Metabolite Biomarkers of Response (BoRs) To Optimize Metastatic Breast Cancer Treatment

Cancer and Evolution Symposium
Elizabeth O’Day, MPhil, PhD
Oct. 16th, 2020
Disclosures

I am the CEO and Founder of Olaris, Inc. Olaris is a private company developing “Biomarkers of Response” (BoR) to optimize treatment decisions. I receive financial support and equity from the company.
Despite access to therapies, **less than 22% of metastatic breast cancer (mBC) patients** survive 5+ years.
**RESISTANCE** Leads to Poor Outcomes

**Today**
- ER+ metastatic BC patients are prescribed CDK4/6 inhibitors
- 20% of patients are *intrinsically* resistant
- All patients acquire resistance

**The Future**
- Each patient is screened prior to treatment
- "resistant" patients receive alternative treatment
- "responders" receive CDK4/6 inhibitor

**RESULTS**
- Poor outcomes
- Increased adverse effects
- Increased healthcare costs
- Improved outcomes
- Reduced adverse effects
- Reduced healthcare costs
Why Metabolites?

Responder (R)

DNA
RNA
Protein

R-Metabolites

Non-Responder (NR)

DNA
RNA
Protein

NR-Metabolites

Biomarkers:

**DNA/RNA:** what **could** happen

**Protein:** what **makes it** happen

**Metabolites:** what **is** happening
Factors Beyond Genetics Influence Drug Response

Metabolomics provides a fingerprint of disease
Not Your Grandfather’s Metabolomics

Pioneering methods using NMR and MS increase metabolome coverage in a highly **reproducible** manner. Proprietary **BoR** algorithm combines top features from multiple ML algorithms to create more **accurate** classifications.

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**OPLS-DA**  
**Random Forest**

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**Evaluation of Non-Uniform Sampling 2D $^1$H–$^{13}$C HSQC Spectra for Semi-Quantitative Metabolomics**

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Received: 20 March 2020; Accepted: 12 May 2020; Published: 16 May 2020

**Abstract:** Metabolomics is the comprehensive study of metabolism, the biochemical processes that sustain life. By comparing metabolites between healthy and disease states, new insights into disease mechanisms can be uncovered. NMR is a powerful analytical method to detect and quantify metabolites. Standard one-dimensional (1D) $^1$H-NMR metabolite profiling is informative but challenged by significant chemical shift overlap. Multi-dimensional NMR can increase resolution, but the required long acquisition times lead to limited throughput. Non-uniform sampling (NUS) is a well-accepted mode of acquiring multi-dimensional NMR data, enabling either reduced acquisition times or increased sensitivity in equivalent time. Despite these advantages, the technique is not widely applied to metabolomics. In this study, we evaluated the utility of NUS $^1$H–$^{13}$C heteronuclear single quantum coherence (HSQC) for semi-quantitative metabolomics. We demonstrated that NUS improved sensitivity compared to uniform sampling (US). We verified that the NUS measurement maintains linearity, making it possible to detect metabolite changes across samples and studies. Furthermore, we calculated the lower limit of detection and quantification (LOD/LOQ) of common metabolites. Finally, we demonstrate that the measurements are repeatable on the same system and across different systems. In conclusion, our results detail the analytical capability of NUS and, in doing so, empower the future use of NUS $^1$H–$^{13}$C HSQC in metabolomic studies.
Case Study: CDK4/6 BoR
Inhibiting the cell cycle is a long-standing strategy for cancer therapy
New “selective” CDK4/6 Inhibitors Block Cell Cycle Progression

<table>
<thead>
<tr>
<th>Structure</th>
<th>Drug</th>
<th>CDK IC50</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ibrance</td>
<td>CDK1: &gt;10μM</td>
</tr>
<tr>
<td></td>
<td>(palbociclib)</td>
<td>CDK2: &gt;10μM</td>
</tr>
<tr>
<td></td>
<td>Pfizer</td>
<td>CDK4: 9-11nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDK6: 15nM</td>
</tr>
<tr>
<td></td>
<td>Kisqali</td>
<td>CDK1: &gt;100μM</td>
</tr>
<tr>
<td></td>
<td>(ribociclib)</td>
<td>CDK2: &gt;50μM</td>
</tr>
<tr>
<td></td>
<td>Novartis</td>
<td>CDK4: 10nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDK6: 39nM</td>
</tr>
<tr>
<td></td>
<td>Verzenio</td>
<td>CDK1: &gt;1μM</td>
</tr>
<tr>
<td></td>
<td>(abemaciclib)</td>
<td>CDK2: &gt;500nM</td>
</tr>
<tr>
<td></td>
<td>Eli Lilly</td>
<td>CDK4: 2nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDK6: 5nM</td>
</tr>
</tbody>
</table>
Critical Need to Identify CDK4/6 NR

- **87% of Responders** [patients who saw tumors decrease within first 6 months of treatment] are **alive + 2 years**
- **90% of Non-Responders** [patients who saw tumors increase within first 6 months of treatment] are **deceased** within 12-15 months

