GS01 0163
Analysis of Microarray Data

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Lecture 4: Cell Lines, Chemo, and Repro

Microarrays let us simultaneously measure the mRNA expression levels of thousands of genes in a sample of interest.

Can we figure out what’s going wrong in cancer? Finding patterns of aberrant gene expression

Can we figure out who to treat? Disease identification and Disease subtyping
Making Research “Translational”

Can we figure out how to treat them?

Long term: *How should I plan to treat patients 5 years from now?* Develop drugs targeting specific abnormalities.

Short term: *How should I treat the patient in my office today?* Figure out which types of available treatments (chemotherapeutic regimens) are likely to be effective.

What do we know about drug effectiveness?
Cancer, Chemo, and Cell Lines

1955 – Cancer Chemotherapy National Service Center (CCNSC) established. One goal: test drugs as anticancer agents. Candidate drugs, assigned an NSC number, were tested for efficacy in leukemic mice.

1976-82 – CCNSC incorporated into Developmental Therapeutics Program (DTP); Human tumors in mice.

1985-90 – Human tumor cell line panel (NCI60) established as first line test.

Today – Tens of thousands of cytotoxic agents have been evaluated for activity against the standard panel.

Using the NCI60 to Predict Sensitivity

Genomic signatures to guide the use of chemotherapeutics

Anil Potti¹,², Holly K Dressman¹,³, Andrea Bild¹,³, Richard F Riedel¹,², Gina Chan⁴, Robyn Sayer⁴, Janiel Cragun⁴, Hope Cottrill⁴, Michael J Kelley², Rebecca Petersen⁵, David Harpole⁵, Jeffrey Marks⁵, Andrew Berchuck¹,⁶, Geoffrey S Ginsburg¹,², Phillip Febbo¹⁻³, Johnathan Lancaster⁴ & Joseph R Nevins¹⁻³


The main conclusion is that we can use microarray data from cell lines (the NCI60) to define drug response “signatures”, which can be used to predict whether patients will respond.

They provide examples using 7 commonly used agents.

This got people at MDA very excited.
The Other Exciting Bit...

All the analyses were performed on publicly available data sets.

The drug response data is publicly available, and maintained by the NCI.

The microarray profiles of the cell lines are publicly available, and at least some are available from the NCI.

Microarray profiles of patient and cell line samples that did and did not respond to various drugs are available from public repositories (esp GEO).

We should be able to do it ourselves!
How it Works

1. Identify and collect the data sets.

2. Using the sensitivity information for a drug of interest (docetaxel) select the extreme cell lines.

3. Using the array profiles of these cell lines, select the features (genes) that best distinguish sensitive from resistant.

4. Use array values for the chosen features to train a binary model distinguishing sensitive from resistant cell lines.

5. Test the model for its ability to make accurate predictions using expression data sets from patient tumors.
Gathering Data

1. Drug response: assays on NCI60 from DTP at NCI
   (http://dtp.nci.nih.gov/docs/cancer/cancer_data.html)

2. Training: Affymetrix U95Av2 arrays on NCI60, performed in triplicate by Novartis (http://dtp.nci.nih.gov/mtargets/download.html)

Identifying the Drugs

The paper gives the *names* of the drugs profiled, but response data is indexed by NSC number. How would you find these numbers? **Google!** (NCI Drug Dictionary)

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What Drug Sensitivity Data Looks Like

Docetaxel Assays for COLO 205

Log10(M) Concentration of Docetaxel

Proportion of Growth Seen at Endpoint
Adding Cutoffs

Docetaxel Assays for COLO 205

Proportion of Growth Seen at Endpoint

Log10(M) Concentration of Docetaxel

GI50
TGI
LC50
Selecting Cell Lines for Docetaxel

Use GI50 and TGI; LC50 shows little change.
Building a Model

Following Potti et al., we selected the top 50 genes based on a two-sample t-test between sensitive and resistant cell lines.

The paper uses “metagenes” to construct a predictive model. Metagenes summarize the information present in a chosen set of genes by taking weighted averages. Mathematically, they’re simply the principal components of the chosen matrix.

For the model, they use probit regression with the metagene scores to separate sensitive and resistant groups.
We want the test data to split like this...
But it doesn’t. Did we do something wrong?
Forensic Bioinformatics

In the outline above, we tried to follow their qualitative approach. Now, we’re going to try to figure out exactly how this worked.

- What cell lines were used?
- What features were selected?
- How were the models built?
- What were the predictions?
What Cell Lines Were Used?

We asked about this, to be sure we were working with the right data.

They responded, but not with precisely what we asked for.

They sent us a giant Excel table.
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Does this answer the question?
The Novartis Data

Some of the first few rows of the Novartis “individual” file at the NCI:

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Using this approach, we can match the cell lines used for 6 of the drugs examined (not cytoxan). The “A” series replicates were used throughout.
How Are Cell Lines Chosen?

