Issues in the Use of High-Throughput "Omics" Assays

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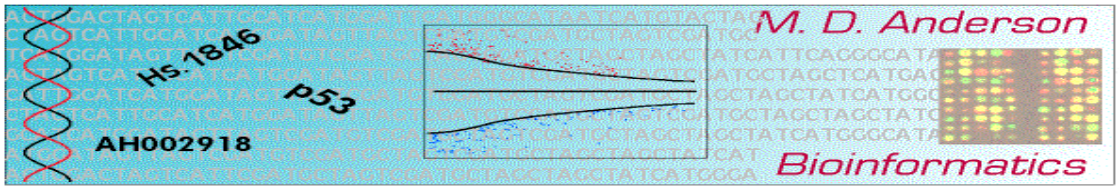
UAB Metabolomics, Jun 3, 2014



Common High-Throughput Issues

If we're looking at thousands of things at the same time, does a p-value of 0.05 sound that persuasive?

Bigger tests require more samples or more precisely formulated hypotheses.

Multiple testing needs to be explicitly addressed, and will affect sample size and power calculations.

Assays are often in flux, so we need to mention what we'll be using, and roughly how we might process the resulting data.

Other "Omics" Issues

Our intuition about what "makes sense" is very poor in high dimensions.

To use "omics-based signatures" as biomarkers, we need to know they've been assembled correctly.

Without documentation, we may need to employ (lengthy!) *forensic bioinformatics* to infer what was done.

Let's look at examples in the context of two case studies involving two different technologies.

A Proteomics Case Study

MECHANISMS OF DISEASE

Mechanisms of disease

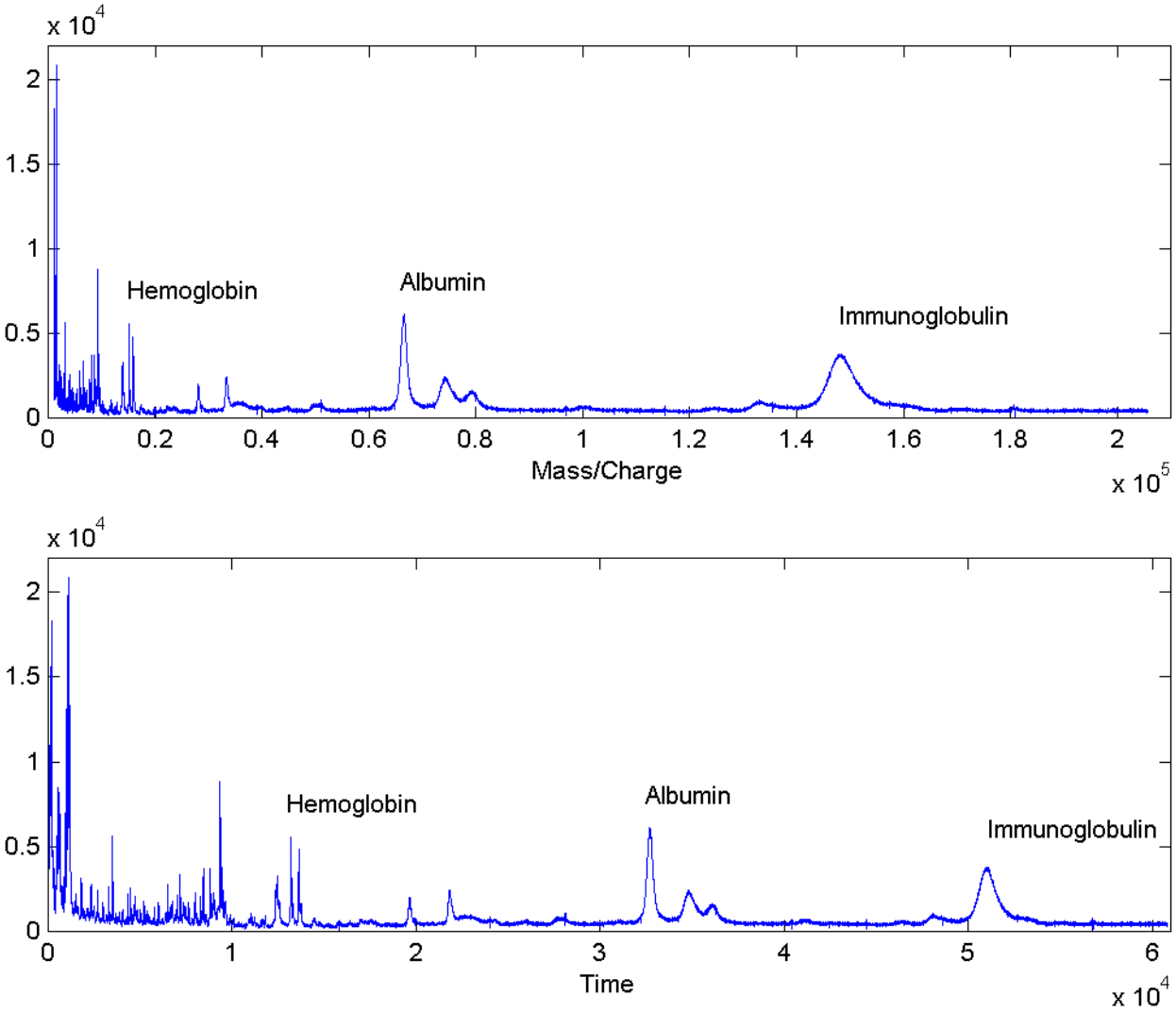
Use of proteomic patterns in serum to identify ovarian cancer

Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta

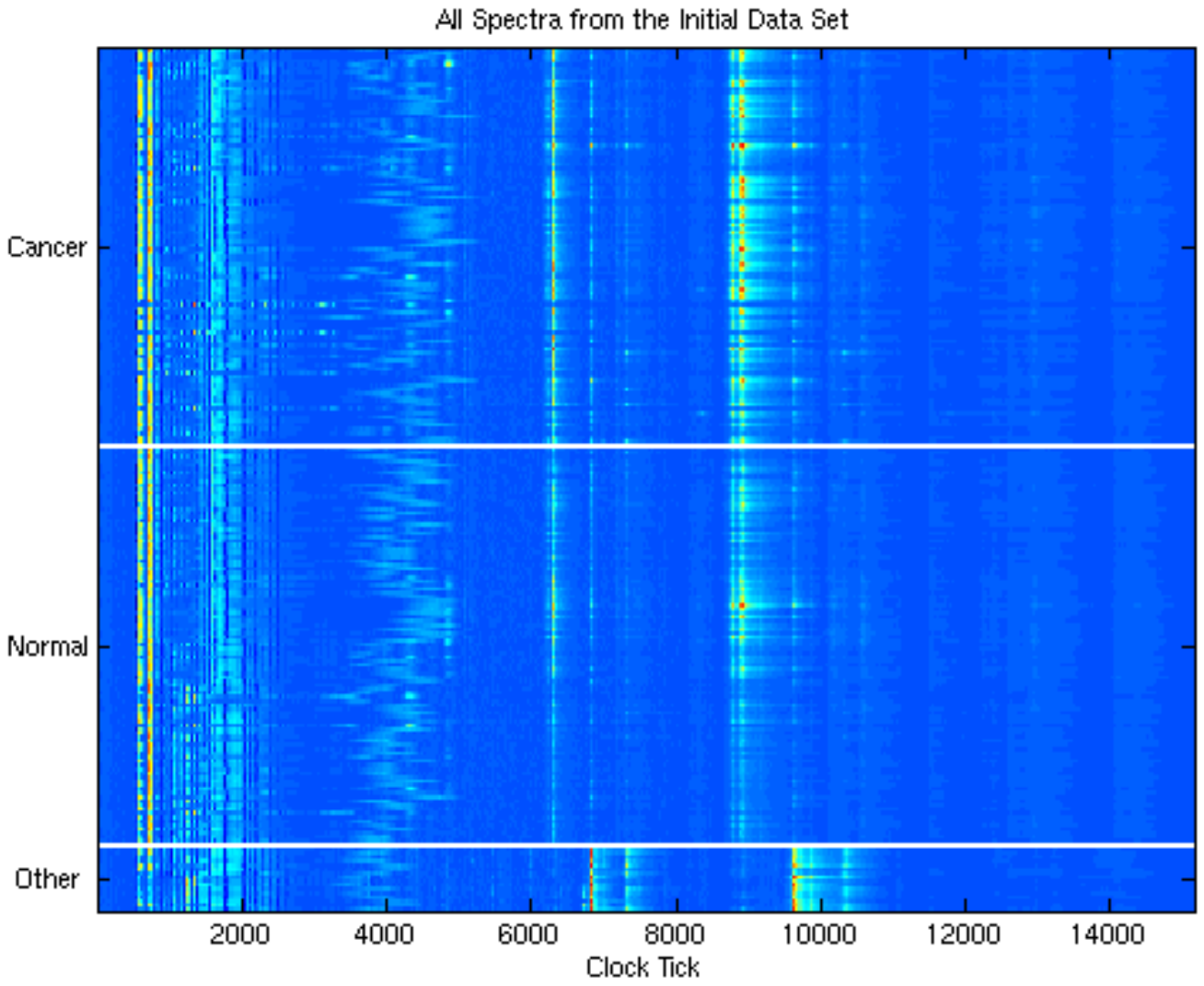
- 100 ovarian cancer patients
- 100 normal controls
- 16 patients with “benign disease”

