

The Importance of Reproducibility in High-Throughput Biology: Case Studies in Forensic Bioinformatics

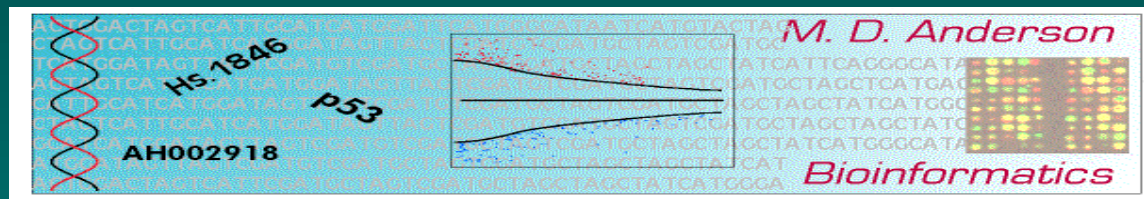
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UT M. D. Anderson Cancer Center

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TCGA Analysis Group, 8 September 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 Chan⁴, Robyn Sayer⁴,
Janiel Cragun⁴, Hope Cottrill⁴, Michael J Kelley², Rebecca Petersen⁵, David Harpole⁵, Jeffrey Marks⁵,
Andrew Berchuck^{1,6}, Geoffrey S Ginsburg^{1,2}, Phillip Febbo¹⁻³, Johnathan Lancaster⁴ &
Joseph R Nevins¹⁻³

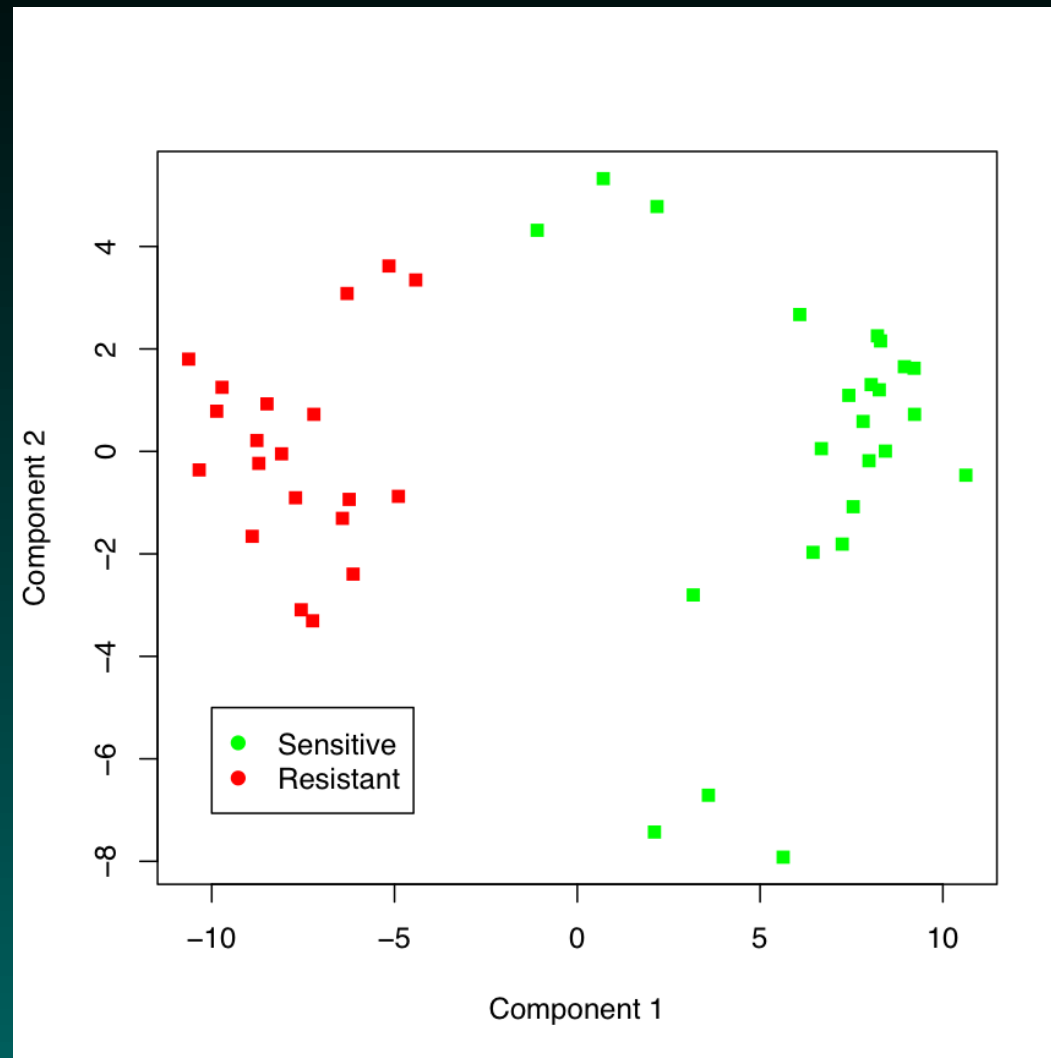
Potti et al (2006), *Nature Medicine*, 12:1294-1300.

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.

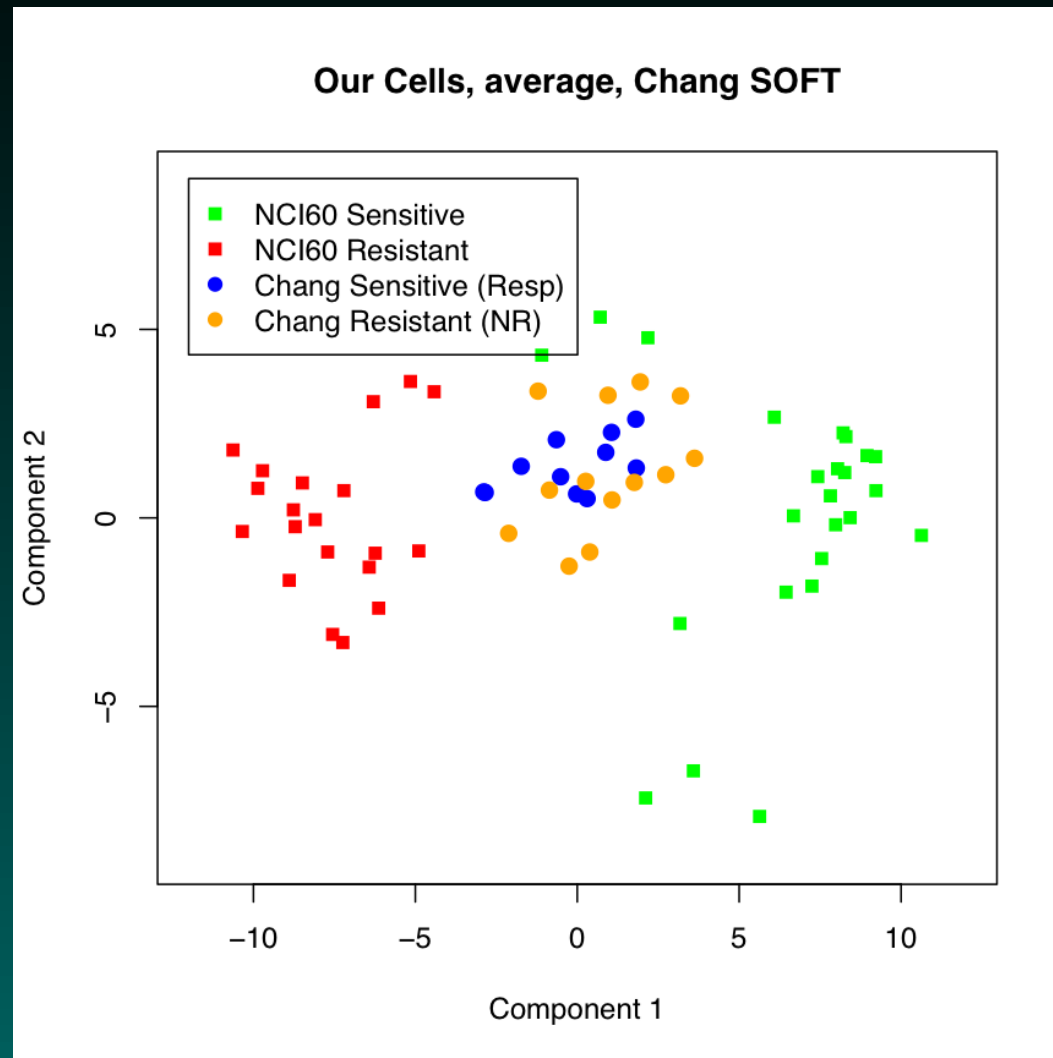
This got people at MDA very excited.

