not permitted except for patients in cohort B who were treated with RIB + fulvestrant (1 prior ET for ABC allowed)
Methods (2 of 3)

• X2107 enrolled postmenopausal patients with HR+/HER2– MBC
  – Patients were recruited to dose-escalation and -expansion phases
  – In the dose-escalation phase, any number of prior lines of ET was permitted; cytotoxic therapy was limited to 1 prior line for ABC
  – In the dose-expansion phase, prior systemic therapy for ABC was not permitted
  – Patients were treated with RIB + LET (arm 1), alpelisib + LET (arm 2), RIB + alpelisib + LET (arm 3), or RIB + alpelisib + LET (arm 4)
  – Patients in arms 1 and 3 received RIB on a 3 weeks on/1 week off schedule, while those in arm 4 received RIB on a continuous schedule
• Tumor samples were collected at baseline and cycle 1 day 15 (C1D15)
• Best overall response (BOR) was evaluated, and patients were classified as having stable disease (SD), partial response (PR), progressive disease (PD), or unknown (UNK)
Methods (3 of 3)

• The nCounter PanCancer IO 360 Panel (NanoString) was used to quantify expression of 770 genes in paired tumor samples from A2115C and X2107

• Pairwise differential gene expression analysis of individual genes and previously published immune-related gene signatures was conducted\(^2\)
Results (1 of 12)

Patients and BOR

- This analysis included 7 patients (Table 1)
  - 5 patients from A2115C
  - 2 patients from X2107
- BORs were: 1 patient with PR, 2 patients with SD, 2 patients with PD, and UNK for 2 patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Combination and Dose</th>
<th>Cohort/Arm</th>
<th>BOR</th>
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<tr>
<td>A2115C</td>
<td>RIB 600 mg + LET 2.5 mg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A</td>
<td>SD</td>
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<tr>
<td>A2115C</td>
<td>RIB 600 mg + LET 2.5 mg&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>B</td>
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<tr>
<td>X2107</td>
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<td>1</td>
<td>PR</td>
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</tbody>
</table>

<sup>a</sup>Dose-expansion phase.
BOR, best overall response; LET, letrozole; PD, progressive disease; PR, partial response; RIB, ribociclib; SD, stable disease; TAM, tamoxifen; UNK, unknown.

Table 1. Patients included in this analysis

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Results (2 of 12)

Gene expression profile by BOR

Pairwise differential gene expression at baseline and C1D15 by BOR is shown in Figure 1

Figure 1. Pairwise differential gene expression profile

Each pixel represents the log2 ratio of differential gene expression between C1D15 and baseline.

BS, baseline; BOR, best overall response; C1D15, cycle 1 day 15; PD, progressive disease; PR, partial response; SD, stable disease; UNK, unknown.
Results (3 of 12)

Significant downregulation of proliferation gene signature with RIB + ET

• Expression of a gene signature related to proliferation was suppressed with RIB + ET (Figure 2A)
  – Expression decreased from baseline to C1D15 in patients who had a clinical benefit with RIB + ET (Figure 2B)

• A similar trend was observed for individual genes within the proliferation gene signature (Figure 2C)
Results (4 of 12)

Figure 2. Proliferation gene signature significantly downregulated with RIB + ET (1 of 2)

A. Gene signature associated with proliferation

B. Expression of gene signature by clinical benefit

FDR = 1.65e-08

Each pixel represents the log2 ratio of differential gene expression between C1D15 and baseline.

BS, baseline; BOR, best overall response; C1D15, cycle 1 day 15; CCNB1, cyclin B1; ET, endocrine therapy; FANCA, FA complementation group A; FDR, false discovery rate; MKI67, marker of proliferation Ki-67; MYC, MYC proto-oncogene; PD, progressive disease; PR, partial response; RAD50, double-strand break repair; RIB, ribociclib; RRM2, ribonucleotide reductase, regulatory subunit M2; SD, stable disease; TYMS, thymidylate synthetase; UNK, unknown.
Results (5 of 12)

Figure 2. Proliferation gene signature significantly downregulated with RIB + ET (2 of 2)

C. Expression of genes within proliferation signature by clinical benefit

C1D15, cycle 1 day 15; CCNB1, cyclin B1; ET, endocrine therapy; FANCA, FA complementation group A; MKI67, marker of proliferation Ki-67; MYC, MYC proto-oncogene; RAD50, double-strand break repair; RIB, ribociclib; RRM2, ribonucleotide reductase, regulatory subunit M2; TYMS, thymidylate synthetase; UNK, unknown.
Results (6 of 12)

Significant upregulation of T-cell inflammation gene signature with RIB + ET

- RIB + ET increased expression of a gene signature associated with immune response and indicative of a T-cell–inflamed microenvironment (Figure 3A)
  - Two of 3 patients who experienced a clinical benefit with RIB + ET had an increase in expression from baseline to C1D15 (Figure 3B)
  - Two patients who experienced no clinical benefit with RIB + ET had decreased expression from baseline to C1D15 (Figure 3B)