Use 50 cancer and 50 normal spectra to train a classification method; test the algorithm on the remaining samples.

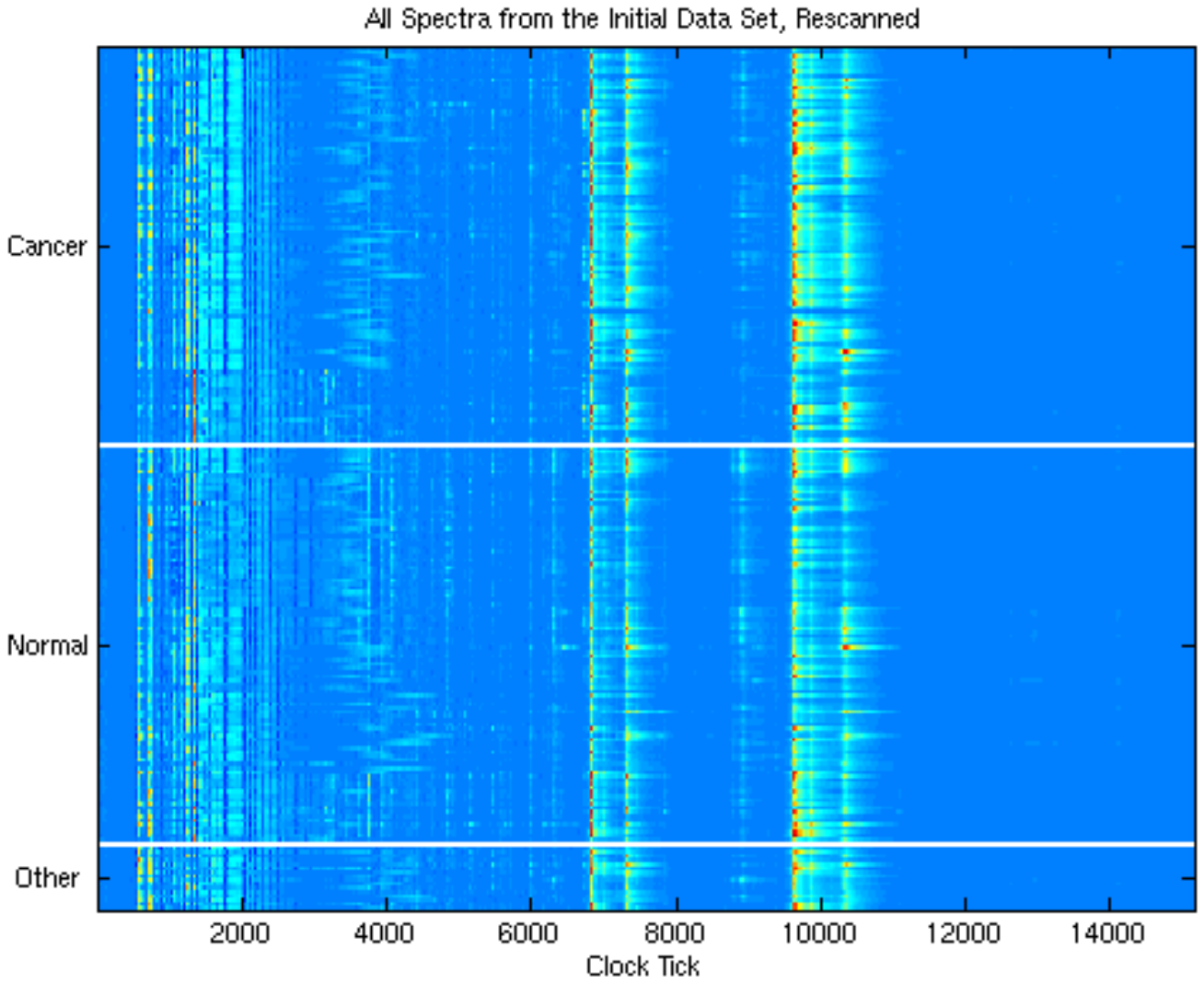
What Do the Data Look Like?