## Current CDK4/6i Biomarkers Fail to Predict R vs NR


<table>
<thead>
<tr>
<th>Proposed Biomarker</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ positivity</td>
<td>In phase 1 study, abemaciclib monotherapy (N=47), only 11 of 36 ER+ patients experienced clinical benefit. In PALOMA-2 and PALOMA-3 trials, benefit from palbociclib did not differ by ER IHC expression.</td>
</tr>
<tr>
<td>Luminal gene expression, Rb status, Cyclin E/CDK2 amplification</td>
<td>Most breast cancer cell lines with luminal gene expression are sensitive to palbociclib (some are ER-). Cell lines with low Rb levels are less sensitive to CDK4/6 inhibitors. CDK2 can substitute for CDK4/6. Cyclin E and CDK2 can phosphorylate Rb to escape block. Each marker needs to be tested in clinic and can be difficult to measure and set “cut-offs”.</td>
</tr>
<tr>
<td>Cyclin D1 amplification and/or loss of p16</td>
<td>In PALOMA-1 (N=165) and separate phase II (N=37) neither cyclin D1 nor p16 were predictive of benefit or PFS with palbociclib</td>
</tr>
</tbody>
</table>
Protein Sialylation Regulates a Gene Expression Signature that Promotes Breast Cancer Cell Pathogenicity

Rebecca A. Kohnz, Lindsay S. Roberts, David DeTomaso, Lara Bideyan, Peter Yan, Sourav Bandyopadhyay, Andrei Goga, Nir Yosef, and Daniel K. Nomura
Metabolites Are Influenced by the environment


Metabolic Insights Can Uncover Disease Mechanisms

Learning From Current Patients To Help Future Patients

- Receive baseline (BL) and 2 months post treatment (2M) plasma sample
- Isolate metabolites
- Detect and quantify Jane’s & Jill’s metabolites using NMR & MS
- Correlate differential metabolites with clinical outcomes

- Jane on AI + CDK 4/6 inhibitor
  Jane’s tumor shrinks (R)
  N=15

- Jill on AI + CDK 4/6 inhibitor
  Jill’s tumor did not shrink (NR)
  N=9

Metabolic Profile of a Responder

Metabolic Profile of a Non-Responder
Identify differential grids for R vs. NR

Volcano plot for variables for comparison of NR vs. R.

Violin plots for variables with lowest p-values for comparison of NR vs. R.

146 R vs NR (p <0.05)
14 expected by chance
"Learning" from Machine Learning → Clear Box ML

**Perform predictive modeling**

**Identify most significant features**
CDK4/6 BoR Differentiates R vs. NR

CDK4/6 BoR differentiates R vs. NR with 95.2% predictive accuracy

We need to collaborate & conduct multi-site studies to uncover meaningful biomarkers

We are working with world-leading breast oncologists across the globe to validate & test CDK4/6 BoR
Why study metabolism?

Metabolism dictates phenotype

Healthy cell

DNA
RNA
Protein
Metabolites

Diseased cell from patient X

DNA
RNA
Protein
Metabolites

DNA/RNA: what could happen
Protein: what makes it happen
Metabolites: what is actually happening

Altered metabolism is linked to many common diseases

- Metabolites provide diagnostics
- Metabolic enzymes provide therapeutic targets
Future Of Medicines

Awesome New Drug

MoA: BoR:

BoR on every drug product
Acknowledgements

Bo Zhang, PhD
Chandra Honrao, PhD
Chen Dong
Srihari Rao
Dejan Juric, MD (MGH)
Christopher Pinto (MGH)
Sarah-Jane Dawson, MD, PhD (PeterMac)
Massimo Cristofanilli, MD (NW)
Qiang Zhang, MD, PhD (NW)
Lorenzo Gerratana, MD (NW)
Cynthia Ma MD, PhD (Washington University in St Louis)
Matt Goetz, MD (Mayo)
Ciara O’Sullivan, MB BCh/BAO (Mayo)
Yap Yoon Sim, MD (National Cancer Center Singapore)
Thank You

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Metabolites Are Powerful Biomarkers

Let’s avoid another vitamin C missed opportunity
Metabolites Are Powerful Biomarkers

**Cell**

**Metabolic Dynamics and Prediction of Gestational Age and Time to Delivery in Pregnant Women**

*Graphical Abstract*

- **Authors**
  - Liang Liang, Marie-Louise Hee Rasmussen
  - Brian Piening, ..., Hanya Mads Morsbrokov Hammelby

- **Correspondence**
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  - mmelbye@stanford.edu

- **In Brief**
  - Identification of blood metabolites in pregnant women that can predict gestational age and time to delivery insights into pregnancy outcomes undetected by ultrasound.

**Cell**

**Molecular Choreography of Acute Exercise**

*Graphical Abstract*

- **Authors**
  - Kevin Contrepois, Si Kegan J. Moneghetti
  - Francois Haddad, M

- **Correspondence**
  - fhaddad@stanford.edu
  - mpsnyder@stanford.edu

- **In Brief**
  - Longitudinal multi-omics characterization of the molecular physiology associated with acute exercise.