- A similar trend was observed for individual genes within the T-cell–inflamed gene signature (Figure 3C)

C1D15, cycle 1 day 15; ET, endocrine therapy; RIB, ribociclib.
Results (7 of 12)

Figure 3. T-cell inflammation gene signature significantly upregulated with RIB + ET (1 of 2)

A. Gene signature associated with T-cell–inflamed microenvironment

B. Expression of gene signature by clinical benefit

FDR = 9.43e-03

Each pixel represents the log2 ratio of differential gene expression between C1D15 and baseline

BS, baseline; BOR, best overall response; C1D15, cycle 1 day 15; CCL5, C-C motif chemokine ligand 5; CD3D, CD3d molecule; CXCL10, C-X-C motif chemokine ligand 10; ET, endocrine therapy; FDR, false discovery rate; HLA-DRA, major histocompatibility complex, class II, DR alpha; HLA-E, major histocompatibility complex, class I, E; IL2RG, interleukin 2 receptor subunit gamma; NKG7, natural killer cell granule protein 7; PD, progressive disease; PR, partial response; RIB, ribociclib; SD, stable disease; STAT1, signal transducer and activator of transcription 1; UNK, unknown.
Results (8 of 12)

Figure 3. T-cell inflammation gene signature significantly upregulated with RIB + ET (2 of 2)

C. Expression of genes within proliferation signature by clinical benefit

C1D15, cycle 1 day 15; CCL5, C-C motif chemokine ligand 5; CD3D, CD3d molecule; CXCL10, C-X-C motif chemokine ligand 10; ET, endocrine therapy; HLA-DRA, major histocompatibility complex, class II, DR alpha; HLA-E, major histocompatibility complex, class I, E; IL2RG, interleukin 2 receptor subunit gamma; NKG7, natural killer cell granule protein 7; RIB, ribociclib; STAT1, signal transducer and activator of transcription 1; UNK, unknown.
Results (9 of 12)

Expression of proliferation, cell cycle regulation, T-cell marker, and immune checkpoint genes

- Lower expression of BRCA2, CENPF, BIRC5, CCND1, CKD2, CDKN1A, and DNMT1 was observed with RIB + ET (Figure 4)
- Expression of LAG3 and HAVCR2 increased, while CD4 expression remained similar with RIB + ET (Figure 5)
Results (10 of 12)

Figure 4. Expression of genes associated with proliferation and cell cycle regulation by clinical benefit

BIRC5, baculoviral IAP repeat containing 5; BRCA2, BRCA2 DNA repair associated; CCND1, cyclin D1; C1D15, cycle 1 day 15, CDK2, cyclin-dependent kinase 2; CDKN1A, cyclin-dependent kinase inhibitor 1A; CENPF, centromere protein F; DNMT1, DNA methyltransferase 1; UNK, unknown.
Figure 5. Expression of T-cell marker and immune checkpoint genes by clinical benefit

C1D15, cycle 1 day 15; CD4, CD4 molecule; HAVCR2, hepatitis A virus cellular receptor 2; LAG3, lymphocyte activating 3; UNK, unknown.
Results (12 of 12)

Limitations

• The sample size used in this analysis was small, and thus is considered hypothesis-generating and warrants further investigation

• Bulk RNA from tumor biopsies was used in this analysis; consequently, we could not distinguish whether changes in immune gene signatures reflected increased infiltration by immune cells or endogenous interferon response in cancer cells
  – The interferon response could be triggered by the observed suppression of DNMT1, which promotes T-cell–mediated immunity by increasing antigen presentation and chemokine production in cancer cells

DNMT1, DNA methyltransferase 1.
Conclusions

• Gene expression analysis was performed on patients from 2 phase I clinical trials of RIB + ET in MBC using paired biopsies from baseline and C1D15
  – Cell cycle and cell proliferation markers were robustly suppressed by RIB + ET, indicating on-target pharmacodynamic suppression by a CDK4/6 inhibitor
  – RIB + ET increased expression of immune-related genes, which occurred preferentially in patients who experienced favorable clinical outcomes

• This analysis is considered hypothesis generating and suggests that an immunomodulatory effect may contribute to the survival outcomes and carryover effects reported for the 3 phase III MONALEESA clinical trials; however, the sample size was small and further investigation is needed

• To the best of our knowledge, this is the first report of immune biomarker activation by a CDK4/6 inhibitor in the treatment of patients with HR+/HER2– MBC in clinical trials

C1D15, cycle 1 day 15; CDK4/6, cyclin-dependent kinase 4/6; ET, endocrine therapy; HER2–, human epidermal growth factor receptor 2-negative; HR+, hormone receptor-positive; MBC, metastatic breast cancer; RIB, ribociclib.
References

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