GS01 0163
Analysis of Microarray Data

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September 9, 2010

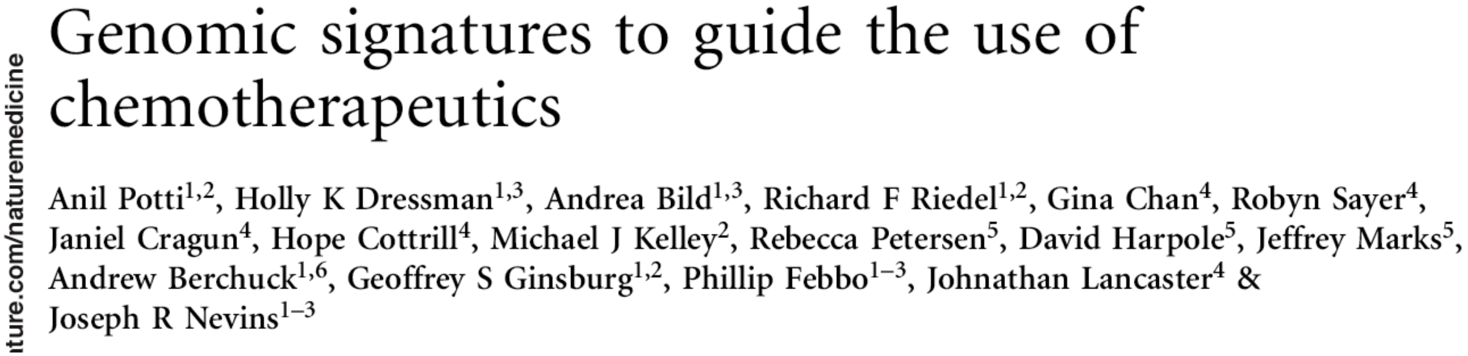
Why is RR So Important in H-TB?

Our intuition about what “makes sense” is very poor in high dimensions. To use “genomic signatures” as biomarkers, we need to know they’ve been assembled correctly.

Without documentation, we may need to employ *forensic bioinformatics* to infer what was done to obtain the results.

Let’s examine some case studies involving an important clinical problem: *can we predict how a given patient will respond to available chemotherapeutics?*

Using the NCI60 to Predict Sensitivity



ture.com/naturemedicine

Genomic signatures to guide the use of chemotherapeutics

Anil Potti[1,2], Holly K Dressman[1,3], Andrea Bild[1,3], Richard F Riedel[1,2], Gina Chan[4], Robyn Sayer[4], Janiel Cragun[4], Hope Cottrill[4], Michael J Kelley[2], Rebecca Petersen[5], David Harpole[5], Jeffrey Marks[5], Andrew Berchuck[1,6], Geoffrey S Ginsburg[1,2], Phillip Febbo[1–3], Johnathan Lancaster[4] & Joseph R Nevins[1–3]

Potti et al (2006), Nature Medicine, 12:1294-1300.

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.

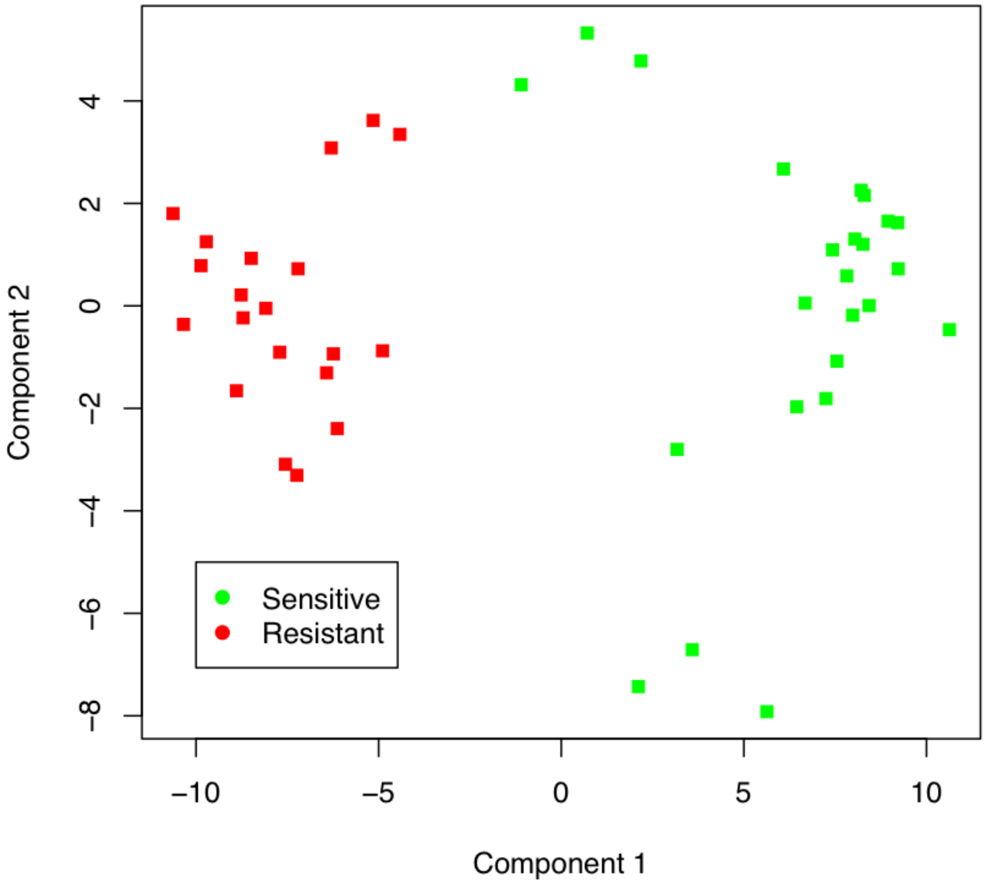
Gathering Data

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

2. Training (NCI60): Affy U95Av2, triplicate runs (`http://dtp.nci.nih.gov/mtargets/download.html`)

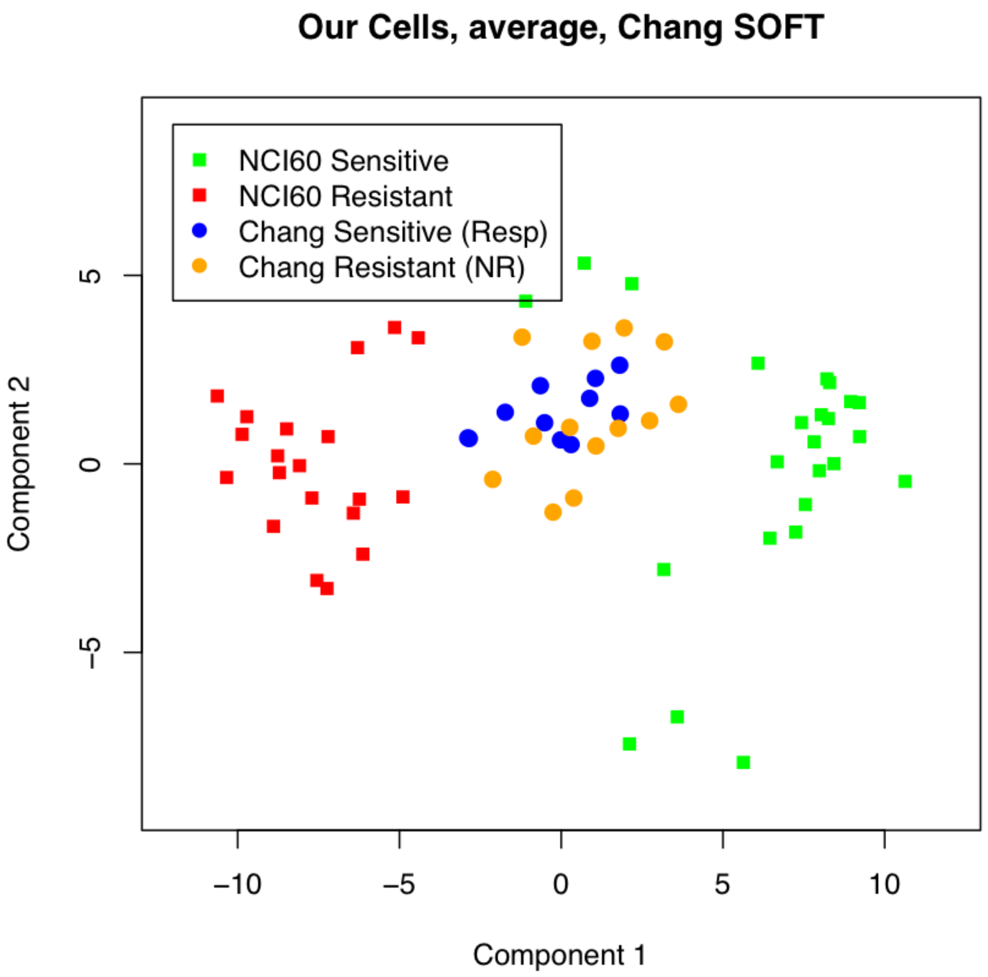
3. Testing: 24 breast tumors on U95Av2; Chang et al (2003) Lancet, 362:362-9. GSE349, GSE350 from GEO. (GSM4913 should be "sensitive". Pers comm.)

Fit Training Data



We want the test data to split like this...

Fit Testing Data



But it *doesn't.* Did we do something wrong?

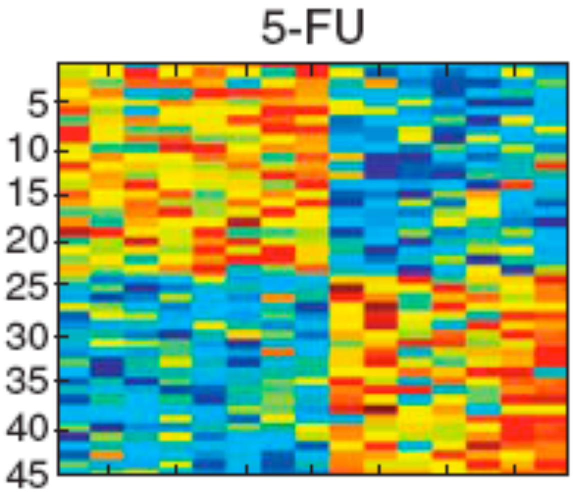
Examining Signatures

Lists of probesets used were given in a supplementary table.

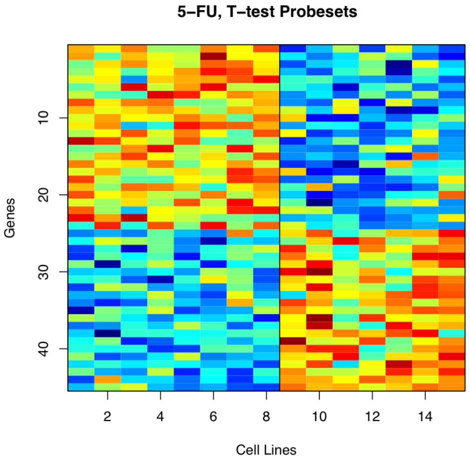
The paper explains why many of these genes make sense.

How were the genes found? Supplementary methods:
"a variance fixed t-test was used to calculate significance".

