Are *in vitro*-based predictive signatures ready for clinical use in breast cancer?

Jae K. Lee, PhD
Division of Biostatistics and Epidemiology
University of Virginia

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The 2nd TBCI Correlative Sciences Workshop
Collaborators

- **Lee** bioinformatics/biostatistics
  - Group at UVA
    - Feng Cheng, PhD
    - Sang-Hoon Cho, PhD
    - Young-chul Kim, PhD
    - Annamalai Muthai, MS
    - Paul Williams, PhD

- **UVA Theodorescu** Clinical Lab
  - Dima Havaleshko, MD
  - Kihyuck Moon, MD
  - Hyeon Jung, MD

- **UVA Computer Science**
  - Andrew Grimshaw, PhD
  - John Kaporvich, PhD

- **UVA Clinical Oncology Group**
  - Chris Thomas, MD

- **UVA Pathology**
  - Chris Moskaluk, MD

- **MD Anderson**
  - Charles Coutant, PhD
  - Yuan Qi, PhD
  - W Fraser Symmans, MD
  - Keith Baggerly, PhD
  - Lajos Pusztai, MD

- **UT San Antonio HSC**
  - Rong Li, PhD
  - Anand Karnad, MD
Predictive models of therapeutic response have been made using previously treated patient data, but they…

- Are very costly & take 5+ yrs for each regimen
- Can predict only for the exact combination therapy used in the previous human patient studies and trials
- No individual compound response predictability for better therapeutic options and/or novel combinations for heterogeneous BC tumors

If works, *in vitro*-based predictive models can

- Provide predictions of heterogeneous BC tumors to individual compounds → True personalized therapy
- Be efficiently made for many drugs and their combinations, e.g. >100 doublet, or even triplet combinations among ~15 therapeutic compounds currently used for breast cancer
Why multi-gene expression predictors work?

- **Tumor Heterogeneity**: Often a large number of alternative genes and networks are relevant to cancer patients’ prognosis and therapeutic responses
  → Individual gene information is frequently variable and noisy

- **Tumor Biology**: Need to understand functional mechanisms of these gene networks
  → e.g. epigenetics & environmental interactions
  (Books in library don’t make actions, but books we take out, read, and interpret make actions!)

- **Technical Advance**: Unbiased genome-wide functional survey with accurate quantification
  → Need to summarize their consistent, consensus network gene actions, avoiding statistical over-fitting!
COXEN: Genomic Based Personalized Chemotherapeutics

“Co-eXpression ExtrapolatioN”  (WWW.COXEN.ORG)

NCI60 Panel

Drug Screen (DTP, NCI)

Expression Profiling

Biomarker networks & Statistical Bioinformatics

COXEN

Human Tumor Expression Profiling

Personalized Chemotherapeutic Response Prediction On Human Cancer

- Lee et al. (PNAS, 2007; Predicting the chemosensitivity of human cancers and its application to drug discovery)
- Havaleshko et al. (Mol Cancer Ther 2007; Prediction of drug combination chemosensitivity in human bladder cancer)
Initial proof-of-principle *in vitro*-based Gene Expression Model (GEM) applications

- Chemosensitivity prediction of paclitaxel and cisplatin on bladder 40 cell lines (BLA-40)
- Novel anticancer drug discovery on BLA-40 and Validation
- Chemotherapeutic response prediction of breast cancer docetaxel (DOC-24) and tamoxifen (TAM-60) trials
Six COXEN Steps for Bladder Cancer Prediction

Step 1: NCI-60 cancer cell lines
- Drug activity profiles of cisplatin and paclitaxel in NCI’s Public Drug Database

Step 2: mRNA expression profiles (Hu133A&B)

Step 3: Drug sensitivity predictor probes (191 probes for cisplatin; 105 for paclitaxel)

Step 4: BLA-40 human bladder cancer cell lines
- mRNA Expression profiles (Hu133A)

Step 5: Drug sensitivity probes “co-expression extrapolated” between the NCI-60 and BLA-40
(18 probes for cisplatin; 13 for paclitaxel - Table S1)

Step 6: MiPP prediction model development
- Training set (internal cross-validation)
- Test set (external cross-validation)

- No Bladder cancer in NCI-60
- Completely prospective prediction!

Independent Validation of COXEN predictions on BLA-40
- COXEN Scores of cisplatin and paclitaxel activity in the BLA-40
- Independent comparison
- In vitro evaluation of cisplatin and paclitaxel activity in BLA-40 cells

NCI-60 Microarray Profiling (HG133A&B), collaboration with John Weinstein, NCI & Eric Kaldjian, Gene Logic

BLA-40 Array & Validation
Theodorescu Lab, UVA
Hierarchical clustering before or after COXEN biomarker selection
Can we predict patient treatment outcome in *breast cancer* clinical trials?