Supplementary Methods: “[W]e chose cell lines . . . that would represent the extremes of sensitivity to a given chemotherapeutic agent (mean GI50 ± 1 SD). . . . [T]he log transformed TGI and LC50 dose . . . was then correlated with the respective GI50 data . . . Cell lines with low GI50 . . . also needed to have a low LC50 and TGI . . .”

So, how clearly separated are the lines used?
Values *overlap*? This holds for all drugs tested. The cell lines don’t make sense. What about the features?
They Reported the Features!

Lists of the probesets used were supplied in supplementary table 1, and at the website named in the supplementary methods document: http://data.cgt.duke.edu/Combo1.php (now NatureMedicine.php). The paper explains why many of these genes make sense.

How were these found? According to the supplementary methods: “a variance fixed t-test was used to calculate significance”.

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5-FU Heatmaps

Nat Med Paper  Our t-tests  Reported Genes

Something isn’t quite right...
Their List and Ours

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> temp <- cbind(
    sort(rownames(pottiUpdated)[fuRows]),
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> colnames(temp) <- c("Theirs", "Ours");
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© Copyright 2009, KA Baggerly, KR Coombes
Offset P-Values: 5FU

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5–FU List+1 Pvalues

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Offset P-Values: Other Drugs

- Topotecan+1
- Etoposide+1
- Adriamycin+1
- Paclitaxel+1
- Docetaxel+1
- Cytoxan+1

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Using Their Software

Their software requires two files (and parameter values):

1. a quantification matrix, genes by samples, with a header row giving the classification (0 = Resistant, 1 = Sensitive, 2 = Test)

2. a list of probeset ids in the same order as the quantification matrix. *The list of probeset ids should not have a header row.*

What do we get?
Heatmaps Match Exactly for Docetaxel!

From Potti et al, Figure 1

From the software

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Heatmaps Match Exactly for 5 Others!

From the paper:

![Heatmap Images](image1.png)

From the software:

![Heatmap Images](image2.png)

We match heatmaps but not gene lists?

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The Software Also Gives Predictions...

So, how good are the predictions?

How good are the ones they report?
Predicting Docetaxel (Chang 03)

Accuracy: 22/24 (91.6%)

Sensitivity:
- Docetaxel sensitive
- Docetaxel resistant

Probability of docetaxel resistance

P < 0.001

Sensitivity:
- Docetaxel sensitive
- Docetaxel resistant

% residual tumour

Sensitive tumours
Resistant tumours

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Predicting Adriamycin (Holleman 04)

Accuracy: 99/122 (81%)

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What’s Going On?

One area for potential mixup is in labeling samples as “0” or “1” instead of “Sensitive” and “Resistant”.

Another is that the software does something odd in computing metagenes:

the metagenes are taken from an SVD applied to both training and test data.
Docetaxel Test Set Predictions

- Training & Test.
  The “combined” predictions are different. This doesn’t mean they’re right. Now, if we choose genes using only the training data, combining is mostly standardizing. But...

- Training Only.
There Were Other Genes...

The 50-gene list for docetaxel has 19 “outliers”.

The initial paper on the test data (Chang et al) gave a list of 92 genes that separated responders from nonresponders.

Entries 7-20 in the Chang et al list (a contiguous bloc) comprise 14 of the 19 outliers.

The other 5 are ERCC1, ERCC4, ERBB2, BCL2L11, and TUBA3. These are the genes named to explain the biology.
Predictions With Random Lines

Proportions Using Random Cell Lines

Proportion of Resp Cases called Sensitive

1 – Proportion of NR Cases called Resistant
So, Do We Think it Works?

Pause here for dramatic tension...

No.

Actually, we might be more surprised if it did.

- the cell lines are from a variety of different tumor types with known differences in reponsiveness.

- the training and test sets were run at different times and under different conditions with different array platforms and definitions of sensitivity.

We think it appears to work due to poor bookkeeping and documentation.
Don’t Take My Word For It!


Try it yourselves! All of the raw data, documentation, and code is available from our web site:

http://bioinformatics.mdanderson.org/Supplements/ReproRsch-Chemo; All of our code and documentation is likewise available from Nature Medicine.
Potti/Nevins Reply (Nat Med 13:1277-8)

Labels for Adria are correct – details on their web page. (*)

They’ve gotten the approach to work again. (Twice!)*

Pharmacogenomic Strategies Provide a Rational Approach to the Treatment of Cisplatin-Resistant Patients With Advanced Cancer


Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial

Hervé Bonnefoi, Anil Potti, Mauro Delorenzi, Louis Mauriac, Mario Campone, Michèle Tubiana-Hulin, Thierry Petit, Philippe Rouanet, Jacek Jassem, Emmanuel Blot, Véronique Becette, Pierre Farmer, Sylvie André, Chaitanya R Acharya, Sayan Mukherjee, David Cameron, Jonas Bergh, Joseph R Nevins, Richard D Iggo
...and Even More...