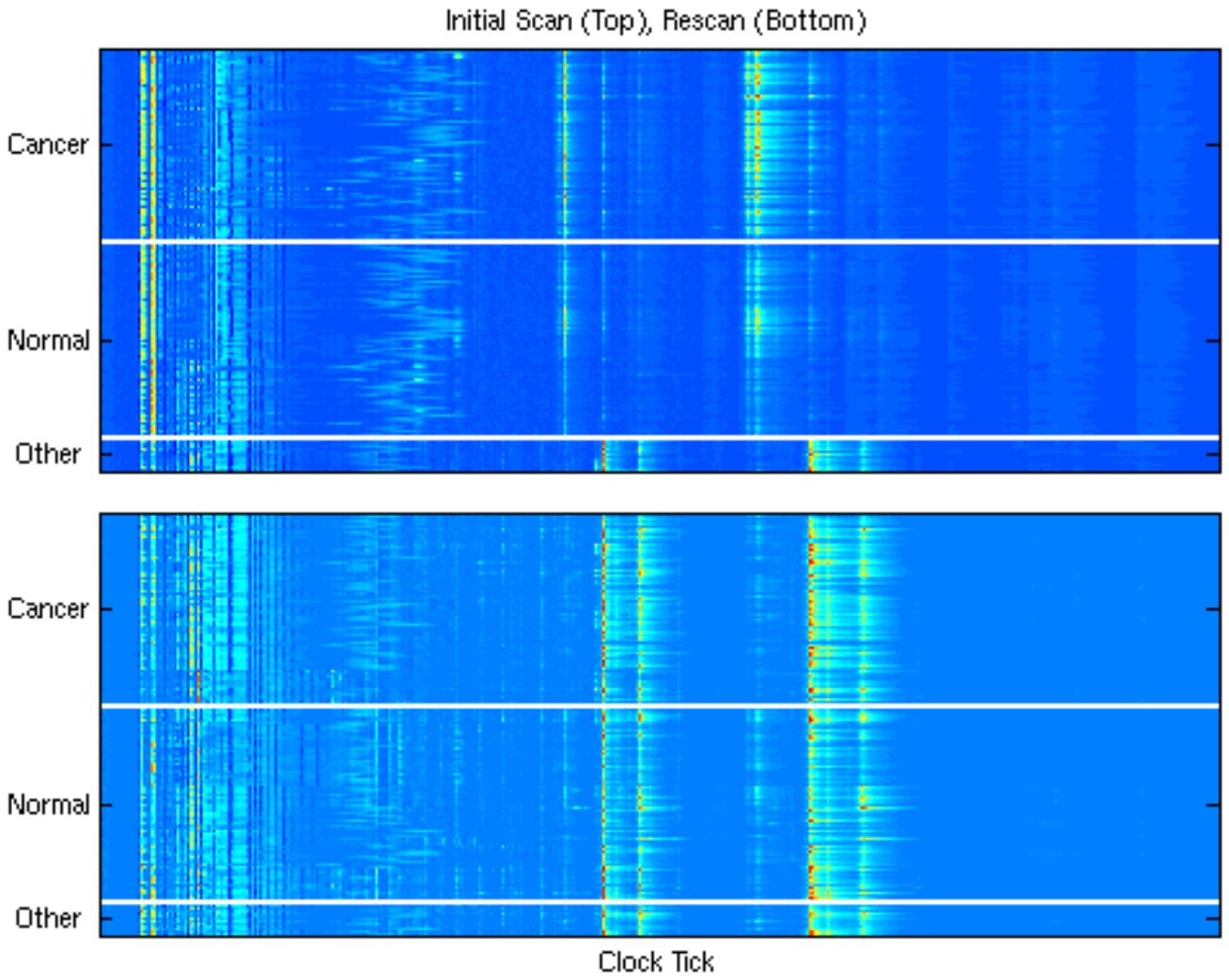
Which Group is Different?