- Chang et al (Lancet, 2003)
- Ma et al (Cancer Cell, 2004)

**NCI-60 cancer cell lines**

**Drug activity profiles of Docetaxel and Tamoxifen**

**Gene Microarray**

**Validation of COXEN predictions in Breast Cancer patients**

- Treatment outcome indices:
  - DOC-24: tumor residual size
  - TAM-60: disease-free survival time

**COXEN Score of clinical outcome for docetaxel and tamoxifen breast cancer trials**

**Comparison of COXEN Score to treatment outcome indices**
In vitro model prediction on single-compound breast cancer clinical trials

Primary tumor response to neoadjuvant doxcetaxel (DOC-24)

- Responder: Tumor size
- Sensitive: COXEN Prediction
- Non-responder: Tumor size
- Non-responder: COXEN Prediction

\[ p\text{-value} = 0.033 \]

Survival following adjuvant tamoxifen (TAM-60)

- Predicted responders
- Predicted non-responders

\[ p\text{-value} = 0.021 \]

Fraction Disease-Free

Time (months)
Consistent prediction on combination chemotherapies for diverse patient sets?

Wasn’t the prediction useful only for each patient set?

Are we ready to use unaltered in vitro-based models to forecast & guide both clinical response and survival of diverse patient sets treated by combination chemotherapy?
## COXEN Prediction of Response to Combination Chemotherapy in Paired Trials

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Study Name</th>
<th>Agent or Combination</th>
<th>Score probability Responders (mean±/2 Std)</th>
<th>Score probability Non-Responders (mean±/2 Std)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>BL-MVAC-Jap</td>
<td>Methotrexate</td>
<td>0.625 +/- 0.071</td>
<td>0.371 +/- 0.071</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vinblastine</td>
<td>0.594 +/- 0.071</td>
<td>0.358 +/- 0.079</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adriamycin</td>
<td>0.552 +/- 0.078</td>
<td>0.402 +/- 0.090</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cisplatin</td>
<td>0.570 +/- 0.076</td>
<td>0.364 +/- 0.080</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AC</td>
<td>0.947 +/- 0.022</td>
<td>0.794 +/- 0.054</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>BL-MVAC-Den</td>
<td>Methotrexate</td>
<td>0.534 +/- 0.122</td>
<td>0.506 +/- 0.168</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vinblastine</td>
<td>0.602 +/- 0.192</td>
<td>0.490 +/- 0.166</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adriamycin</td>
<td>0.756 +/- 0.121</td>
<td>0.441 +/- 0.154</td>
<td>0.071</td>
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<tr>
<td></td>
<td></td>
<td>Cisplatin</td>
<td>0.783 +/- 0.075</td>
<td>0.442 +/- 0.148</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AC</td>
<td>0.969 +/- 0.003</td>
<td>0.643 +/- 0.140</td>
<td>0.038</td>
</tr>
<tr>
<td>Ovarian</td>
<td>OV-Plat</td>
<td>Carboplatin</td>
<td>0.715 +/- 0.074</td>
<td>0.563 +/- 0.141</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taxol</td>
<td>0.429 +/- 0.087</td>
<td>0.223 +/- 0.114</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>0.826 +/- 0.061</td>
<td>0.638 +/- 0.132</td>
<td>0.007</td>
</tr>
<tr>
<td>Breast</td>
<td>BR-TFAC-MDA</td>
<td>Taxol</td>
<td>0.568 +/- 0.130</td>
<td>0.345 +/- 0.072</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(Hess-133)</td>
<td>5-FU</td>
<td>0.549 +/- 0.119</td>
<td>0.472 +/- 0.077</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adriamycin</td>
<td>0.326 +/- 0.135</td>
<td>0.164 +/- 0.061</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
<td>0.340 +/- 0.138</td>
<td>0.218 +/- 0.071</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FAC</td>
<td>0.721 +/- 0.116</td>
<td>0.576 +/- 0.072</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>BR-GED</td>
<td>Gemcitabine</td>
<td>0.219 +/- 0.046</td>
<td>0.154 +/- 0.033</td>
<td>0.014</td>
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<tr>
<td></td>
<td></td>
<td>Epirubicin</td>
<td>0.430 +/- 0.113</td>
<td>0.324 +/- 0.057</td>
<td>0.055</td>
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<tr>
<td></td>
<td></td>
<td>Docetaxel</td>
<td>0.804 +/- 0.076</td>
<td>0.726 +/- 0.042</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GED</td>
<td>0.919 +/- 0.034</td>
<td>0.859 +/- 0.023</td>
<td>0.003</td>
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<tr>
<td>Survivor</td>
<td>OV-CT</td>
<td>Carboplatin</td>
<td>0.498 +/- 0.253</td>
<td>0.202 +/- 0.212</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taxol</td>
<td>0.397 +/- 0.227</td>
<td>0.114 +/- 0.136</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>0.721 +/- 0.213</td>
<td>0.310 +/- 0.224</td>
<td>0.008</td>
</tr>
<tr>
<td>Breast</td>
<td>BR-FAC-Duke</td>
<td>5-FU</td>
<td>0.514 +/- 0.112</td>
<td>0.301 +/- 0.155</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adriamycin</td>
<td>0.211 +/- 0.107</td>
<td>0.036 +/- 0.042</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
<td>0.263 +/- 0.118</td>
<td>0.114 +/- 0.124</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FAC</td>
<td>0.611 +/- 0.120</td>
<td>0.392 +/- 0.177</td>
<td>0.033</td>
</tr>
</tbody>
</table>
COXEN Prediction of Overall Survival to Combination Chemotherapy in Breast Cancer