An Integrated Genomic-Based Approach to Individualized Treatment of Patients With Advanced-Stage Ovarian Cancer

Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Jonathan Gray, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, and Johnathan M. Lancaster

Genomic and Molecular Profiling Predicts Response to Temozolomide in Melanoma

Christina K. Augustine,1,5 Jin Soo Yoo,1 Anil Potti,2,4 Yasunori Yoshimoto,1,5 Patricia A. Zipfel,1,5 Henry S. Friedman,1 Joseph R. Nevins,3,4 Francis Ali-Osman,1 and Douglas S. Tyler1,5

Is everybody ready?
Mapping Potti to Chang (Reply)

Chang/Duke Correlations > 0.9999

© Copyright 2009, KA Baggerly, KR Coombes
Adriamycin 0.9999+ Correlations (Reply)
Nat Med, Take 2

- Breast data (n = 24).txt (gone)

- Adria_ALL (n = 122).txt (replaced with Adria_ALL_data1_n95.doc)

“In the version ... initially published ... 27 samples were replicated... The authors have reanalyzed ... using only the 95 unique samples...”

“the authors have added two more accession numbers (GSE2351 and GSE649)”
The New Adria Plot

74 resistant, 21 sensitive
The First 20 Files Now Named

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GSM44303 RES</td>
</tr>
<tr>
<td>2</td>
<td>GSM44304 RES</td>
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<td>3</td>
<td>GSM9653 RES</td>
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<td>GSM9653 RES</td>
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</tr>
<tr>
<td>8</td>
<td>GSM9657 RES</td>
</tr>
<tr>
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<td>GSM9658 SEN</td>
</tr>
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<td>10</td>
<td>GSM9658 SEN</td>
</tr>
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</tr>
<tr>
<td>12</td>
<td>GSM9695 RES</td>
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</tr>
<tr>
<td>14</td>
<td>GSM9698 RES</td>
</tr>
<tr>
<td>15</td>
<td>GSM9699 SEN</td>
</tr>
<tr>
<td>16</td>
<td>GSM9701 RES</td>
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<tr>
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<td>GSM9708 RES</td>
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<tr>
<td>18</td>
<td>GSM9708 SEN</td>
</tr>
<tr>
<td>19</td>
<td>GSM9709 RES</td>
</tr>
<tr>
<td>20</td>
<td>GSM9711 RES</td>
</tr>
</tbody>
</table>
The First 20 Files Now Named

<table>
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<td>GSM9711</td>
<td>RES</td>
</tr>
</tbody>
</table>

15 duplicates; 6 are inconsistent.
Summary (Reply)

Looking at the 80 distinct GSM ids, Potti et al class
61 Resistant,
13 Sensitive,
6 Both Ways.

Using LC50 values with specified cutoffs, Holleman et al class
22 Resistant,
48 Sensitive,
10 Intermediate.

One new GEO dataset listed as a source (GSE649) flags samples that are resistant to vincristine, not adriamycin.
Validation 1: Hsu et al

Pharmacogenomic Strategies Provide a Rational Approach to the Treatment of Cisplatin-Resistant Patients With Advanced Cancer


Same approach, using Cisplatin and Pemetrexed.

For cisplatin, U133A arrays were used for the training set, and ERCC1, ERCC4 and DNA repair genes are identified as being “important”.

With some work, we matched the heatmaps.
What About the Gene Lists?

The list for pemetrexed was generated from training data where the sensitive and resistant labels were reversed.

But we can match all 85 of the genes for pemetrexed!

After we account for the off-by-one error.

We can match 41 of the 45 genes for cisplatin, after we account for the off-by-one error.
The 4 We Can’t Match (Reply)

203719_at, ERCC1,
210158_at, ERCC4,
228131_at, ERCC1, and
231971_at, FANCM (DNA Repair).

ERCC1 and/or ERCC4 were outliers in the earlier gene lists for Docetaxel, Paclitaxel, and Adriamycin. We find their frequent recurrence disturbing. Even so, the last two here are special.

These probesets aren’t on the U133A arrays that were used. They’re on the U133B.
Validation 2: Bonnefoi et al

Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial

Hervé Bonnefoi, Anil Potti, Mauro Delorenzi, Louis Mauriac, Mario Campone, Michèle Tubiana-Hulin, Thierry Petit, Philippe Rouanet, Jacek Jassem, Emmanuel Blot, Véronique Becette, Pierre Farmer, Sylvie André, Chaitanya R Acharya, Sayan Mukherjee, David Cameron, Jonas Bergh, Joseph R Nevins, Richard D Iggo

Lancet Oncology, Dec 2007, 8:1071-8. (early access Nov 14)

Similar approach, using signatures for Fluorouracil, Epirubicin (used Adriamycin), Cyclophosphamide, and Taxotere (Docetaxel) to predict response to one of two combination therapies: FEC and TET.