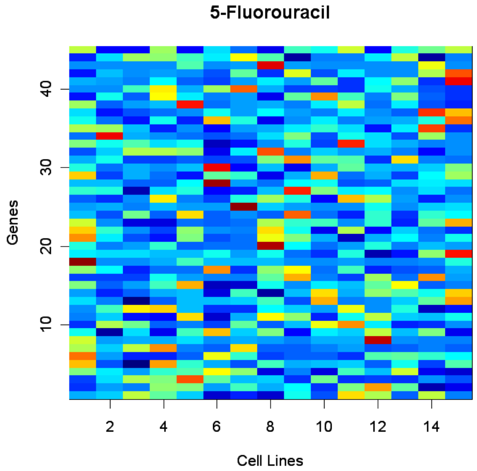
5-FU Heatmaps



Nat Med Paper



Our t-tests

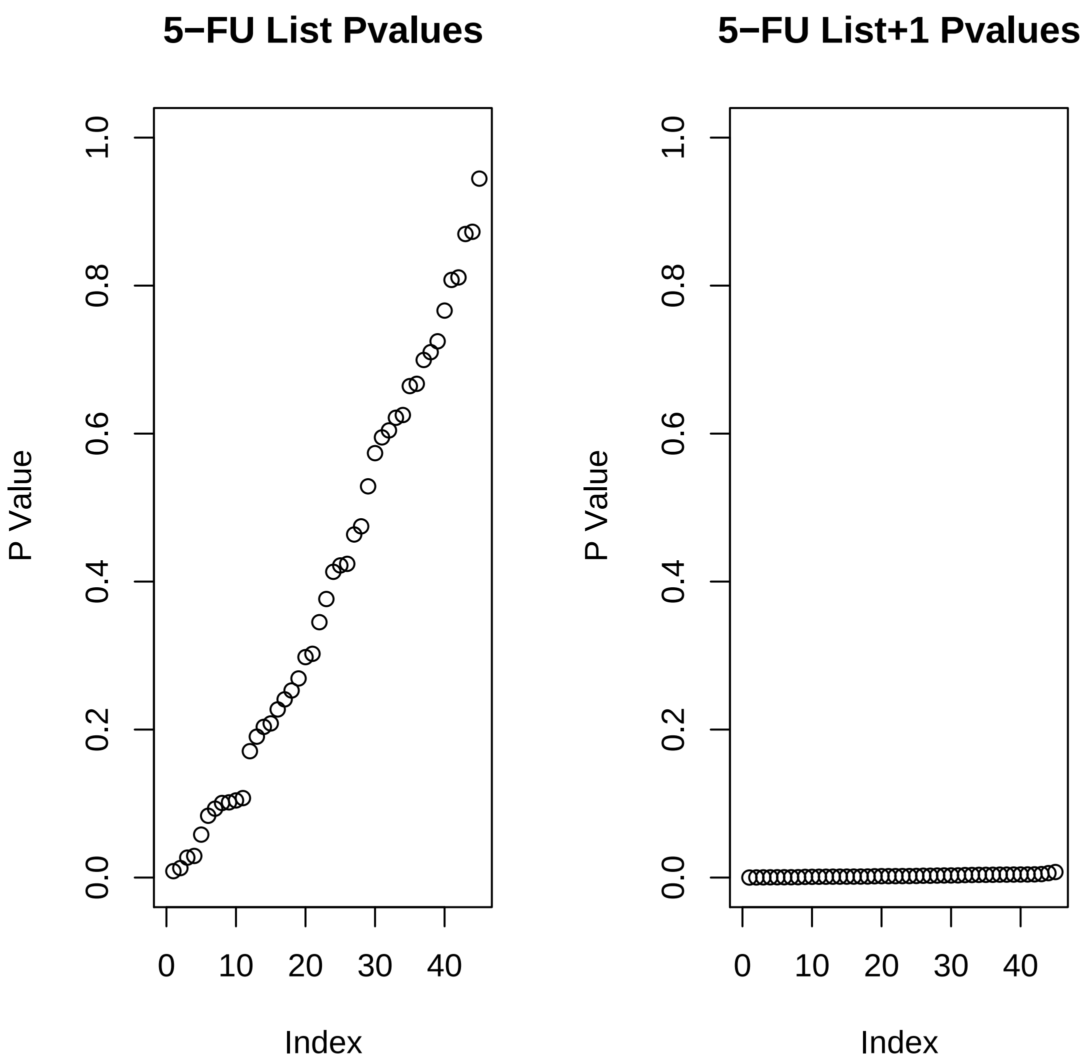


Reported Genes

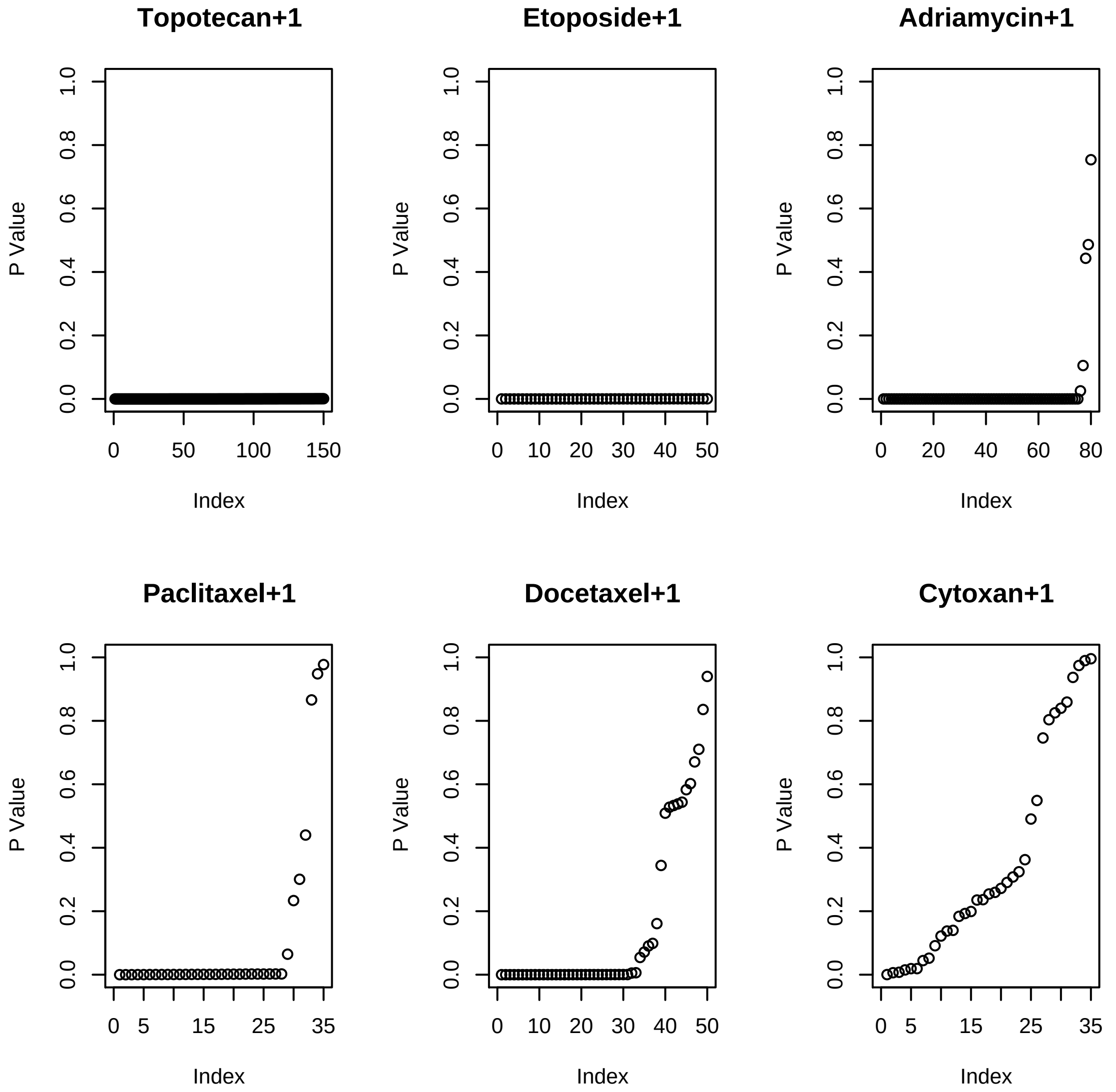
Their List and Ours

```
> temp <- cbind(
    sort(rownames(pottiUpdated)[fuRows]),
    sort(rownames(pottiUpdated)[
         fuTQNorm@p.values <= fuCut]);
> colnames(temp) <- c("Theirs", "Ours");
> temp
     Theirs          Ours
...
[3,] "1881_at"       "1882_g_at"
[4,] "31321_at"      "31322_at"
[5,] "31725_s_at"    "31726_at"
[6,] "32307_r_at"    "32308_r_at"
...
```

Offset P-Values: 5FU



Offset P-Values: Other Drugs



Using Their Software

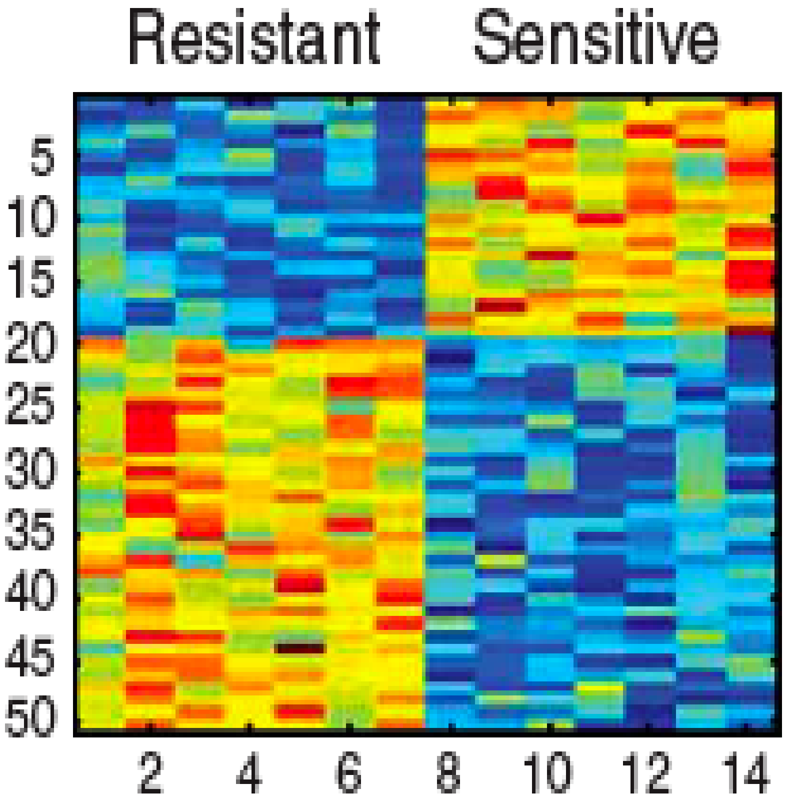
Their software requires two input files:

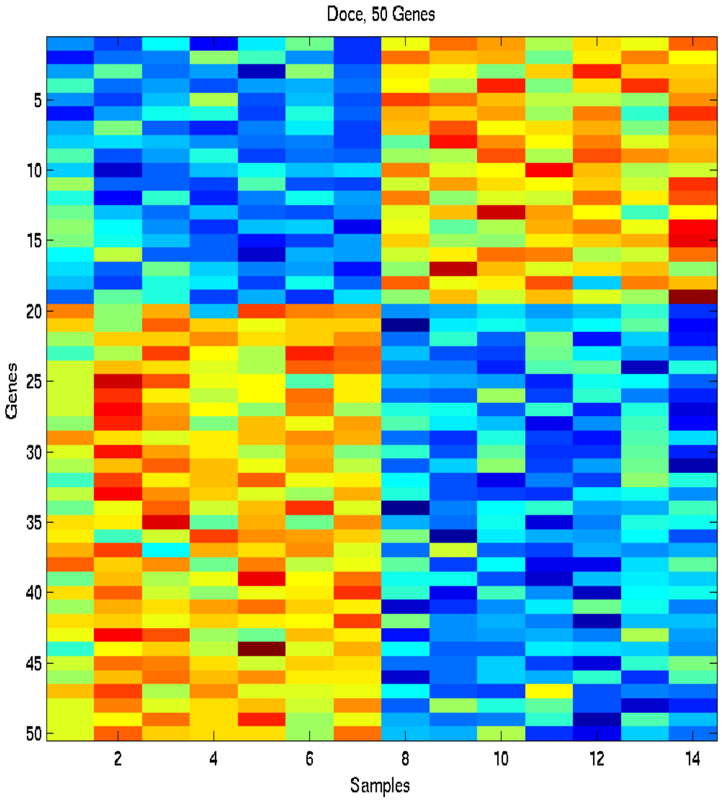
1. *a quantification matrix*, genes by samples, with a header giving classifications (0 = Resistant, 1 = Sensitive, 2 = Test)

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

What do we get?