Fit Training Data



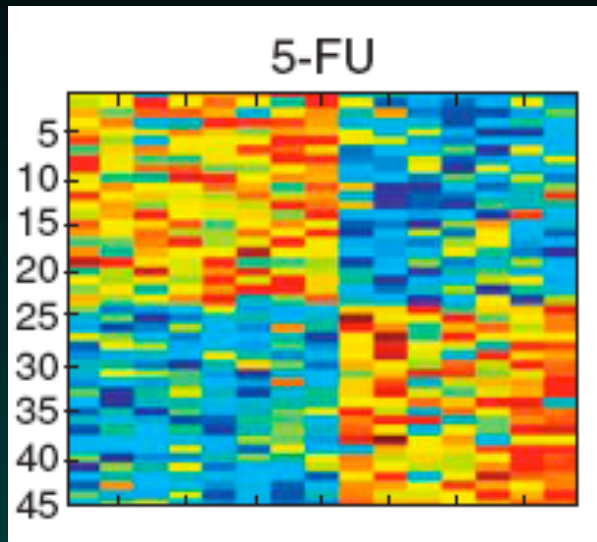
We want the test data to split like this...

Fit Testing Data



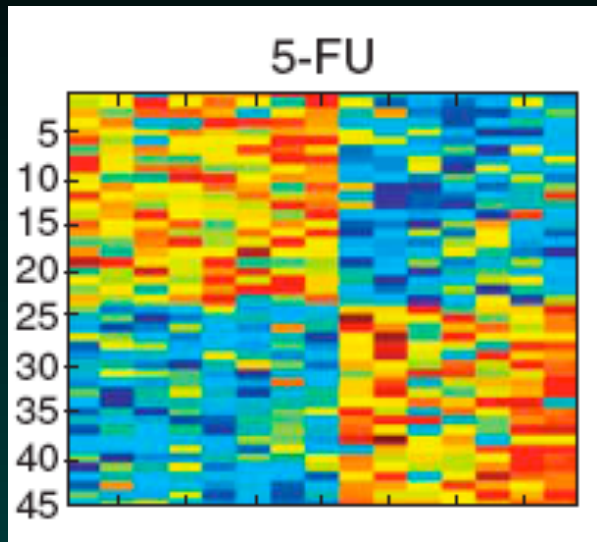
But it *doesn't*. Did we do something wrong?