Really?


All Spectra from the Initial Data Set, Rescanned
Cancer
Normal
Other
2000
4000
6000
8000
10000
12000
14000
Clock Tick

Processing Can Trump Biology: Design!



Some Timeline

2004:

* Early Jan: Correlogic, Quest and LabCorp advertise the forthcoming “OvaCheck” assay at SGO.

* Jan 29: Critiques available online

* Feb 3: New York Times coverage

* Feb-Mar: Letters from FDA to companies involved

* July: FDA rules omics signatures are medical devices and will be regulated accordingly.

2006:

* FDA releases draft guidance on IVDMIAs

* NCI Clinical Proteomic Technologies for Cancer (CPTAC)

Are Things Better Now?

New York Times, 2.3.04

New Cancer Test Stirs Hope and Concern

By ANDREW POLLACK

Jill Doimer's mother died in 2002 from ovarian cancer, detected too late to be effectively treated.

So Ms. Doimer is eagerly awaiting the introduction of a new test that holds the promise of detecting early-stage ovarian cancer far more accurately than any test available now, using only blood from a finger prick.

Not only does she plan to be tested, but an advocacy group she helped found, Ovarian Awareness of Kentucky, also intends to spread the word to women and doctors.

"If it's going to happen to me or anyone I know, I want it to be caught at an early stage," said Ms. Doimer, who lives in Louisville.

The new test, expected to be available in the next few months, could have a big effect on public health if it works as advertised. That is because when ovarian cancer is caught early, when it is treatable by surgery, more than 90 percent of women live five years or longer. But right now, about three-quarters of cases are detected after the cancer has advanced, and then only 35 percent of women survive five years.

The test is also the first to use a new technology that some believers say could revolutionize diagnostics. It looks not for a single telltale protein — like the prostate-specific antigen, or P.S.A., used to diagnose prostate cancer — but rather for a complex fingerprint formed by all the proteins in the blood. Similar tests are being developed for prostate, pancreatic, breast and other cancers. The technique may work for other diseases as well.

"I've been in cancer research for 40 years and I think it's the most important breakthrough in those years," said Dr.

Continued on Page 6

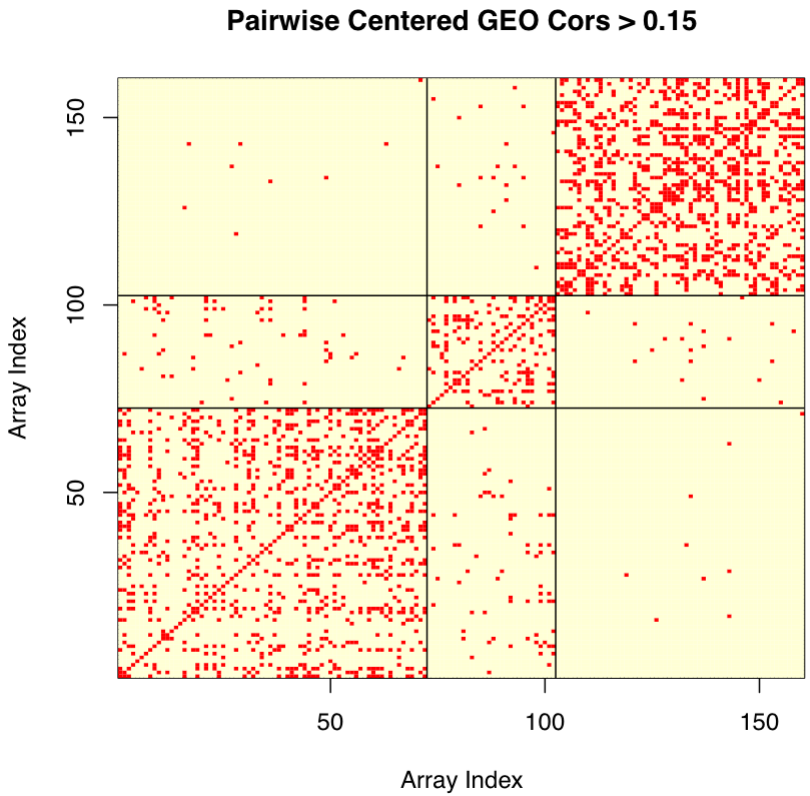
Cancer Test For Women Raises Hope, And Concern