Heatmaps Match Exactly for Docetaxel!



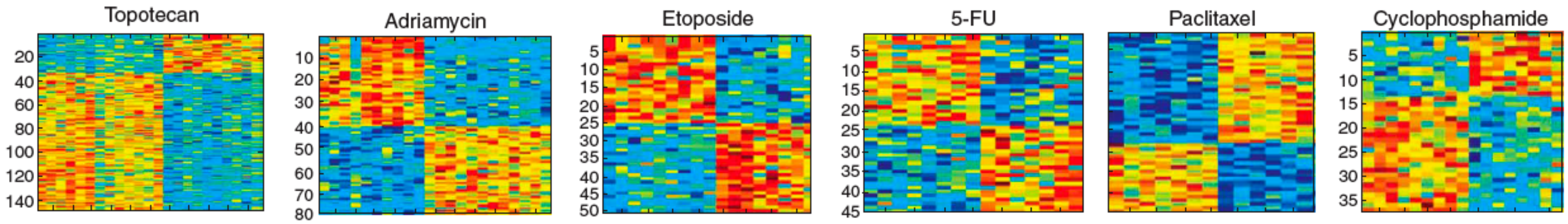


From Potti et al, Figure 1

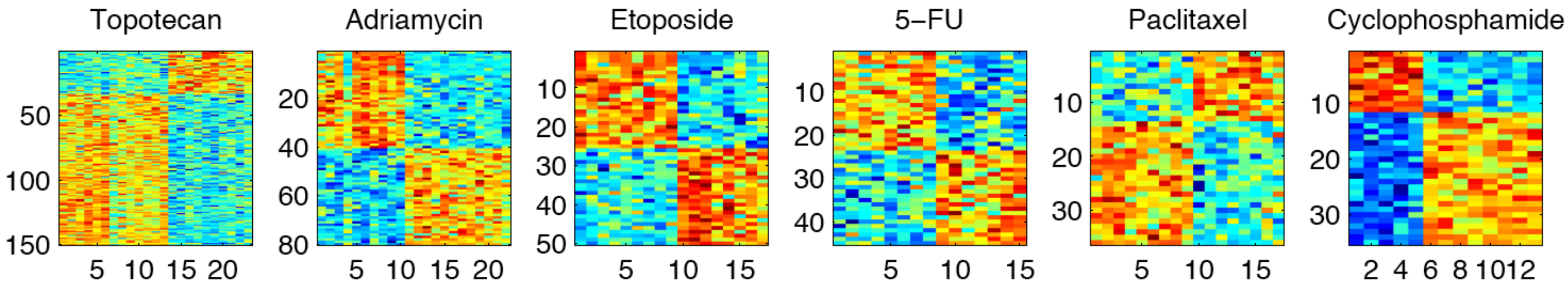
From the software

Heatmaps Match Exactly for 5 Others!

From the paper:

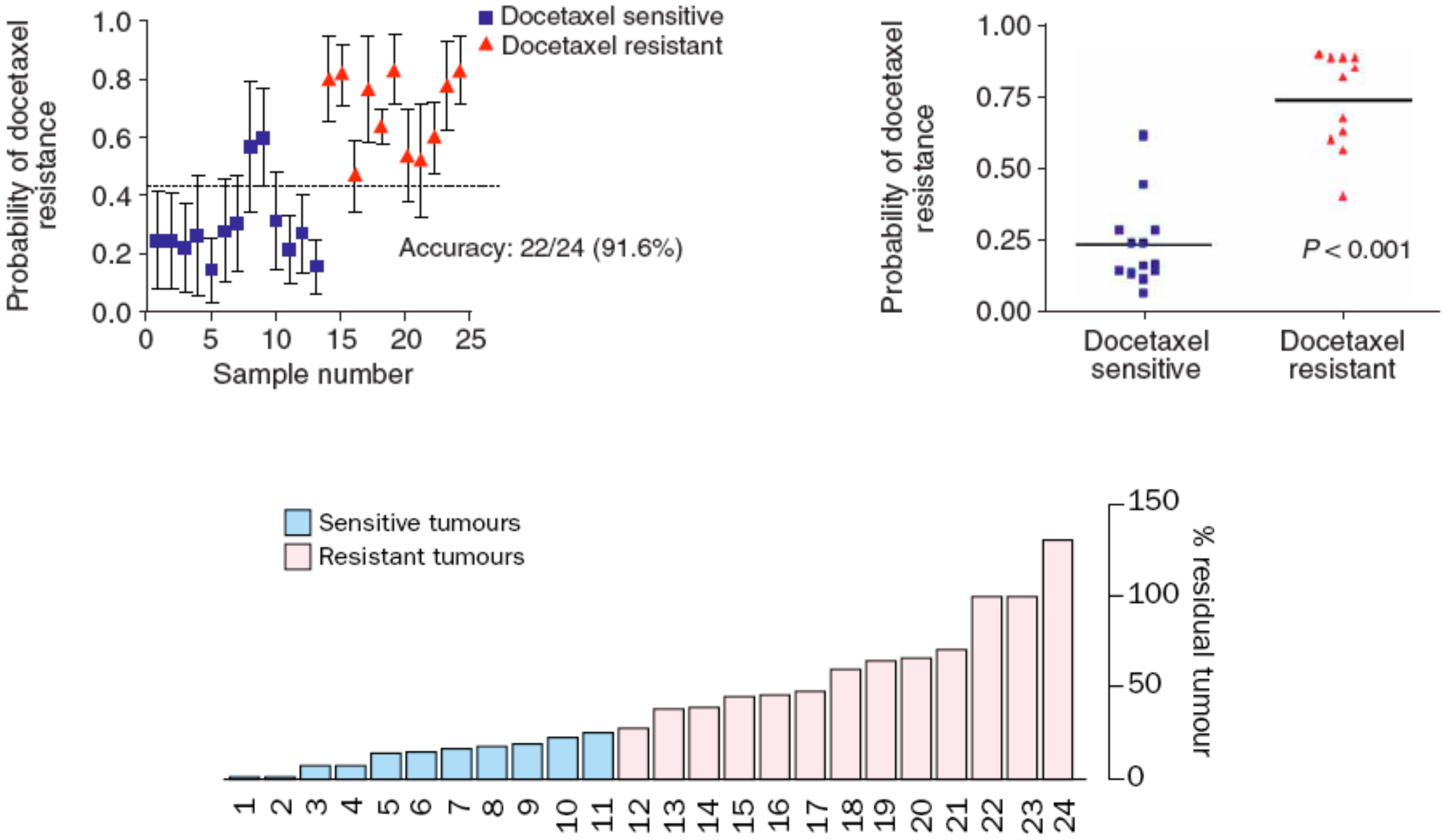


From the software:

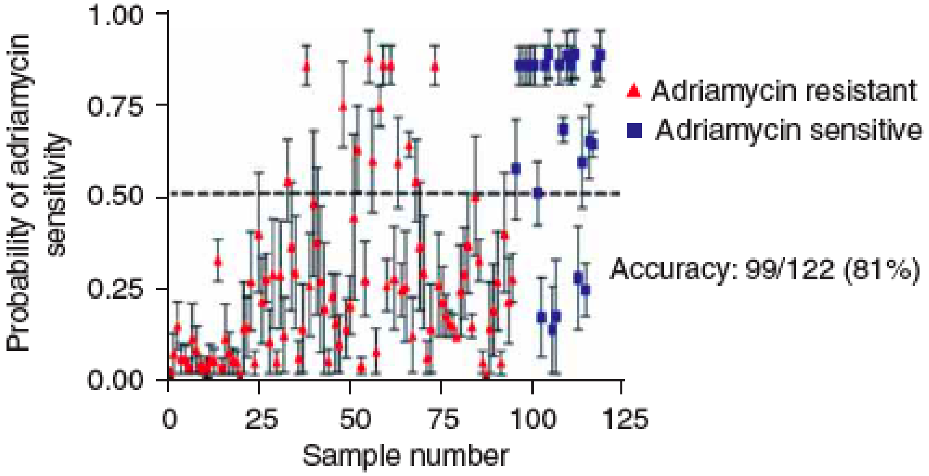


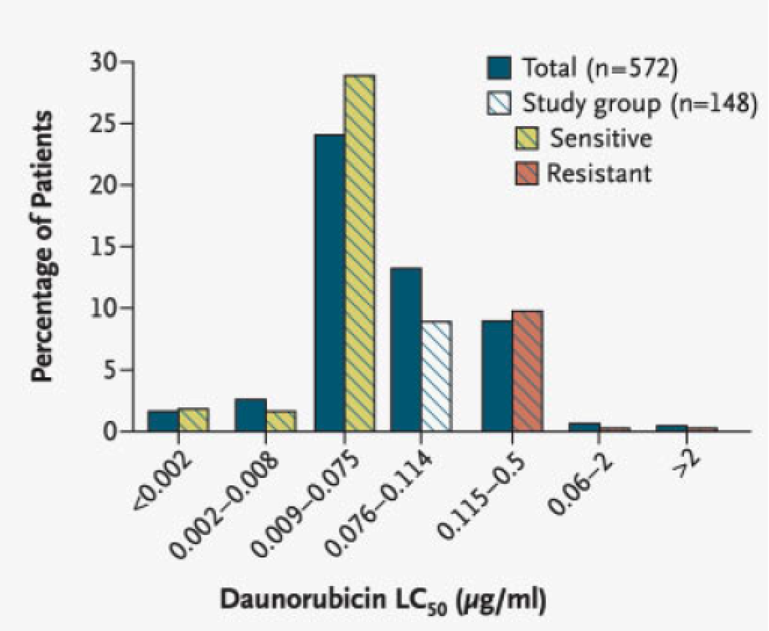
- We match heatmaps but not gene lists? We'll come back to this, because their software also gives *predictions*.

Predicting Docetaxel (Chang 03)



Predicting Adriamycin (Holleman 04)





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 Chang et al’s list comprise 14/19 outliers.