5-FU Heatmaps

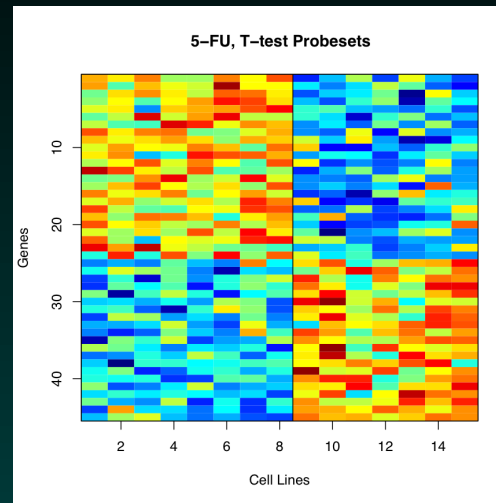


Nat Med Paper

5-FU Heatmaps

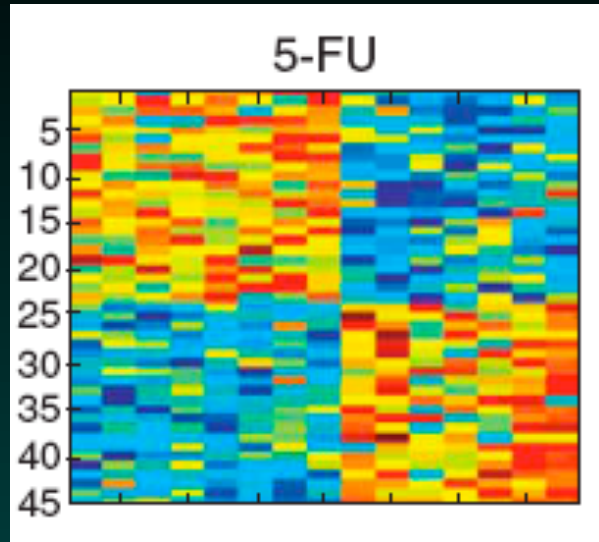


Nat Med Paper

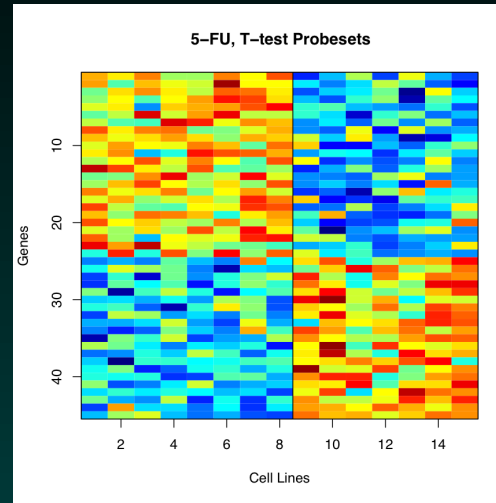


Our t-tests

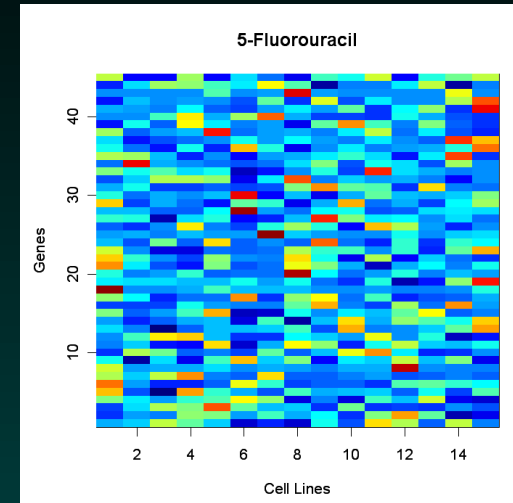
5-FU Heatmaps



Nat Med Paper



Our t-tests

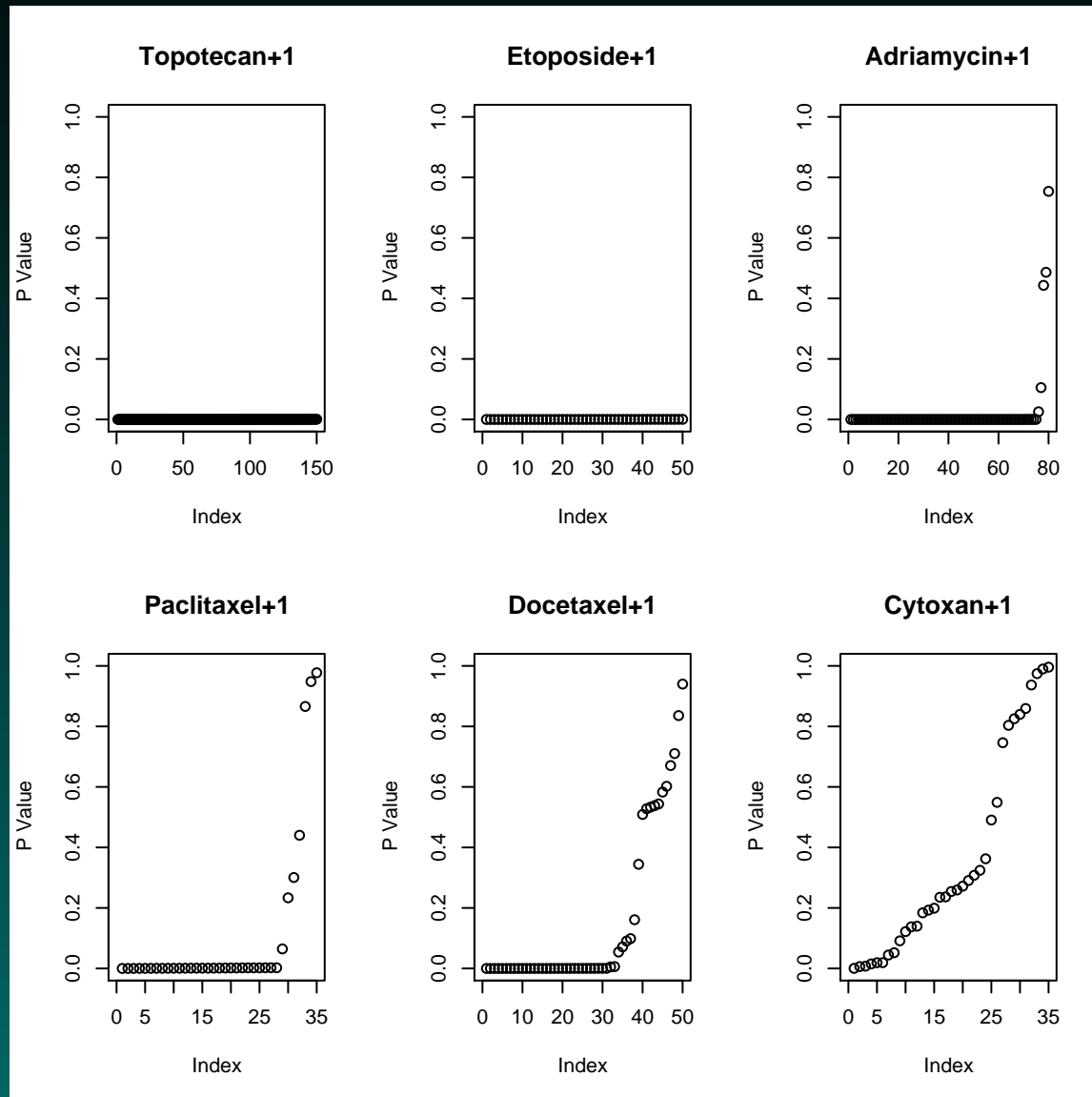


Reported Genes

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: Other Drugs



Using Their Software

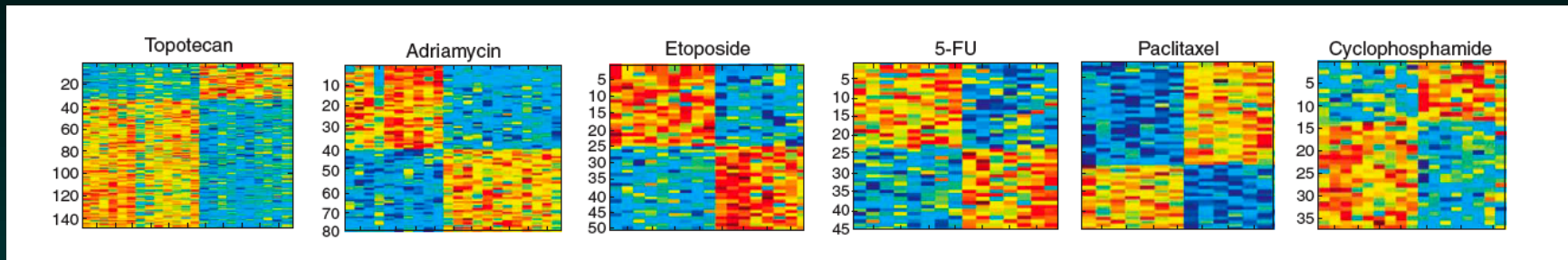
Their software requires two input files:

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.***

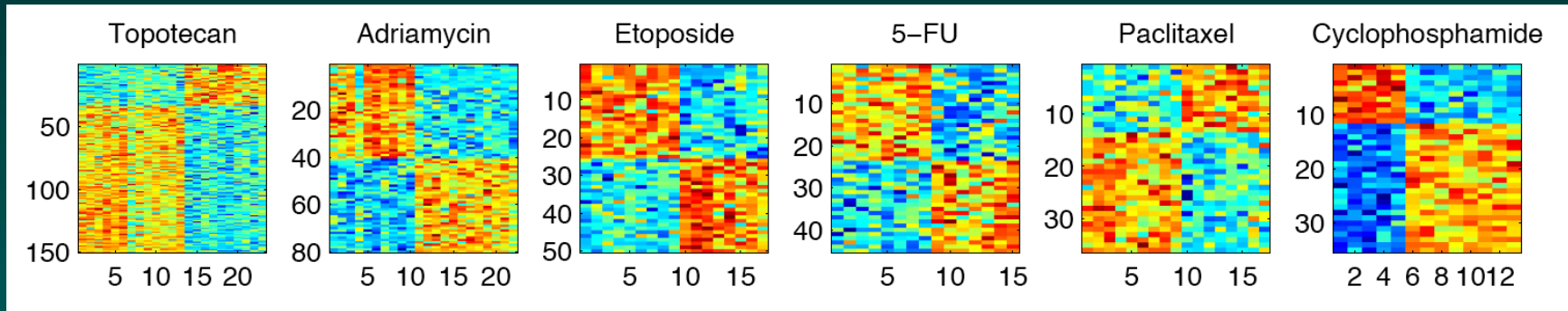
What do we get?

Heatmaps Match Exactly for Most Drugs!