*Image Description*:
- Left panel: Diagram showing weekly blood sampling from pregnancy to childbirth with global metabolomic profiling.
- Right panel: Graphical abstract depicting molecular analysis and changes in metabolites, proteins, and transcripts during exercise and recovery phases.
BoR Report: Reliable Data For Clinicians

Example Patient Report

**ER+/HER2- Metastatic Breast Cancer BoR Report**

**Patient/ID:** Patient, 1603  
**Gender:** Female  
**Date of Birth:** 1/1/1956  
**Diagnosis:** metastatic ER+/HER2- BC

**Report #:**  
**Ordering Physician:** Dr. First Last Name  
**Pathologist:** Dr. First Last Name  
**Specimen Type:** Plasma

Olaris BoR Score uses a metabolite-profiling platform to determine the expression of a panel of metabolites and calculates a score ranging from 0-100.

The findings are applicable to women who have ER+/HER2- metastatic breast cancer.

**Clinical Experience:** The likelihood of response shown below are from a retrospective study that included 21 ER+/HER2- metastatic breast cancer patients who were treated with CDK4/6 inhibitors, Ibrance (palbociclib) or Kisqali (ribociclib).

Your baseline (BL) BoR Score compared to other patient Responders (R) and Non-Responders (NR)

If Olaris BoR Score is:  
- Greater than 0 → R  
- Less than 0 → NR

**BL CDK4/6 class BoR Score:** +7

**BL Ibrance BoR Score:** +4

**BL Kisqali BoR Score:** -1

**BL PI3K BoR Score:**

**BL mTOR BoR Score:**
## Biomarkers Accelerate Drug Development

Biomarkers increase the Probability of Success (PoS) At Every Stage of Clinical Development

<table>
<thead>
<tr>
<th>Therapeutic group</th>
<th>Phase 1 to Phase 2</th>
<th>Phase 2 to Phase 3</th>
<th>Phase 3 to approval</th>
<th>Overall</th>
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<tr>
<td></td>
<td>Total phase transitions</td>
<td>POS$_{1,2}$, % (SE, %)</td>
<td>Total phase transitions</td>
<td>POS$_{2,3}$, % (SE, %)</td>
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<td>4773</td>
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<tr>
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<td>43.5 (1.5)</td>
<td>742</td>
<td>38.8 (1.8)</td>
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<tr>
<td>All</td>
<td>10 485</td>
<td>29.7 (0.4)</td>
<td>5515</td>
<td>20.3 (0.5)</td>
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<td>With biomarker</td>
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<td>57.1 (18.7)</td>
<td>2</td>
<td>50.0 (35.4)</td>
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<td>2050</td>
<td>30.2 (1.0)</td>
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<td>42</td>
<td>54.8 (7.7)</td>
<td>42</td>
<td>28.6 (7.0)</td>
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<tr>
<td>All</td>
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<td>2092</td>
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<td>38.9 (1.0)</td>
<td>2106</td>
<td>25.4 (0.9)</td>
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<tr>
<td>With biomarker</td>
<td>9</td>
<td>55.6 (16.6)</td>
<td>14</td>
<td>35.7 (12.8)</td>
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<tr>
<td>All</td>
<td>2515</td>
<td>39.0 (1.0)</td>
<td>2120</td>
<td>25.5 (0.9)</td>
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<td>34.3 (2.5)</td>
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<tr>
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<td>364</td>
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<td>287</td>
<td>28.9 (2.7)</td>
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<td>1453</td>
<td>34.7 (1.2)</td>
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<tr>
<td>With biomarker</td>
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<td>44.4 (9.6)</td>
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<td>1480</td>
<td>34.9 (1.2)</td>
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<td><strong>Ophthalmology</strong></td>
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<tr>
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<td>52.2 (3.7)</td>
<td>274</td>
<td>34.7 (2.9)</td>
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<tr>
<td>With biomarker</td>
<td>1</td>
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<td>3</td>
<td>33.3 (27.2)</td>
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<tr>
<td>All</td>
<td>181</td>
<td>51.9 (3.7)</td>
<td>277</td>
<td>34.7 (2.9)</td>
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<tr>
<td><strong>Vaccines</strong></td>
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<tr>
<td>(infectious disease)</td>
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<tr>
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<td>761</td>
<td>32.9 (1.7)</td>
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<tr>
<td>With biomarker</td>
<td>0</td>
<td>N.A. N.A.</td>
<td>5</td>
<td>0.0 (0.0)</td>
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<tr>
<td>All</td>
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<td>766</td>
<td>32.6 (1.7)</td>
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<tr>
<td><strong>Overall</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No biomarker</td>
<td>20 042</td>
<td>34.7 (0.3)</td>
<td>14 169</td>
<td>26.6 (0.4)</td>
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<tr>
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<td>840</td>
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</tr>
<tr>
<td>All</td>
<td>21 255</td>
<td>35.2 (0.3)</td>
<td>15 009</td>
<td>27.4 (0.4)</td>
</tr>
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