The others: ERCC1, ERCC4, ERBB2, BCL2L11, TUBA3.
These are the genes named to explain the biology.

RR Theme: Don't Take My Word For It!

Read the paper! Coombes, Wang & Baggerly, Nat Med, Nov 6, 2007, 13:1276-7, author reply 1277-8.

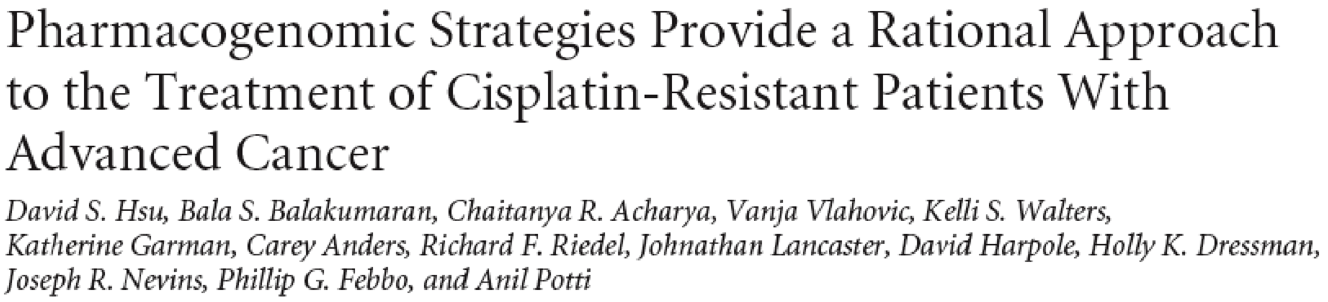
Try it yourselves! All of the raw data, documentation*, and code* is available from our web site (*and from Nat Med):

`http://bioinformatics.mdanderson.org/Supplements/ReproRsch-Chemo`.

Potti/Nevins Reply (Nat Med 13:1277-8)

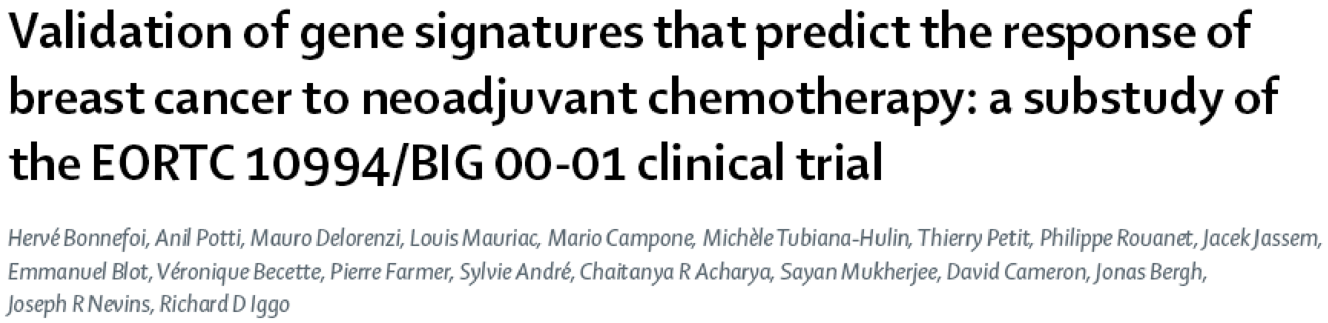
Labels for Adria are correct – details on their web page.

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

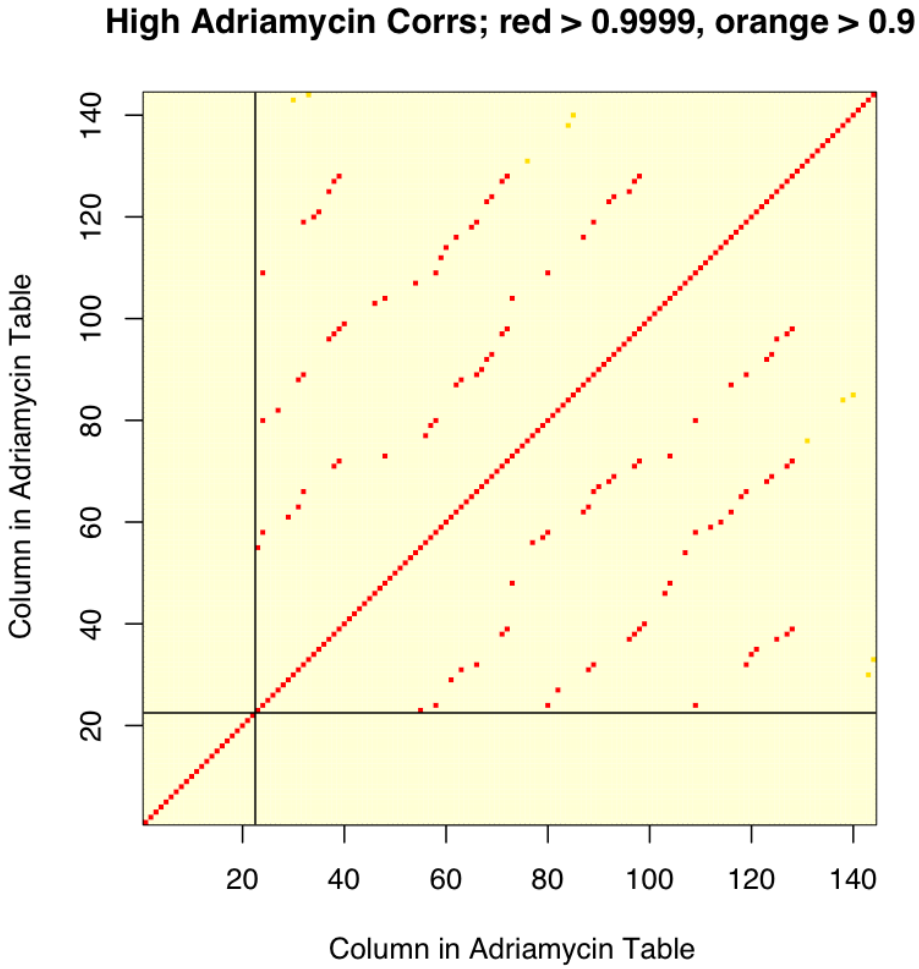
David S. Hsu, Bala S. Balakumaran, Chaitanya R. Acharya, Vanja Vlahovic, Kelli S. Walters, Katherine Garman, Carey Anders, Richard F. Riedel, Johnathan Lancaster, David Harpole, Holly K. Dressman, Joseph R. Nevins, Phillip G. Febbo, and Anil Potti



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

Adriamycin 0.9999+ Correlations (Reply)



Redone in Aug 08, “using only the 95 unique samples”

The First 20 Files Now Named

```
Sample ID  Response
 1 GSM44303   RES          11 GSM9694   RES
 2 GSM44304   RES          12 GSM9695   RES
 3 GSM9653    RES          13 GSM9696   RES
 4 GSM9653    RES          14 GSM9698   RES
 5 GSM9654    RES          15 GSM9699   SEN
 6 GSM9655    RES          16 GSM9701   RES
 7 GSM9656    RES          17 GSM9708   RES
 8 GSM9657    RES          18 GSM9708   SEN
 9 GSM9658    SEN          19 GSM9709   RES
10 GSM9658    SEN          20 GSM9711   RES
```

The First 20 Files Now Named

```
Sample ID  Response
 1 GSM44303   RES        11 GSM9694    RES
 2 GSM44304   RES        12 GSM9695    RES
 3 GSM9653    RES        13 GSM9696    RES
 4 GSM9653    RES        14 GSM9698    RES
 5 GSM9654    RES        15 GSM9699    SEN
 6 GSM9655    RES        16 GSM9701    RES
 7 GSM9656    RES        17 GSM9708    RES
 8 GSM9657    RES        18 GSM9708    SEN
 9 GSM9658    SEN        19 GSM9709    RES
10 GSM9658    SEN        20 GSM9711    RES
```

15 duplicates; 6 inconsistent. (61R, 13S, 6B) vs (22,48,10).

Validation 1: Hsu et al

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

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J Clin Oncol, Oct 1, 2007, 25:4350-7.

Same approach, using Cisplatin and Pemetrexed.

For cisplatin, U133A arrays were used for training. ERCC1, ERCC4 and DNA repair genes are identified as "important".

With some work, we matched the heatmaps. (Gene lists?)

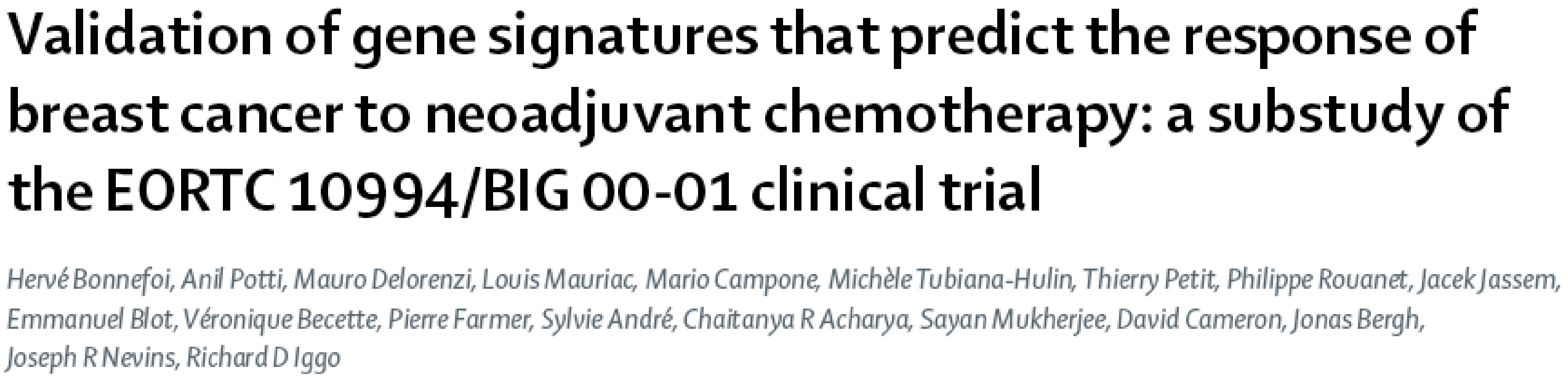
The 4 We Can't Match (Reply)