From the **paper**:

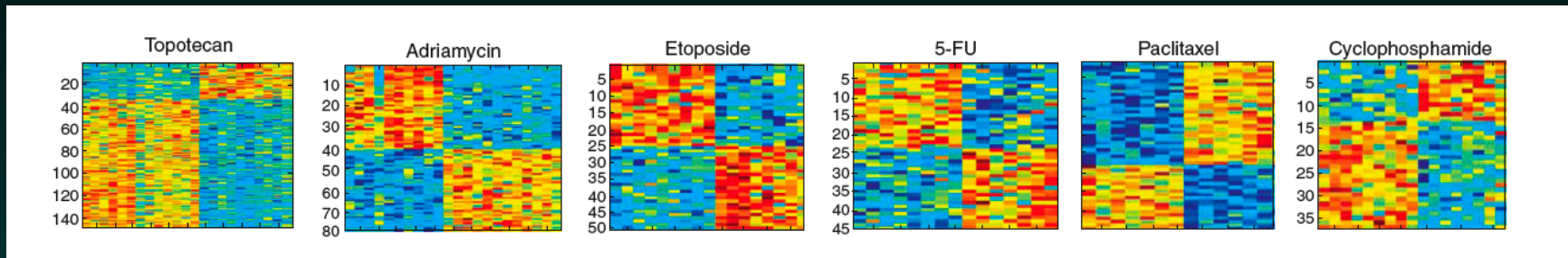


From the **software**:

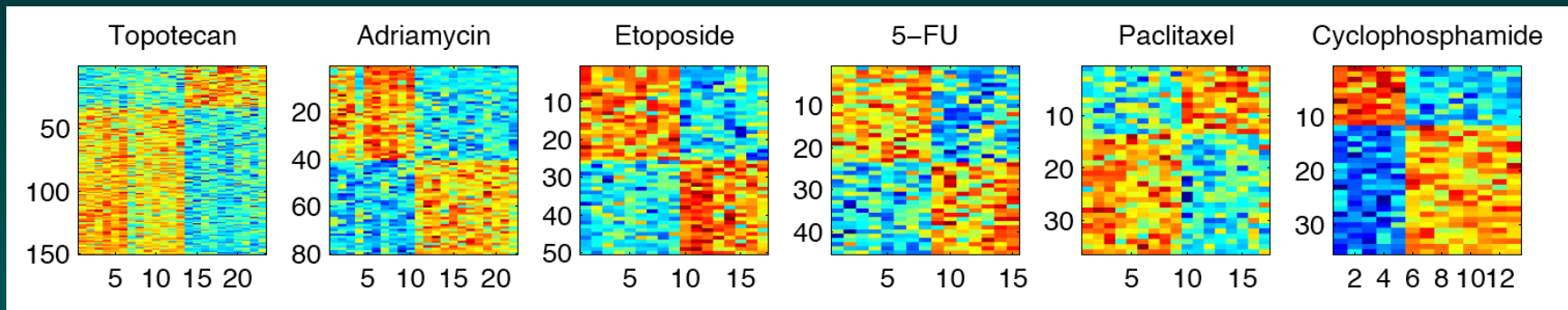


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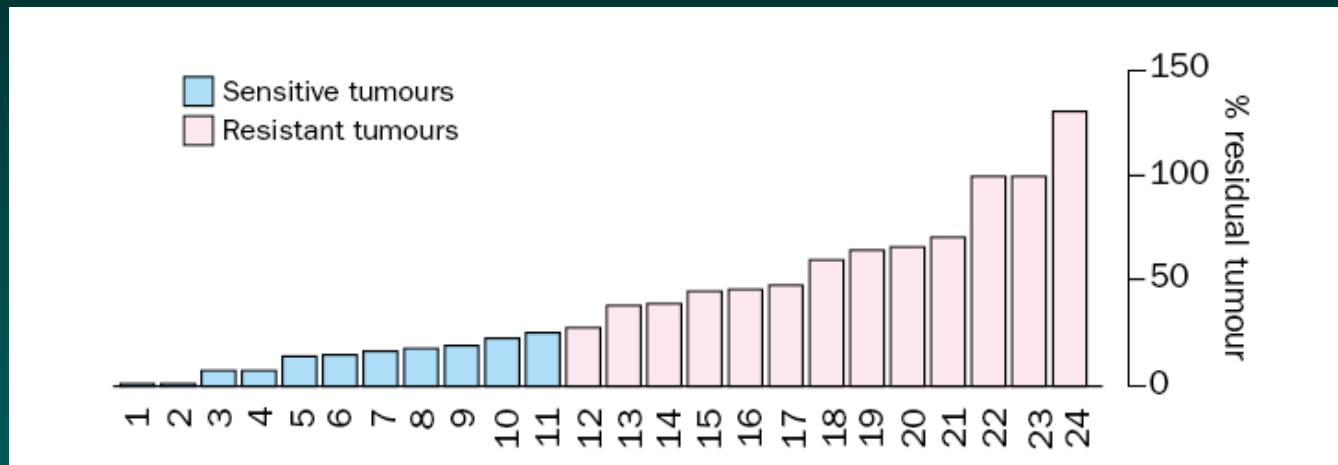
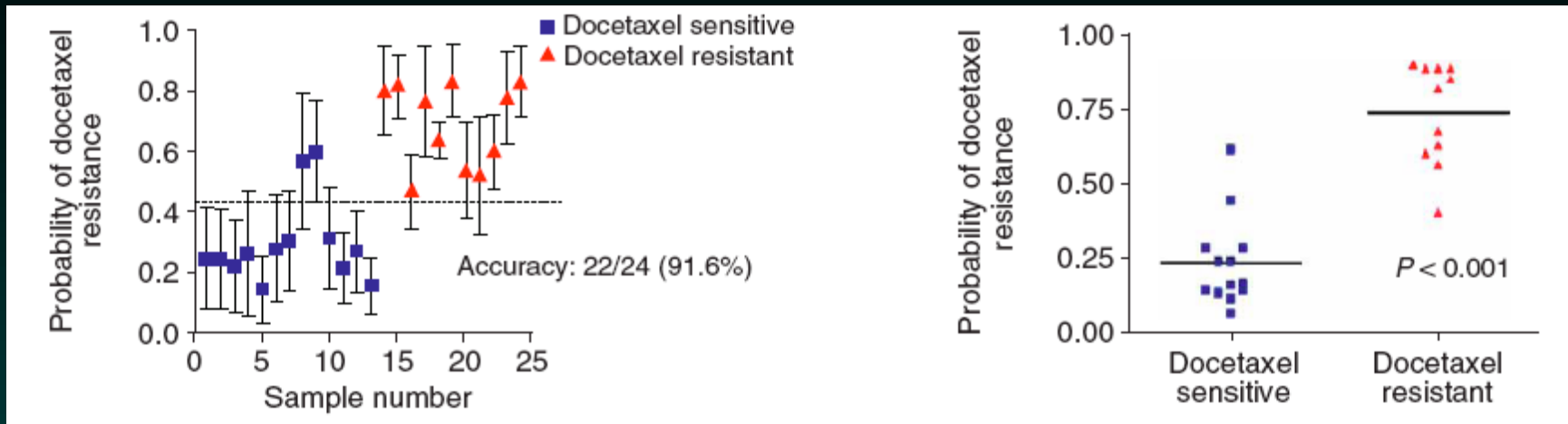


From the **software**:

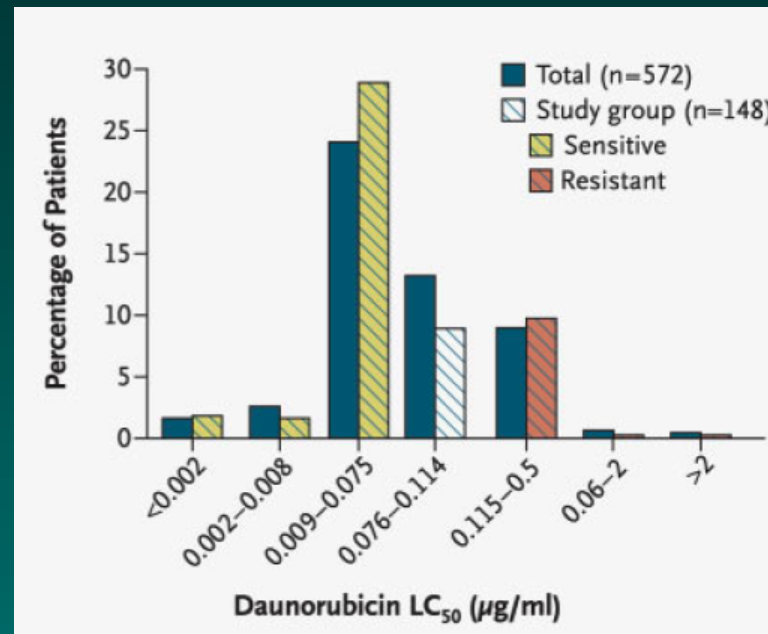
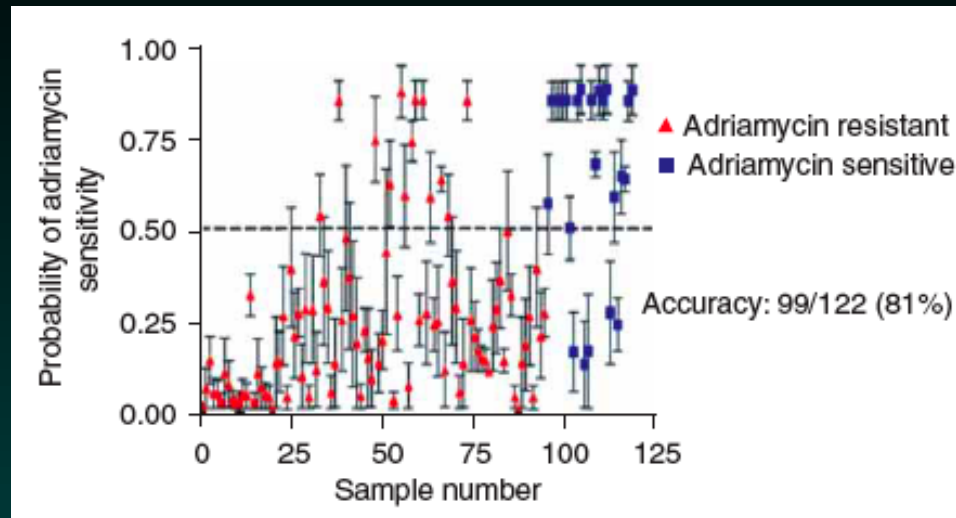


We match heatmaps but not gene lists? We'll come back to this, because their software also gives *predictions*.

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!)

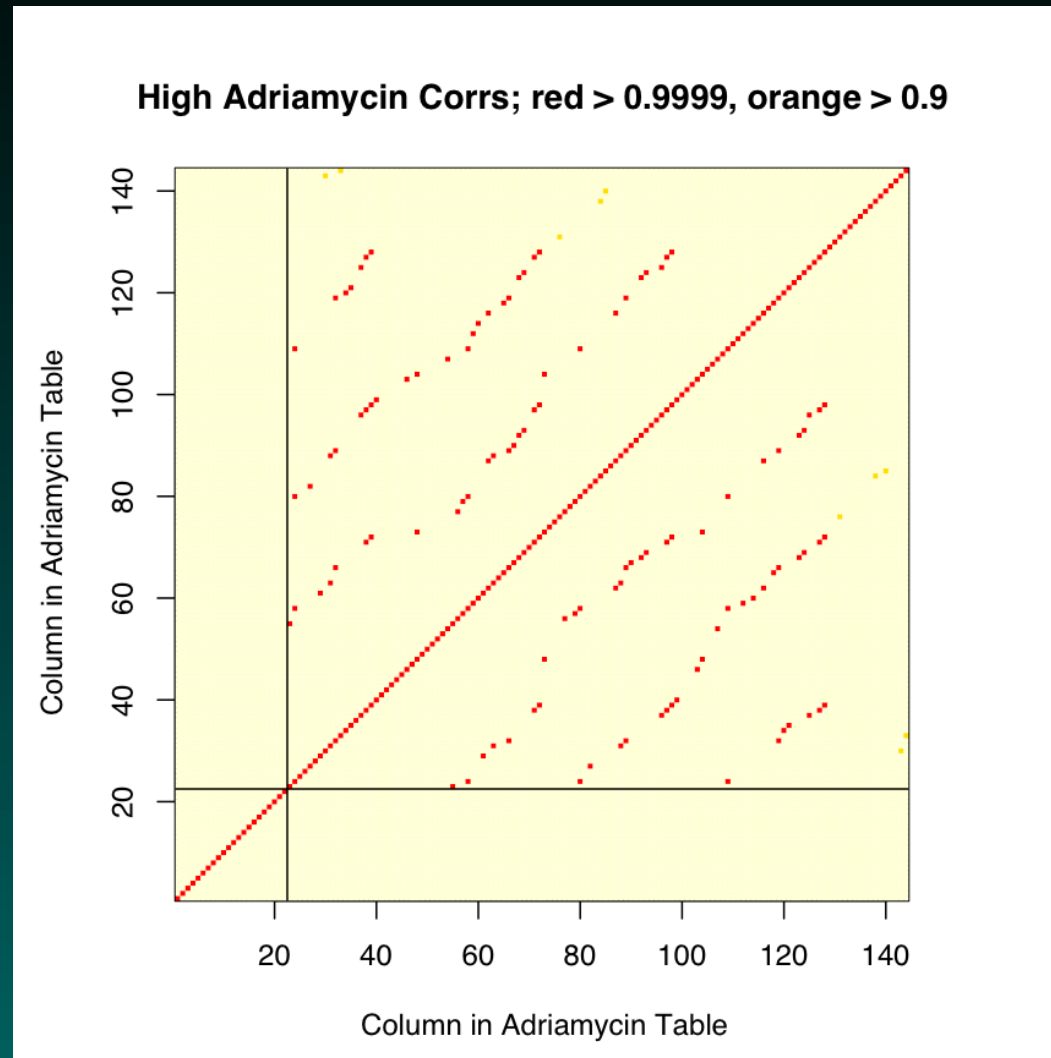
Pharmacogenomic Strategies Provide a Rational Approach to the Treatment of Cisplatin-Resistant Patients With Advanced Cancer