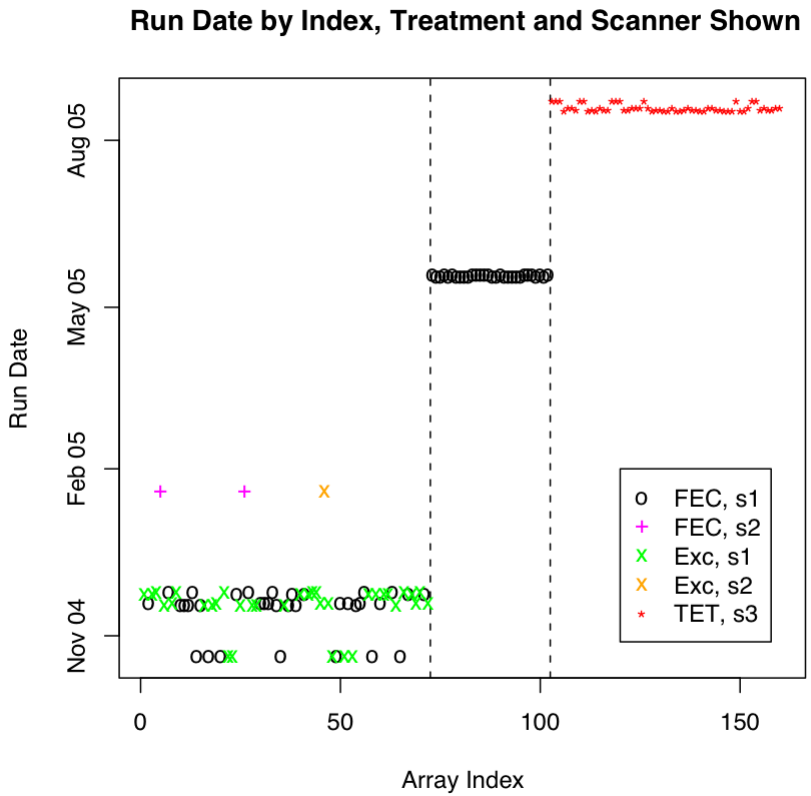
By ANDREW POLLACK

A new blood test aimed at detecting ovarian cancer at an early, still treatable stage is stirring hopes among women and their physicians. But the Food and Drug Administration and some experts say the test has not been proved to work.

New York Times, Aug 26, 2008.

Is This an Isolated Problem?





High Sample Correlations

Array Run Dates

See Leek et al, Nat Rev Gen 2010 for more examples.

Using Cell Lines 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: we can use microarray data from cell lines (the NCI60) to define drug response “signatures”, which can predict whether patients will respond.