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

The last two probesets 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

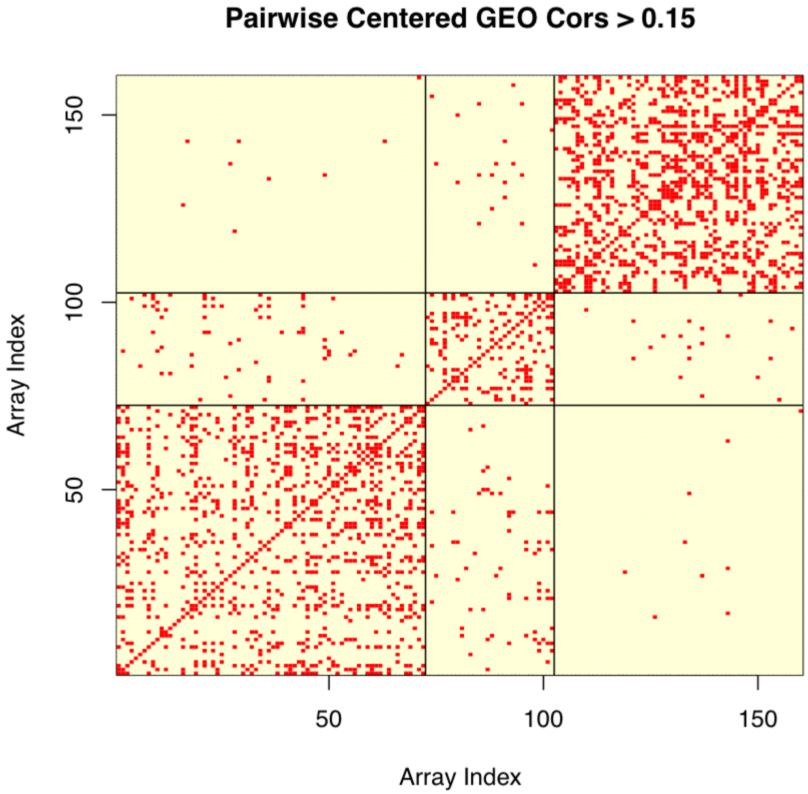
Hervé Bonnefoi, Anil Potti, Mauro Delorenzi, Louis Mauriac, Mario Campone, Michèle Tubiana-Hulin, Thierry Petit, Philippe Rouanet, Jacek Jassem, Emmanuel Blot, Véronique Becette, Pierre Farmer, Sylvie André, Chaitanya R Acharya, Sayan Mukherjee, David Cameron, Jonas Bergh, Joseph R Nevins, Richard D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

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

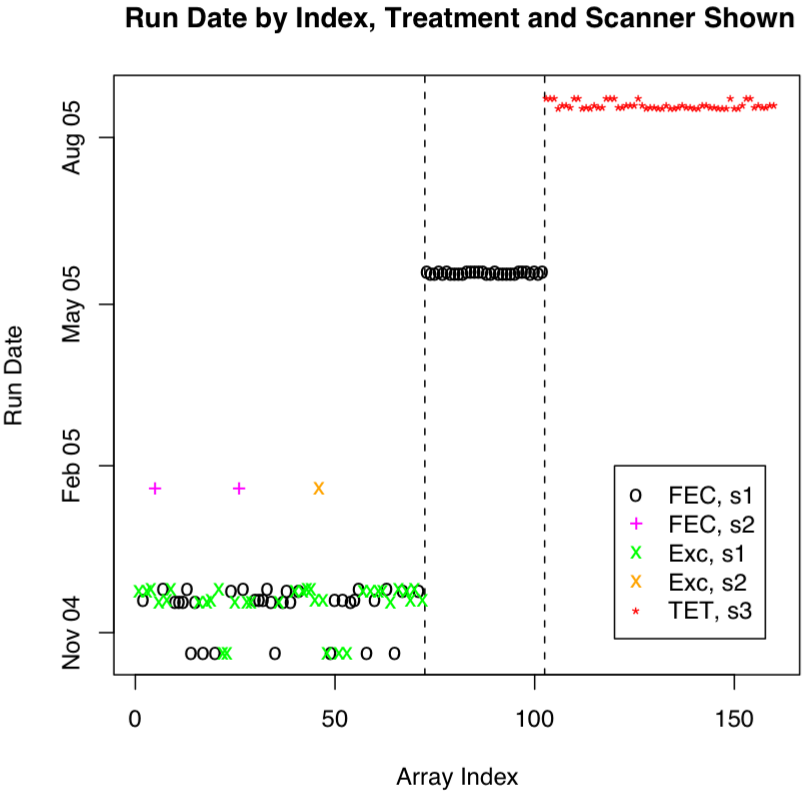
Similar approach, using signatures for Fluorouracil, Epirubicin Cyclophosphamide, and Taxotere to predict response to combination therapies: FEC and TET.

Potentially improves ER- response from 44% to 70%.

We Might Expect Some Differences...


Pairwise Centered GEO Cors > 0.15

High Sample Correlations after Centering by Gene


Run Date by Index, Treatment and Scanner Shown

Array Run Dates

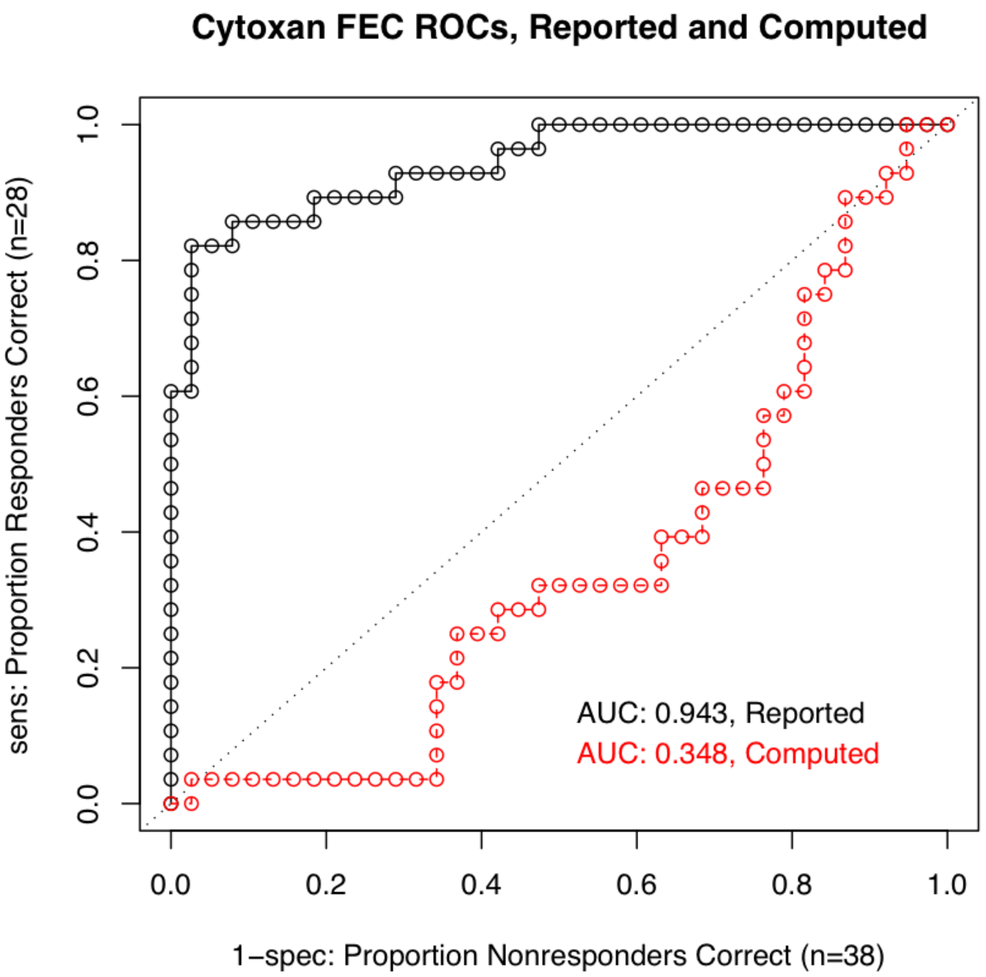
How Are Results Combined?

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

- $$P(TFAC) = P(T)+P(F)+P(A)+P(C)-P(T)P(F)P(A)P(C).$$
- $$P(ET) = \max[P(E), P(T)].$$
- $$P(FEC) = \frac{5}{8}[P(F) + P(E) + P(C)] - \frac{1}{4}.$$

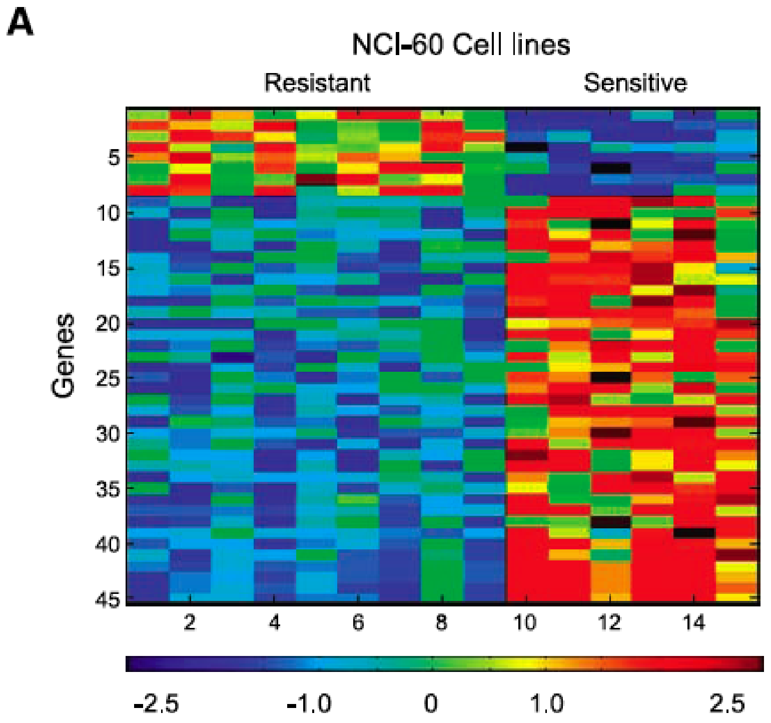
Each rule is different.

Predictions for Individual Drugs? (Reply)

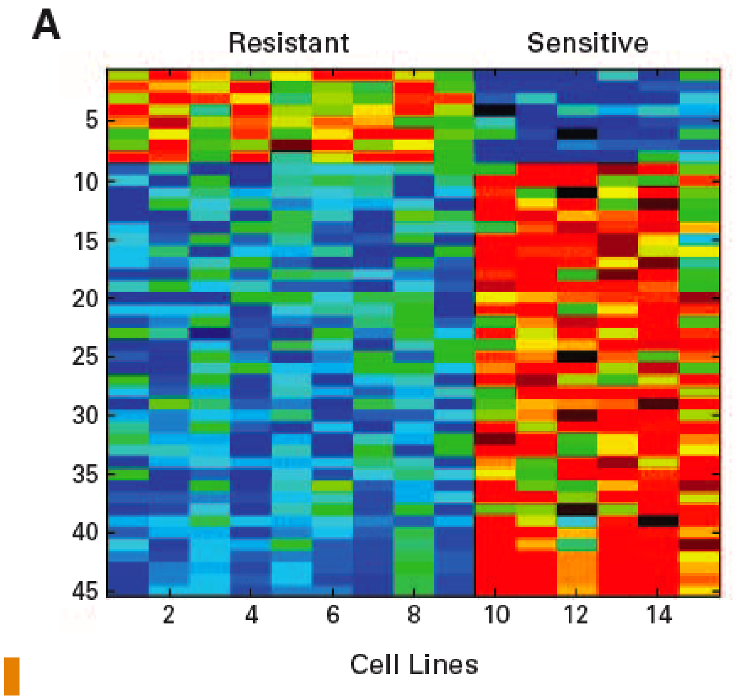


Does cytoxan make sense?

Temozolomide Heatmaps



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 Timeline Here...

Nat Med Nov 06*, Nov 07*, Aug 08.
JCO Lung Oct 07*.
Lancet Oncology Breast Dec 07*.
CCR Temozolomide Jan 09*.
(* errors reported to journals.)
... other more recent papers ...