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

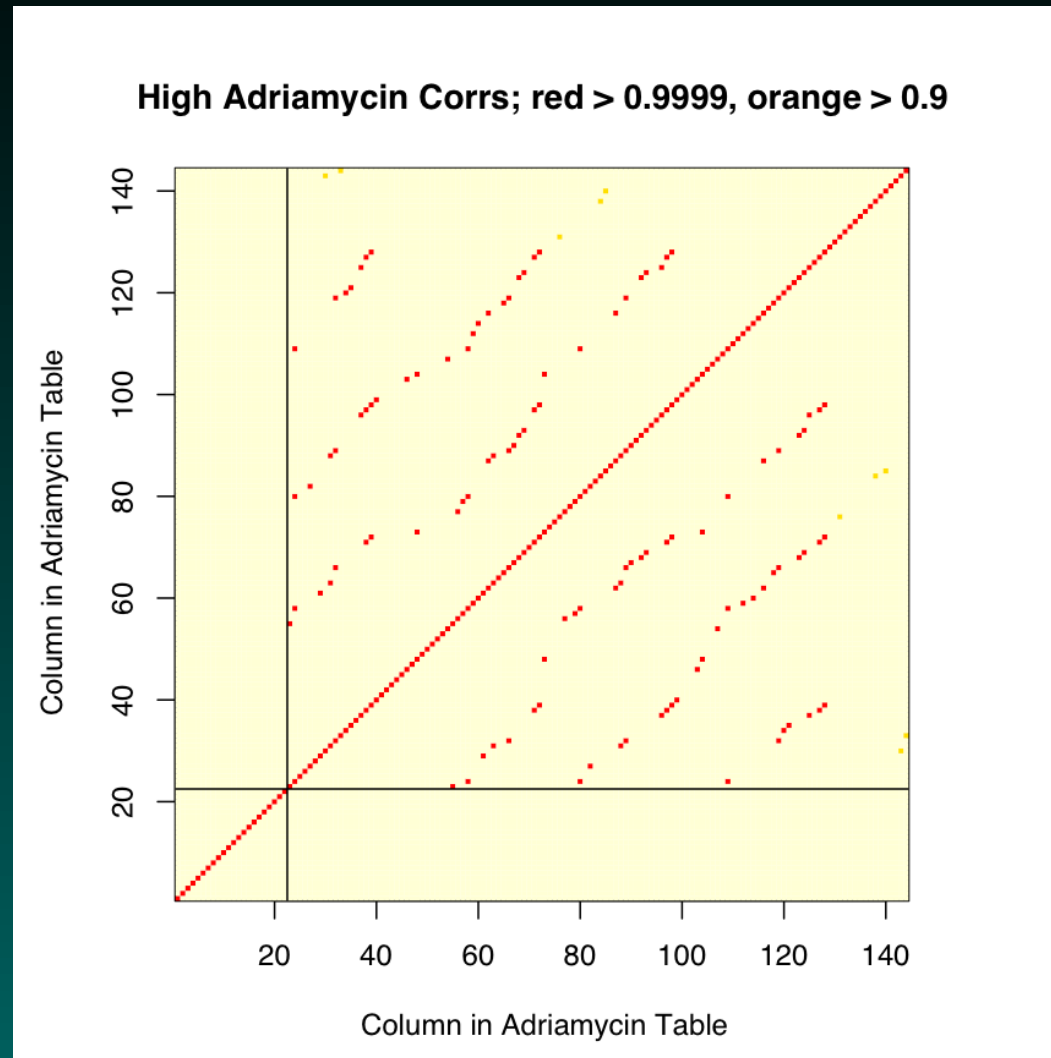
Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial

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)



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

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.*

Some Timeline Here...

Nat Med Nov 06*, Nov 07*, Aug 08. JCO Lung Oct 07*.
Lancet Oncology Breast Dec 07*. (* errors reported)

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May/June 2009: **we learn clinical trials had begun.**

2007: pemetrexed vs cisplatin, pem vs vinorelbine.

2008: docetaxel vs doxorubicin, topotecan vs dox (Moffitt).

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

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

Sep-Oct: Story covered by *The Cancer Letter*, Duke starts internal investigation, suspends trials.

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.

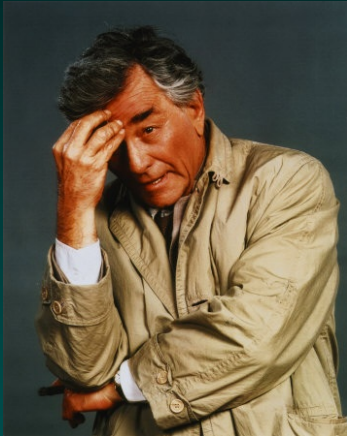
Their investigation's results "*strengthen ... confidence in this evolving approach to personalized cancer treatment.*"

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.

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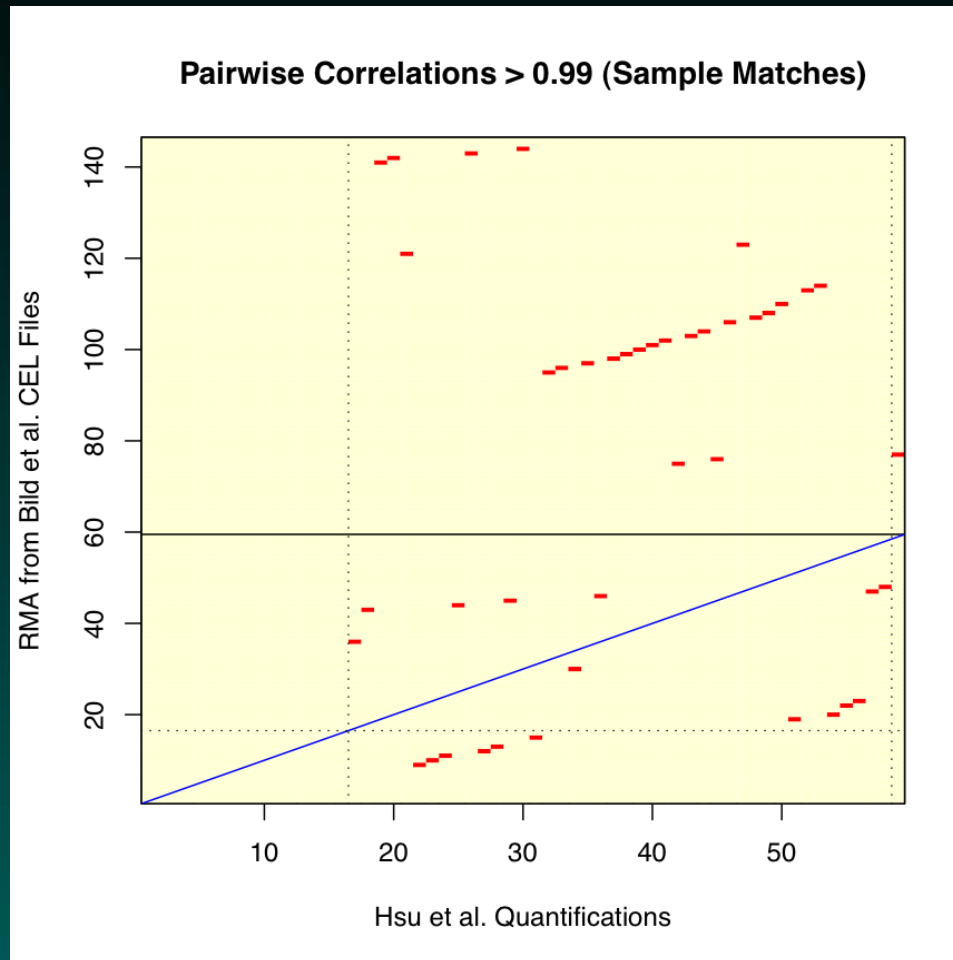


oh, there's just one more thing...

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.

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).

May 3: redacted report supplied.

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“we were unable to identify a place where the statistical methods were described in sufficient detail to independently replicate the findings of the papers.” – **review panel**

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*

(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 Kendziorowski, 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, and a Caveat

Duke announces trials resuspended

NPR blog, Science blog, Nature blog, NYT blog, article

Lancet Oncology issues Expression of Concern

Varmus & Duke request IOM Involvement

Questions raised about NEJM paper

JCO launches investigation

More awards found to be wrong, COI claims

<http://groups.google.com/group/reproducible-research>

Correspondence to Nature

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We've seen problems like these before. CAMDA 2002.

Proteomics 2003-5. TCGA current. Others at MDA.

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.

What Should the Norm Be?

For papers?

Things we look for:

1. Data (often mentioned, given MIAME)
2. Provenance
3. Code
4. Descriptions of Nonscriptable Steps
5. Descriptions of Planned Design, if Used.

For clinical trials?