Potentially improves ER- response from 44% to 70%.
We Might Expect Some Differences...

High Sample Correlations after Centering by Gene

Array Run Dates
What Cell Lines Were Involved?

Supplementary “webpanel” from Lancet Oncology.

<table>
<thead>
<tr>
<th>Genomic Signatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>What Cell Lines Were Involved?</td>
</tr>
<tr>
<td>Supplementary “webpanel” from Lancet Oncology.</td>
</tr>
</tbody>
</table>

The sensitive and resistant categories for docetaxel are reversed relative to Potti et al.
How Are Results Combined?

Potti et al predicted response to TFAC. Bonnefoi et al TET and FEC. Let P() indicate prob sensitive. The rules used are as follows.

\[ P(TFAC) = P(T) + P(F) + P(A) + P(C) - P(T)P(F)P(A)P(C). \]

\[ P(ET) = \max[P(E), P(T)]. \]

\[ P(FEC) = \frac{5}{8}[P(F) + P(E) + P(C)] - \frac{1}{4}. \]

*Each rule is different.*
Predictions for Individual Drugs? (Reply)

Does cytoxan make sense?
Ovarian Cancer and Pathways

An Integrated Genomic-Based Approach to Individualized Treatment of Patients With Advanced-Stage Ovarian Cancer

Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Jonathan Gray, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, and Johnathan M. Lancaster


Looking for pathway deregulation in ovarian cancer.

Using tumor array profiles to predict response to cisplatin.

119 serous tumors, quantifications, CEL files, and clinical information provided.
Looking at the Data

We began by looking at the RMA quantifications that they posted for the various arrays.

For each array, expression values were recorded for 22115 probesets. This is a strange number. There are 22283 total probesets on Affy U133A arrays, of which 68 are “controls” that are not often used in signatures. But $22283-68 = 22215$.

But, they used justRMA, so we could quantify the CEL files ourselves...
CELs vs Tables. We expected better (fewer outliers).
Looking at Their Other Quants

Finding the Best Match, CEL RMA Column 1

Which one would you pick?
Looking at The “Best” Fit

Two RMA Quantifications: 872 From CEL, 2476 From XLS

Same array. *Different* names (2476 from XLS, 872 from CEL).
How Bad is It?

The names match for 32/119 samples. For all but 3 of the others, we get very good correlations but a mismatch in names.

We don’t have a clear “winner” for their quantifications for D1837, M4161, or M444.
More Raw Data

Data from the authors’ web site for an earlier paper in Nature (Bild et al, 2006),
http://data.cgt.duke.edu/oncogene.php, supplies CEL files and clinical information for 146 ovarian tumor samples, a superset of the ones examined by Dressman et al.

Checking the entire Bild set,
XLS M4161 corresponds to D2159
XLS M444 corresponds to D2171
XLS D1837 corresponds to D2247.

Can we see what happened?
Where the Best Fits Are...

Most of the poor fits are 3 names off.
Augustine et al., 2009, *Clin Can Res*, **15**:502-10, Fig 4A. Temozolomide, NCI-60.

Hsu et al., 2007, *J Clin Oncol*, **25**:4350-7, Fig 1A. Cisplatin, Gyorffy cell lines.
Some Observations

The most common mistakes are simple.

Confounding in the Experimental Design

Mixing up the sample labels
Mixing up the gene labels
Mixing up the group labels
(Most mixups involve simple switches or offsets)

This simplicity is often hidden.

Incomplete documentation

Unfortunately, we suspect
The most simple mistakes are common.
Steps Towards Reproducibility at MDA

Literate Programming. For the past two years, we have required the analysts in our department to prepare reports in Sweave, where documentation has been interweaved with the code used to produce the analysis. There’s also odfWeave or SASWeave, if you prefer.

Reusing Templates. For common types of analyses, we’re starting from canonical template analyses that have been assembled in depth. This standardizes and speeds the analysis. We have also produced RATB – the “Report and Analysis Template Builder” – for this purpose.
Steps Towards Clarity at MDA

*Report Structure.* The biologists we work with often don’t want to plow through the code, but they do want to understand. This is aided by following a format they’re familiar with: Introduction, Methods, Results, Conclusions.

*Executive Summaries.* Each report is prefaced by a brief textual description following the same outline.

*Appendices.* Some things we want to know all the time:
1. `SessionInfo`, the libraries used,
2. `Saves`, the data produced, and
3. `File Location`, where the data is.
Acknowledgements

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MDACC Ovarian SPORE, Lung SPORE, Breast SPORE