Things we learned May/June 2009:

clinical trials had begun.
2007: pemetrexed vs cisplatin, pem vs vinorelbine.
2008: docetaxel vs doxorubicin, topotecan vs dox (Moffitt).

More Timeline (2009)

Sep 1. Paper submitted to *Annals of Applied Statistics*.

Sep 14. Paper online at *Annals of Applied Statistics*.

Late Sep. Duke starts internal investigation.
Oct 2. Story covered by *The Cancer Letter*.*
Oct 6. Two Duke clinical trials suspended.
Oct 8. Moffitt trial terminated.
Oct 9. Suspensions covered in *The Cancer Letter*.
Oct 19. Third Duke trial suspended.
Oct 23. Blinded validation discussed in *The Cancer Letter*. *
(Jan/Feb 2010 - *The IMS Bulletin*!)

* Isn't all this moot if it works in a blinded validation?

Well, About That “Blinding”

“Data was made available to us, blinded. All we got was the gene expression data. We ran the predictions and sent it back to the EORTC investigators” – *Joe Nevins, Oct 2.*

```
Sample info supplied:
Arm, Composite label
A, npCR Ep P- T3 N1 HB01 ...
A, pCR Ep Pp T2 N1 HB04
```

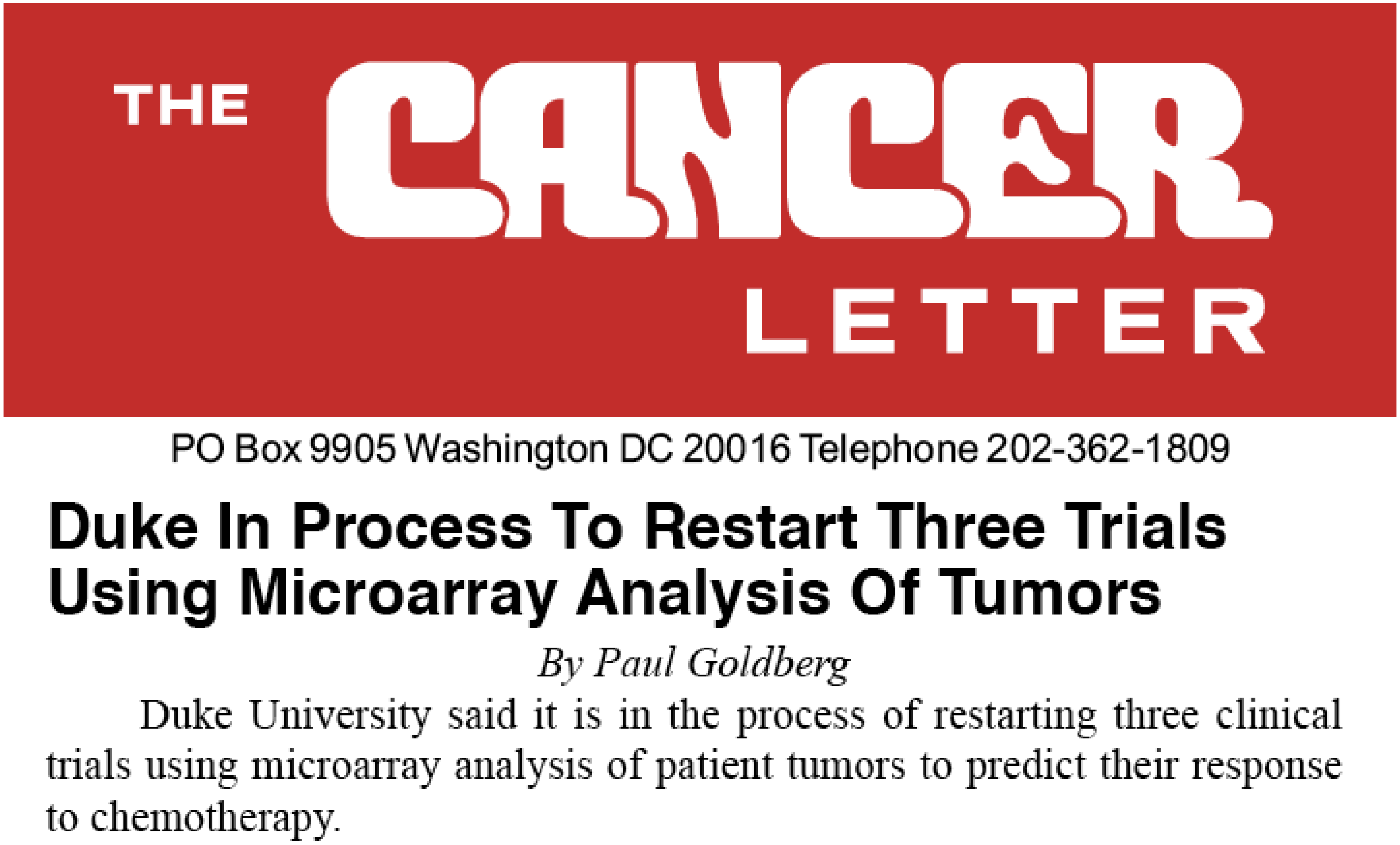
The data weren’t blinded.

“we would not be able to reproduce the reported probabilities with the information we have about how they were obtained.”
– *Mauro Delorenzi, Oct 23.*

Or validated.

So, what happened next?

Jan 29, 2010



THE CANCER LETTER

PO Box 9905 Washington DC 20016 Telephone 202-362-1809

Duke In Process To Restart Three Trials Using Microarray Analysis Of Tumors

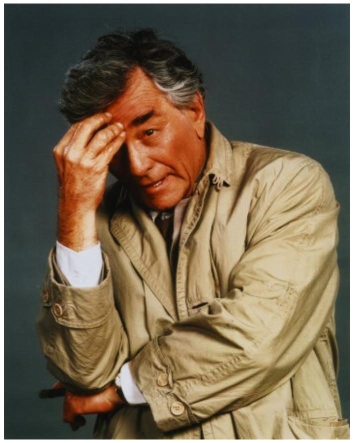
By Paul Goldberg

Duke University said it is in the process of restarting three clinical trials using microarray analysis of patient tumors to predict their response to chemotherapy.

Their investigation's results *"strengthen ... confidence in this evolving approach to personalized cancer treatment."*

Why We're Unhappy...

"While the reviewers approved of our sharing the report with the NCI, *we consider it a confidential document*" (Duke). A *future paper* will explain the methods.

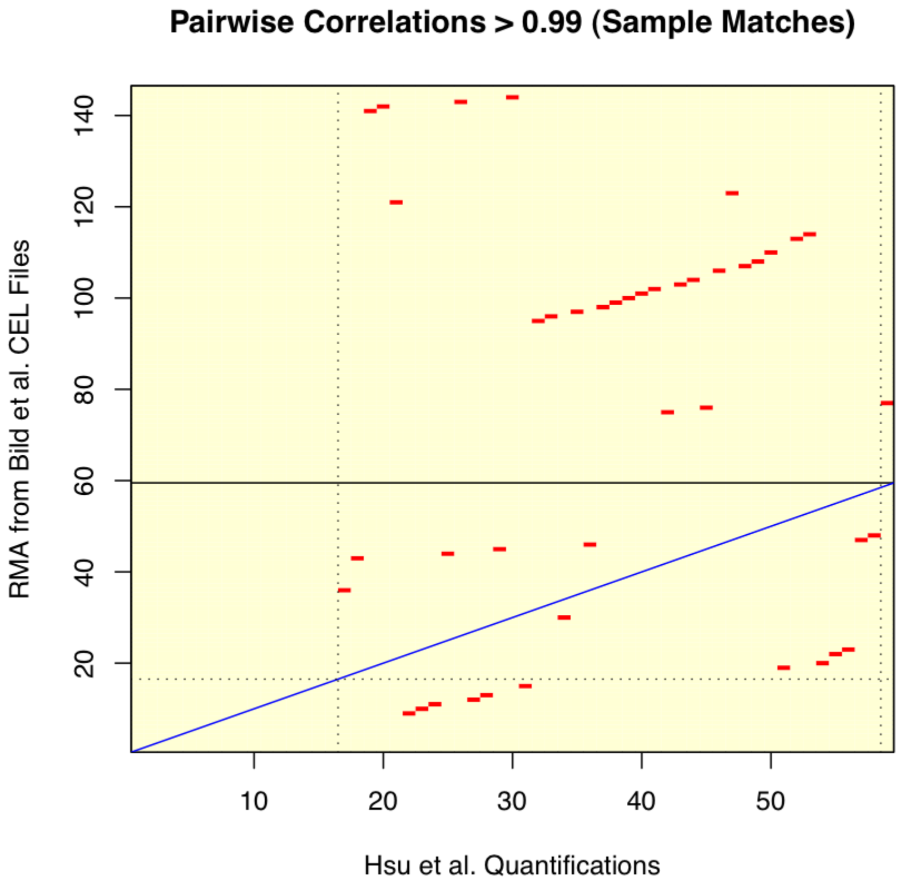


oh, there's just one more thing...

In mid-Nov (mid-investigation), the Duke team posted new data for cisplatin and pemetrexed (in trials since '07).

These included quantifications for 59 ovarian cancer test samples (from GSE3149) used for predictor validation.

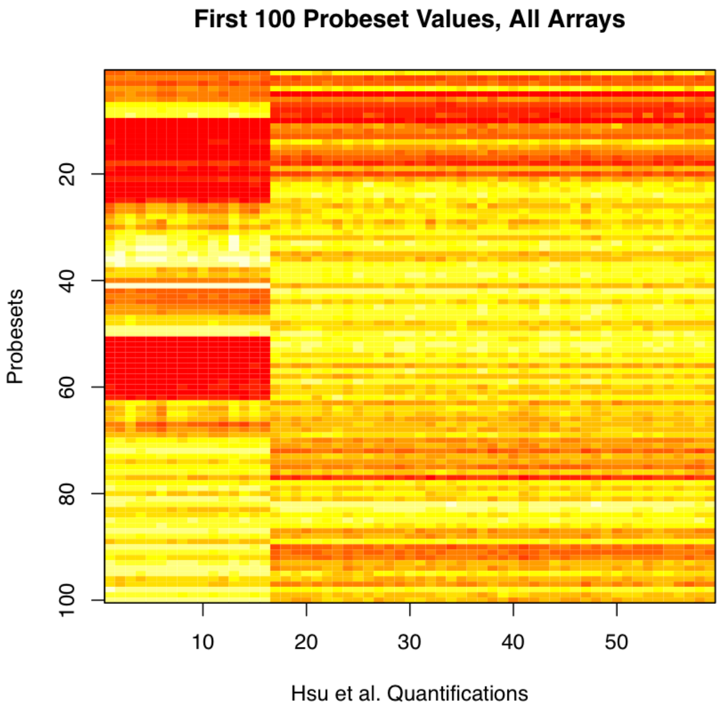
We Tried Matching The Samples



We correlated the 59 vectors with all samples in GSE3149.

43 samples are mislabeled; 16 don’t match at all.

Why Can’t We Match Some At All?



We checked the first 100 probeset intensities across samples.

The first 16 don’t match because the genes are mislabeled.

We reported this to Duke and to the NCI in mid-November.

All data was stripped from the websites within the week.

So, What Next?

The trials resumed.
We waited to see the methods.
We waited.
We tried being patient.

We're not very good at it.