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>

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

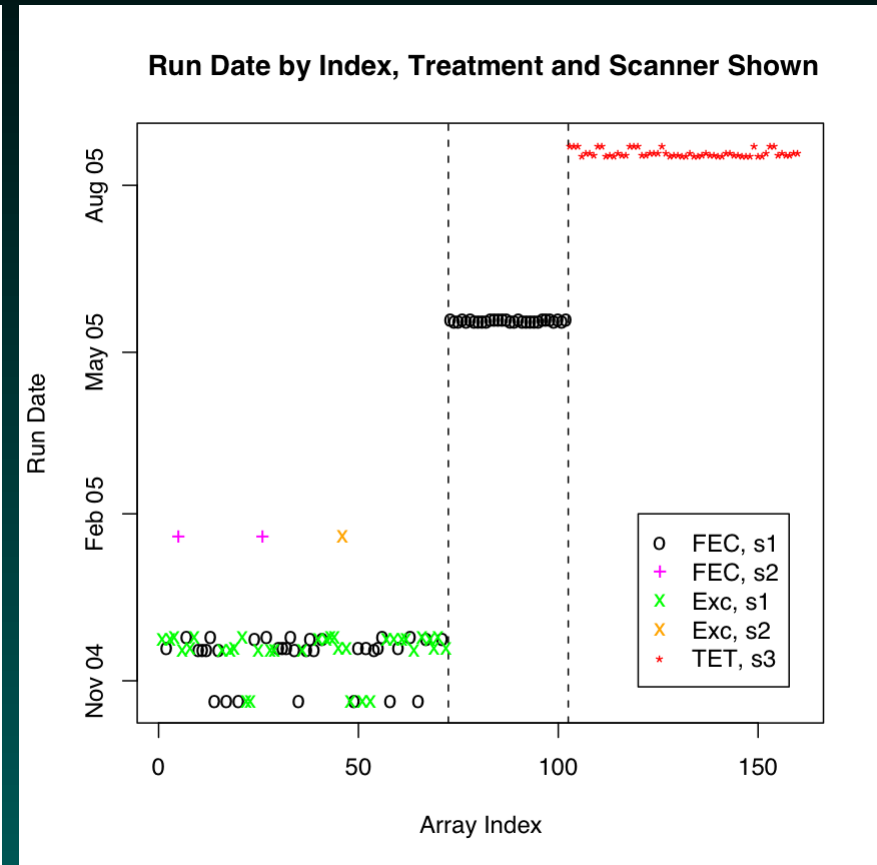
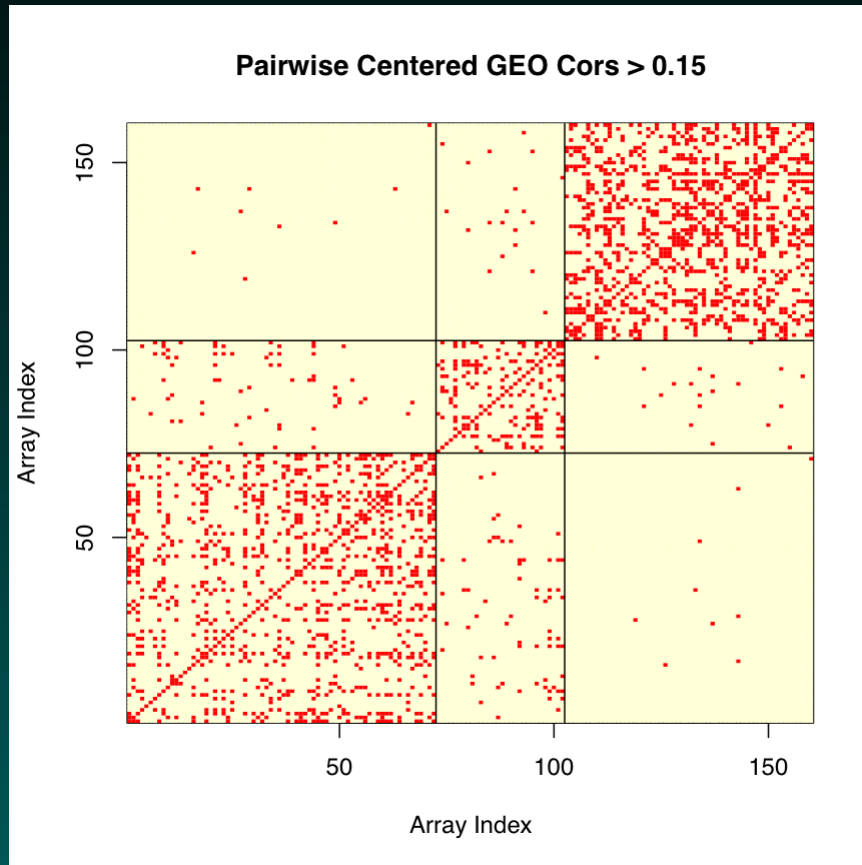
Hervé Bonnefoi, Anil Potti, Mauro Delorenzi, Louis Mauriac, Mario Camponé, Michèle Tubiana-Hulin, Thierry Petit, Philippe Rouanet, Jacek Jassem, Emmanuel Blot, Véronique Becette, Pierre Farmer, Sylvie André, Chaitanya R Acharya, Sayan Mukherjee, David Cameron, Jonas Bergh, Joseph R Nevins, Richard D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



High Sample Correlations
after Centering by Gene

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.

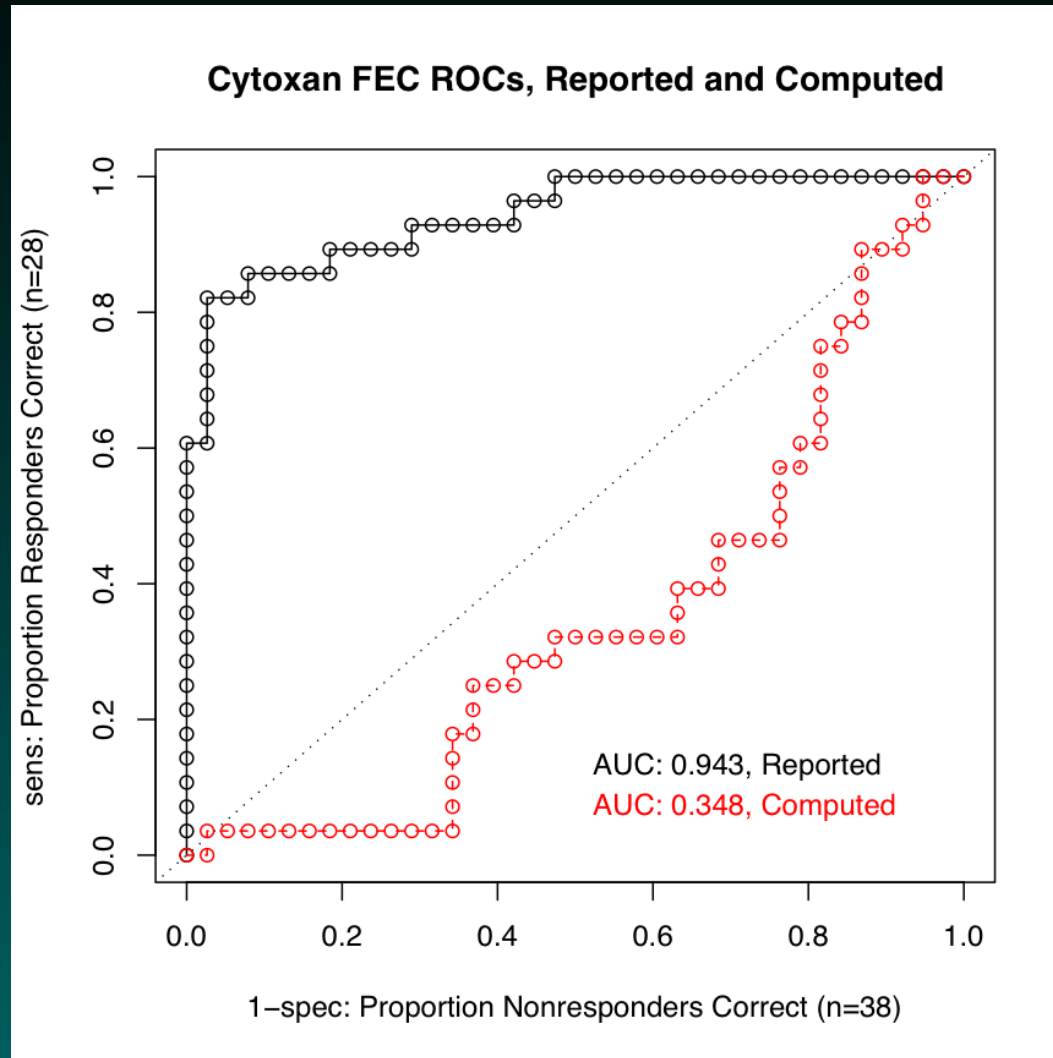
$$P(TFAC) = P(T) + P(F) + P(A) + P(C) - P(T)P(F)P(A)P(C).$$