BR-FAC

P=0.053
(N=45)

Time (month)

Proportion of Survival

- Red: Predicted Responders (19)
- Blue: Predicted Nonresponders (26)
Question: Will this performance be realized in a completely-blinded prospective setting in practice?
Three multi-gene prediction modeling & Blinded prospective application

Model Development

- General Breast Cancer Patient Population (N=251, Miller 2005)
- NCI-60 Panel
  - TFAC Drugs
- COXEN
  - In vitro COXEN GEM
  - T F A C
  - In vivo COXEN GEM
  - T F A C
  - Human GEM
  - TA
  - TFAC

TFAC Treated Breast Cancer Patient Set (N=133, Hess-133)
- Tumor Sample Taken
- Tumor Profiling
- Responders
- Non Responders
- Biomarker & Model Development
- Blinded Prospective Model Application
  - Prospective Applications of Models to TFAC-treated 100 Patients
### Prediction by three multi-gene modeling strategies

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Single Agent or Combination</th>
<th>Responders GEM Scores (mean±/− 95% CI)</th>
<th>Non-Responders GEM Score (mean±/− 95% CI)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COXEN</td>
<td>5-FU</td>
<td>0.447+/−0.229</td>
<td>0.426+/−0.074</td>
<td>0.848</td>
</tr>
<tr>
<td>GEM</td>
<td>Adriamycin</td>
<td>0.168+/−0.170</td>
<td>0.235+/−0.078</td>
<td>0.459</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>0.146+/−0.176</td>
<td>0.160+/−0.061</td>
<td>0.879</td>
</tr>
<tr>
<td></td>
<td>TFAC^</td>
<td>0.659+/−0.192</td>
<td>0.601+/−0.075</td>
<td>0.562</td>
</tr>
<tr>
<td>Human GEM</td>
<td>TFAC^</td>
<td>0.480+/−0.158</td>
<td>0.234+/−0.057</td>
<td><strong>0.006</strong>**</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COXEN</td>
<td>5-FU</td>
<td>0.262+/−0.057</td>
<td>0.254+/−0.023</td>
<td>0.787</td>
</tr>
<tr>
<td>GEM</td>
<td>Adriamycin</td>
<td>0.365+/−0.098</td>
<td>0.239+/−0.037</td>
<td><strong>0.019</strong>*</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>0.366+/−0.135</td>
<td>0.251+/−0.049</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>0.613+/−0.155</td>
<td>0.380+/−0.055</td>
<td><strong>0.002</strong>**</td>
</tr>
<tr>
<td></td>
<td>TFAC^</td>
<td>0.755+/−0.133</td>
<td>0.595+/−0.052</td>
<td><strong>0.028</strong>*</td>
</tr>
</tbody>
</table>
Concordant Prediction Performance of three modeling strategies
Consistent predicted ranks by three models

- COXEN in vitro
- HumanGEM (TFAC)
- COXEN in vivo

- GEM (T)
- GEM (TA)

Correlation coefficients:
- r = 0.65 (p = 3.9e-13)
- r = 0.75 (p = 2.2e-16)
- r = 0.90 (p = 1.2e-19)
Highly encouraging possibility in efficiently developing in vitro-based prediction models for therapeutic response

- Concordant prediction of unaltered *in vitro*-based models on geographically and ethnically diverse patient sets
- Validated by a completely-blinded prospective prediction

**Are *in vitro*-based models ready for clinical use?**

- NOT YET represent validation of a pre-defined predictor with a pre-set threshold to call a case + (responder) or – (non-responder)
- rather shows proof-of-a-concept, illustrating the *in vitro*-based models are truly informative (from each ROC curve) in stratifying patients’ responses

→ A standard diagnosis assay platform and procedure should be defined for routine clinical practice, from which a fixed cutoff value can be defined for a target patient population
Conclusion

- *in vitro*-based models are limited for the compounds that show no relevant drug activities *in vitro*, e.g. anti-angiogenesis compounds

- Validation and validation for many predictive *in vitro*-based models for translation to clinical practice!
  - Validate on historical FFPE patient samples in ethnically, geographically-diverse clinical settings based on standardized diagnosis assay platforms and procedures
  - Prospective clinical trials with, e.g. standard combination chemotherapy arm vs. genomic-guided arms among current equivalent therapeutic options

- Need to establish, maintain, & integrate infrastructure:
  - 1) genomics/proteomics/other molecular databases, 2) patient sample archives, 3) clinical information database

→ Personalize therapies for better cure of breast cancer patients in a near sight!!