We know Duke won't show us the report.
But Duke showed it to the NCI.
Would the NCI show us the report?
Might the NCI *have to* show us the report?

FOI(L)A!

April 7: Paul Goldberg of *the Cancer Letter* requests "access to and copies of the report (and attendant data)" from the NCI under the Freedom of Information Act (FOIA).
"I look forward to your reply within 20 business days, as the statute requires."

April 26: NCI agrees in principle to release the report, redacting only the names of the authors. Duke legal is allowed further redactions to protect trade secrets.

May 3: redacted report supplied.

May 7: other statisticians invited to comment.

May 14: story covered in *the Cancer Letter*.

Some Interesting Things...

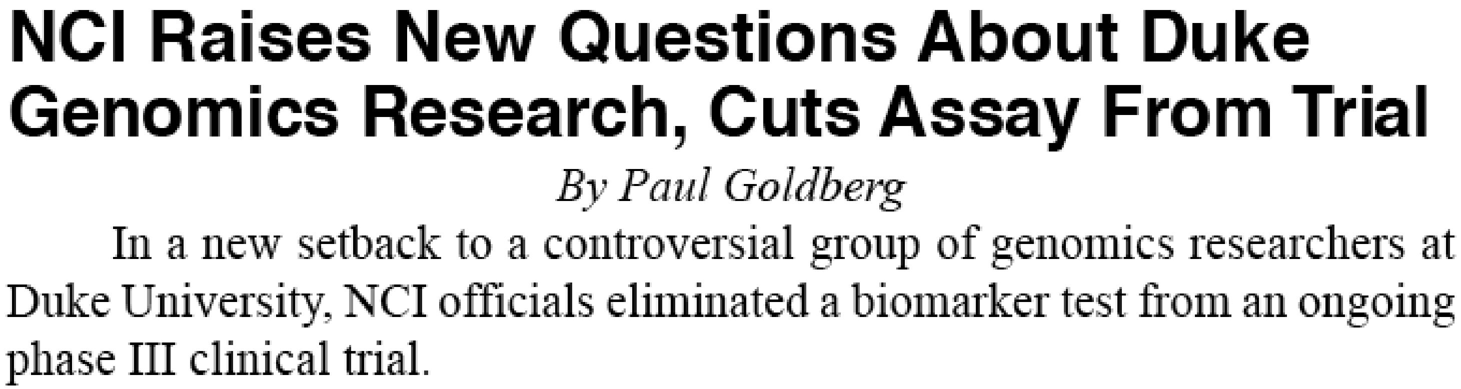
“In our review of the methods... *we were unable to identify a place where the statistical methods were described in sufficient detail to independently replicate the findings of the papers*. Only by examining the R code from Barry were we able to uncover the true methods used.”

The Duke investigators *really need* to work on “clearly explaining ... the specific statistical steps used in developing the predictors and the prospective sample assignments”

The supporting data and code weren’t sent to the NCI.

The report makes no mention of the problems with cisplatin/pemetrexed that arose during the investigation.

May 14, 2010



NCI Raises New Questions About Duke Genomics Research, Cuts Assay From Trial

By Paul Goldberg

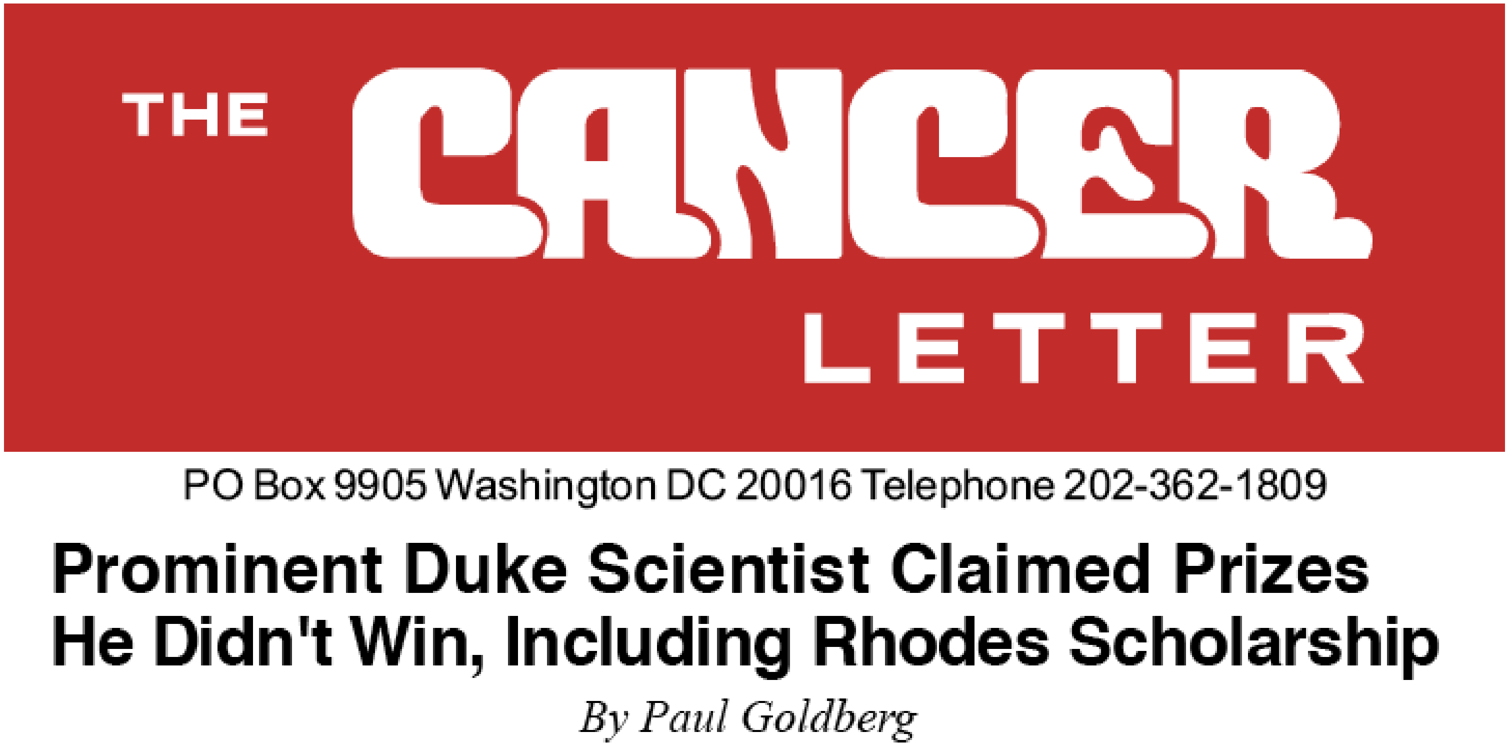
In a new setback to a controversial group of genomics researchers at Duke University, NCI officials eliminated a biomarker test from an ongoing phase III clinical trial.

"We have asked [CALGB] to remove the Lung Metagene Score from the trial, because we were unable to confirm the score's utility" – *Jeff Abrams, CTEP director*

"When the issues came up with the review by Duke of their studies, we decided to review the LMS score in the trial we sponsored" (CALGB 30506).

(The NCI doesn't directly sponsor the resumed trials.)

July 16, 2010



THE CANCER LETTER

PO Box 9905 Washington DC 20016 Telephone 202-362-1809

Prominent Duke Scientist Claimed Prizes He Didn't Win, Including Rhodes Scholarship

By Paul Goldberg

July 19, 2010

“Duke administrators accomplished something monumental: they triggered a public expression of outrage from biostatisticians.”

A Baron, K Bandeen-Roche, D Berry, J Bryan, V Carey, K Chaloner, M Delorenzi, B Efron, R Elston, D Ghosh, J Goldberg, S Goodman, F Harrell, S Hilsenbeck, W Huber, R Irizarry, C Kendziorski, M Kosorok, T Louis, JS Marron, M Newton, M Ochs, G Parmigiani*, J Quackenbush, G Rosner, I Ruczinski, Y Shyr*, S Skates, TP Speed, JD Storey, Z Szallasi, R Tibshirani, S Zeger

Req to Varmus, DoD, ORI, Duke: suspend trials.

Subsequent Events

NPR blog
Duke announces trials resuspended
Science blog, Nature blog
NYT blog, article
Lancet Oncology issues Expression of Concern
NEJM states no questions raised
Varmus & Duke request IOM Involvement
Questions raised about NEJM paper
JCO launches investigation
Science news feature
More awards found to be wrong, COI claims

Scientists for RR

Google group formed
http://groups.google.com/group/reproducible-research

Correspondence to Nature

Working on White Paper Guidelines

It’s Not Just Them

This is a particularly egregious combination, but we’ve seen many of these problems before.

Critical Analysis of Microarray Data (CAMDA) 2002: annotations in the contest dataset were scrambled due to an Excel error.

Proteomics 2003-5: several studies showed effects driven by design confounding; calibration (annotation) and processing inconsistencies.

TCGA (current): label scrambling going from label 1 (raw) to label 2 (processed) data.

Other examples that never left MD Anderson.

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.

Some Lessons

Is our own work reproducible?

Literate Programming. For the past two years, we have required reports to be prepared in *Sweave*.

Reusing Templates.

Report Structure.

Executive Summaries.

Appendices. Some things we want to know all the time: *SessionInfo*, *Saves*, and *File Location*.

The buzz phrase is *reproducible research*.

Some Acknowledgements

Kevin Coombes

Shannon Neeley, Jing Wang

David Ransohoff, Gordon Mills

Jane Fridlyand, Lajos Pusztai, Zoltan Szallasi

MDACC Ovarian SPORE, Lung SPORE, Breast SPORE

Now in the *Annals of Applied Statistics!* Baggerly and Coombes (2009), 3(4):1309-34.
`http://bioinformatics.mdanderson.org/Supplements/ReproRsch-All`

Ovarian Cancer and Pathways

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

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Dressman et al, JCO, Feb 10, 2007.

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.