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

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

Each rule is different.

Predictions for Individual Drugs? (Reply)



Does cytoxan make sense?

What About Blinded Validation?

“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.*

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Sample info supplied:

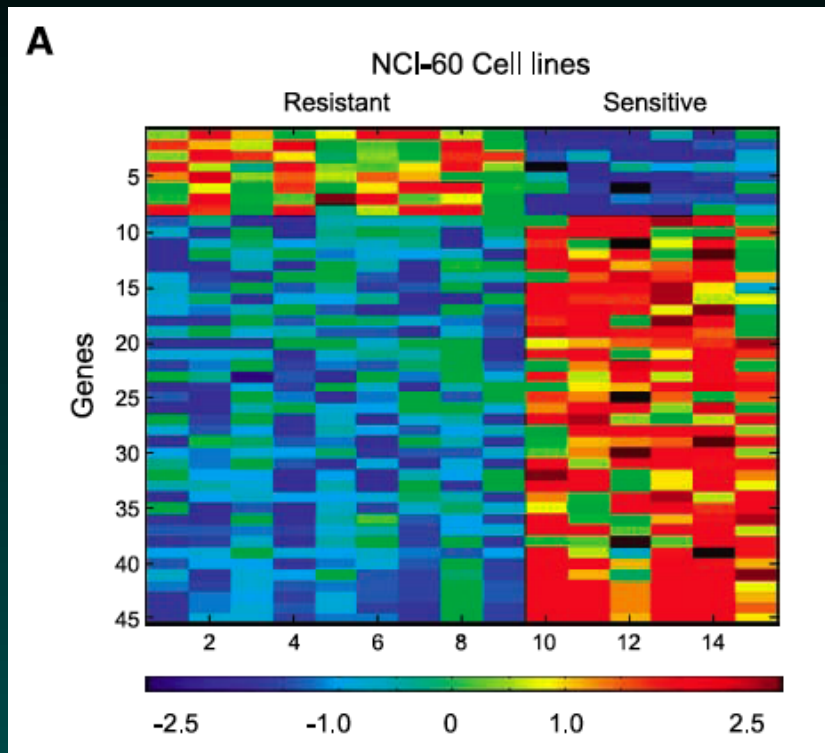
Arm, Composite label

A, npCR Ep P- T3 N1 HB01 ...

A, pCR Ep Pp T2 N1 HB04

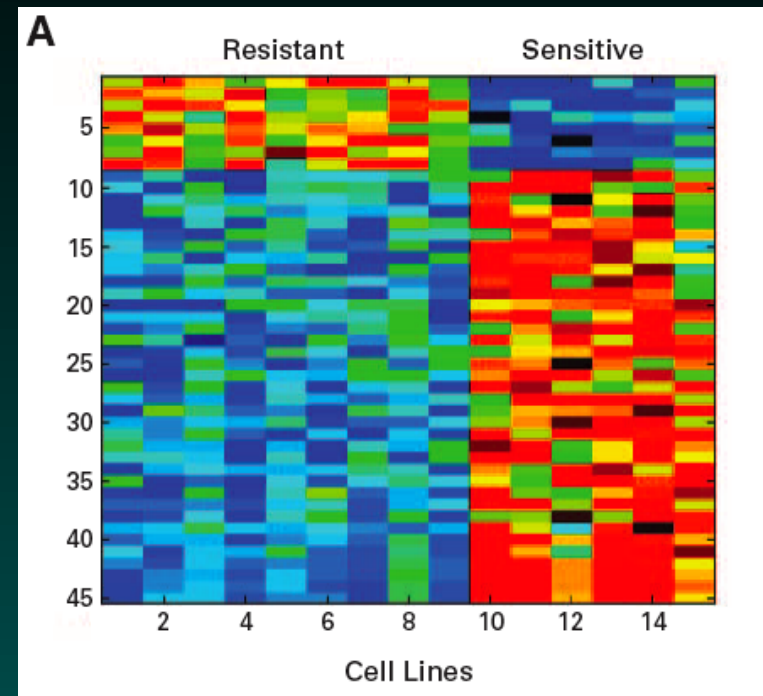
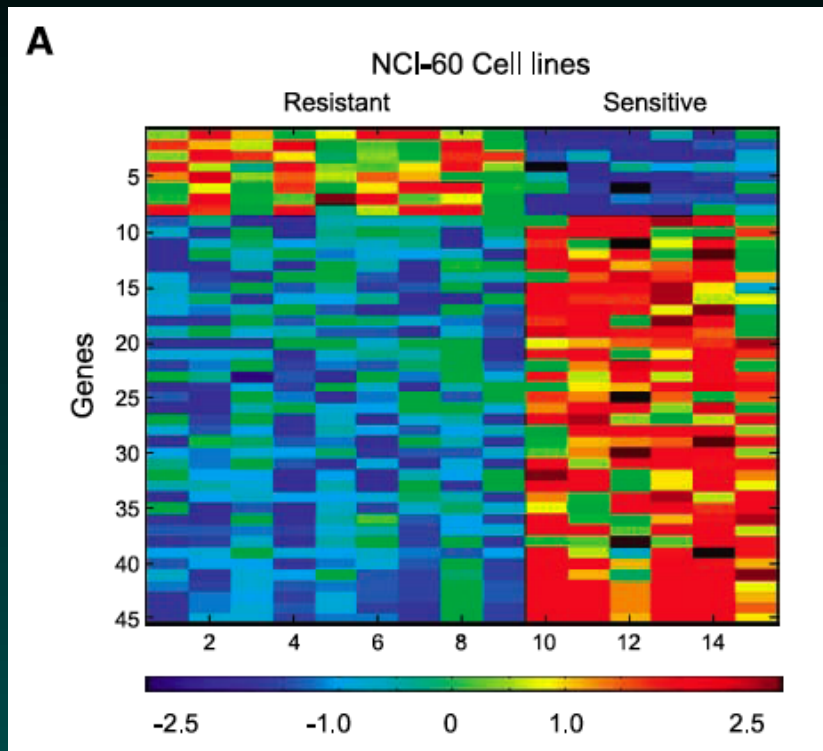
The data weren't blinded.

Temozolomide Heatmaps



Augustine et al., 2009, *Clin Can Res*, 15:502-10, Fig 4A.
Temozolomide, NCI-60.

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, Gyroffy cell lines.

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Title

Cell Line Story

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