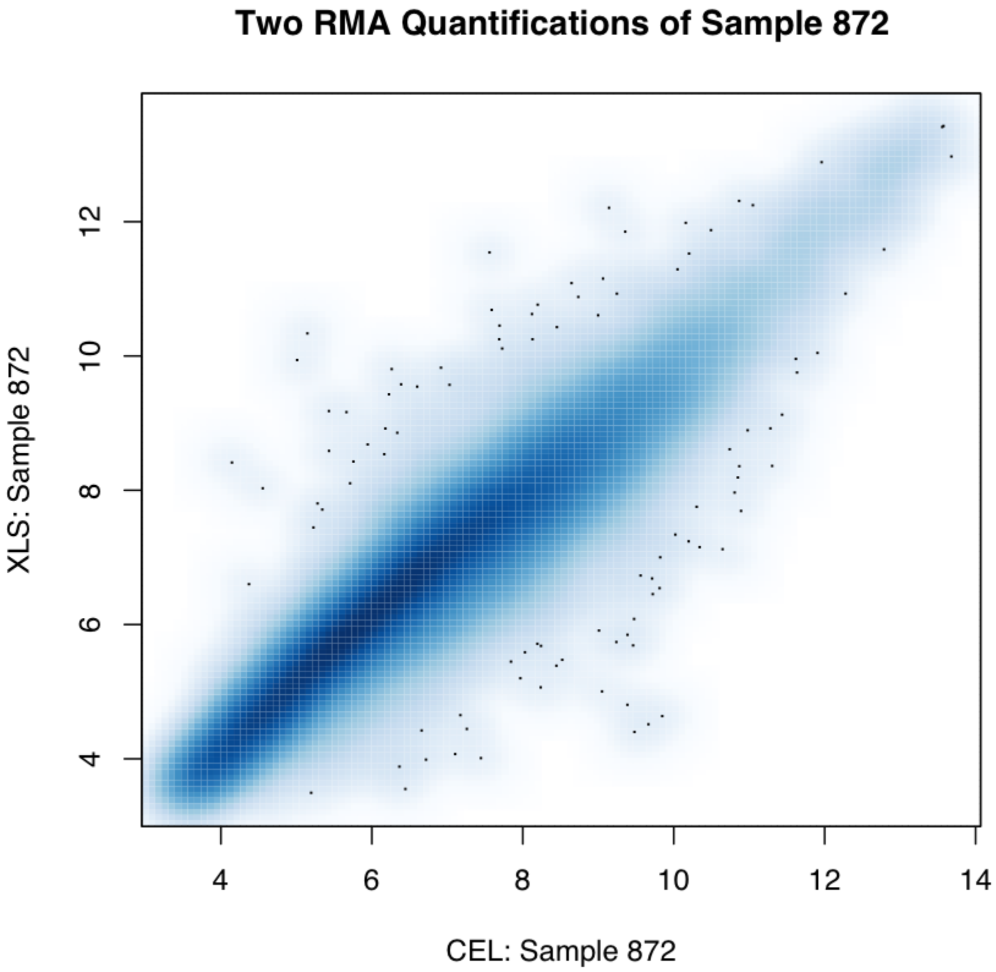
Looking at the Data

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.

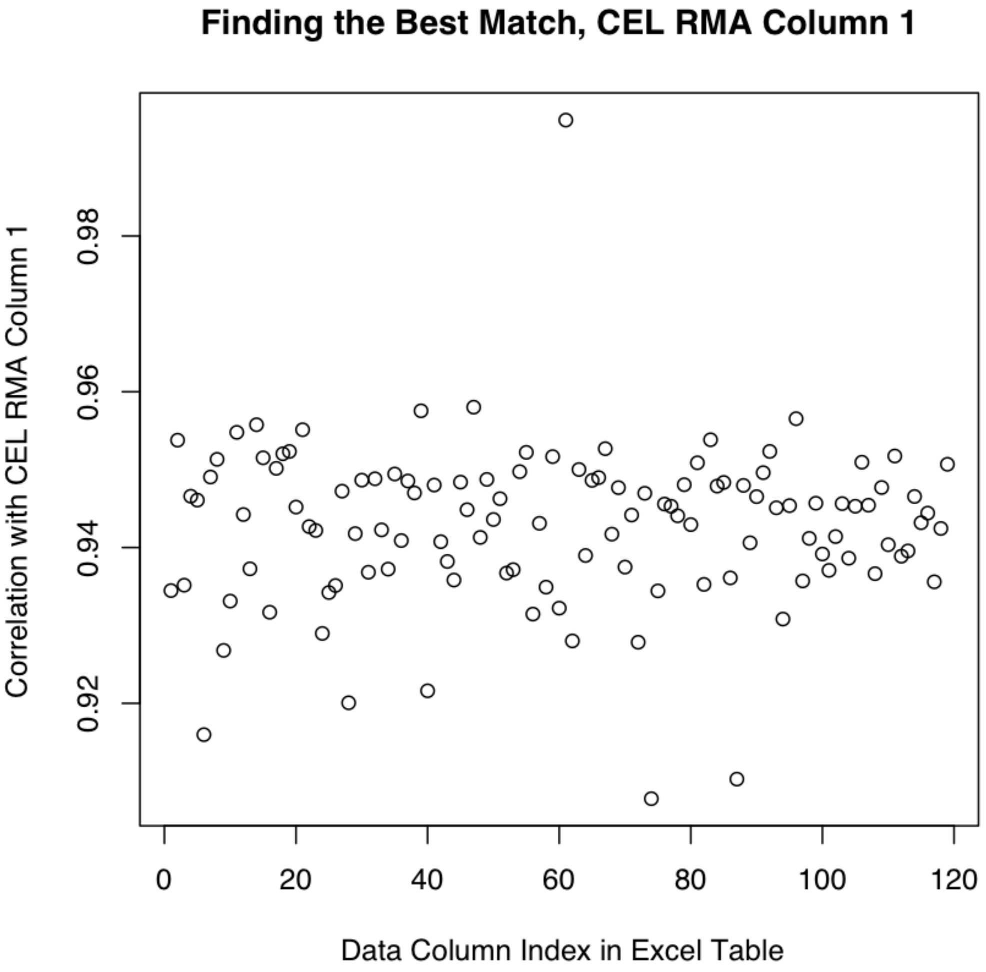
But, they used justRMA, so we could quantify the CEL files ourselves...

Checking Agreement



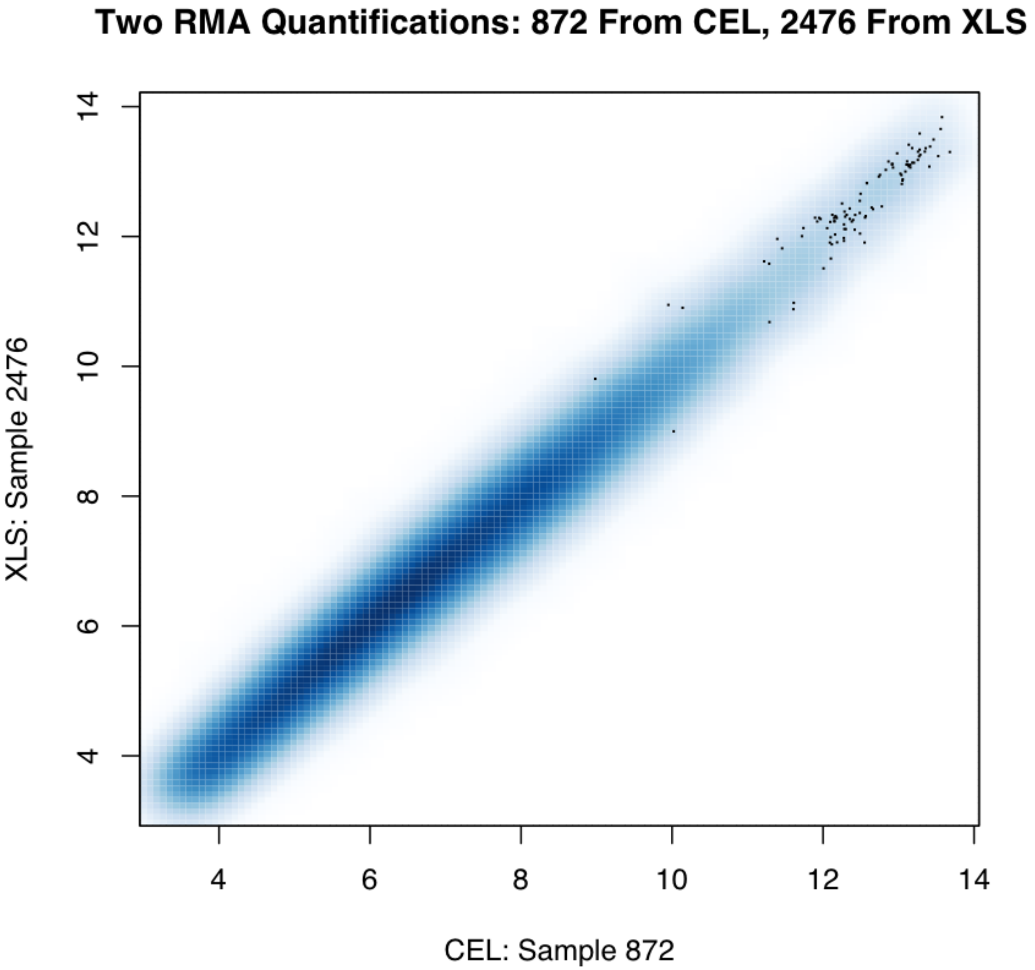
CELs vs Tables. We expected better (fewer outliers).

Looking at Their Other Quants



Which one would you pick?

Looking at The “Best” Fit



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.

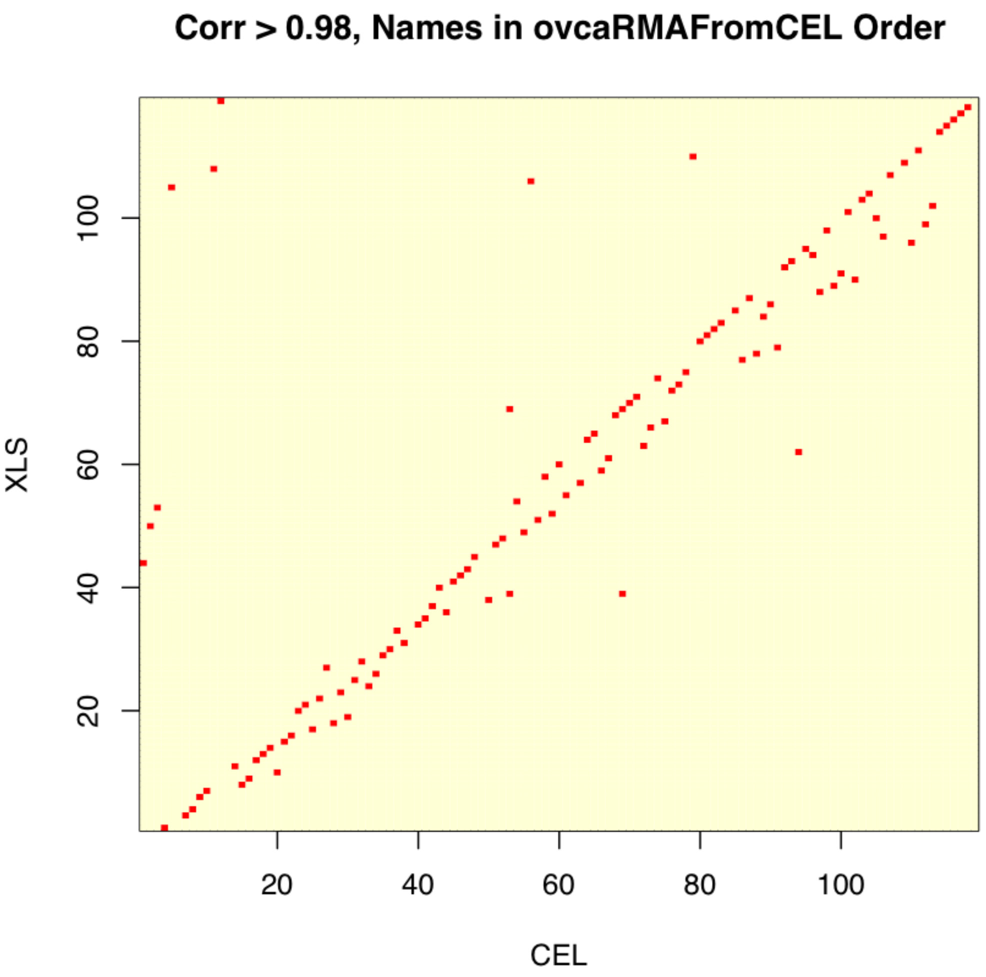
More Raw Data

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 clincial 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.