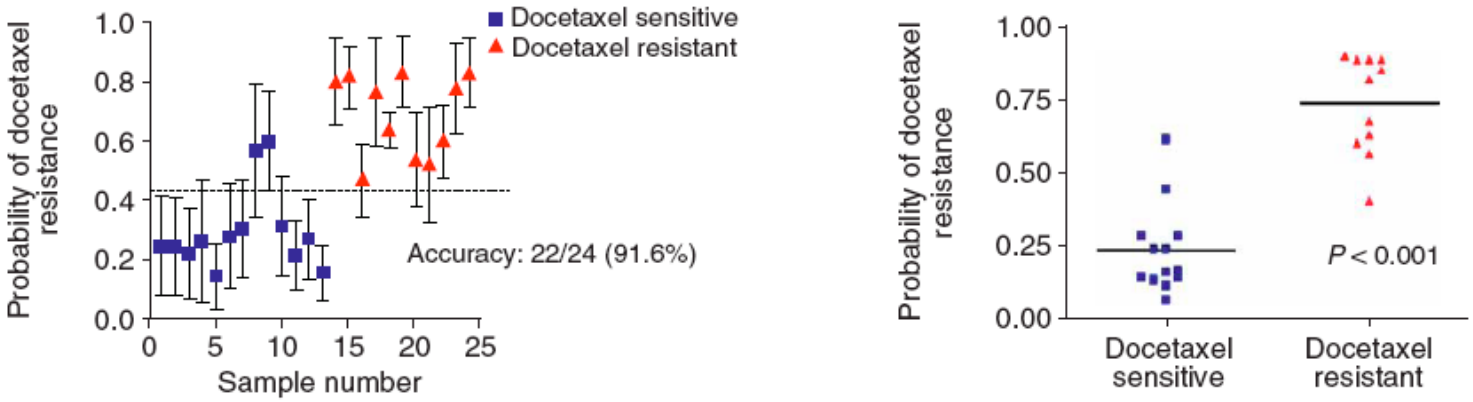
They provide examples using 7 commonly used agents.

This got people at MDA very excited.

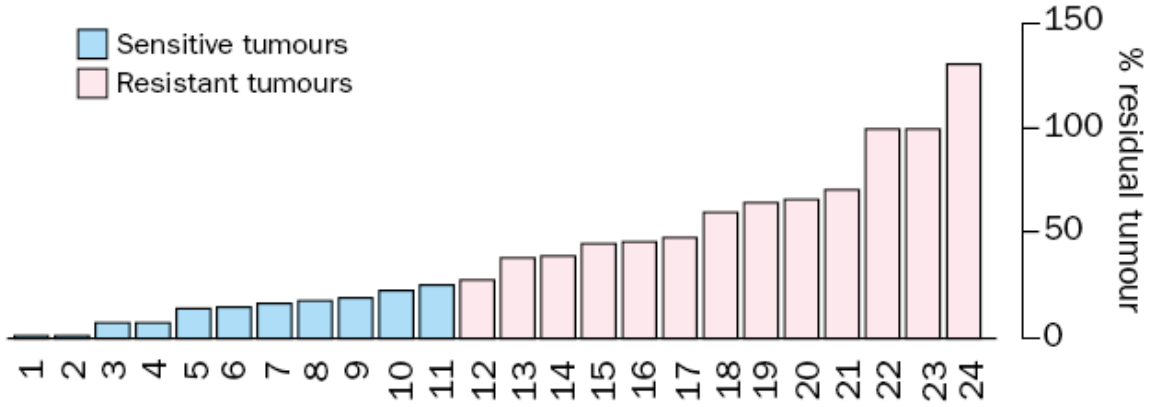
Their Gene List and Ours

```
> 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"
...
```

Predicting Response: Docetaxel

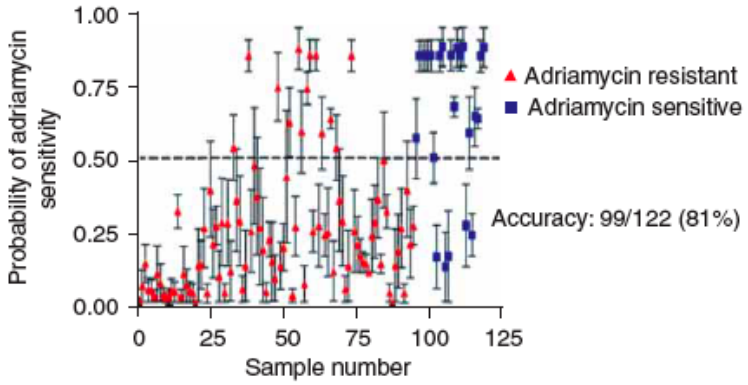


Potti et al, Nat Med 2006, 12:1294-300, Fig 1d

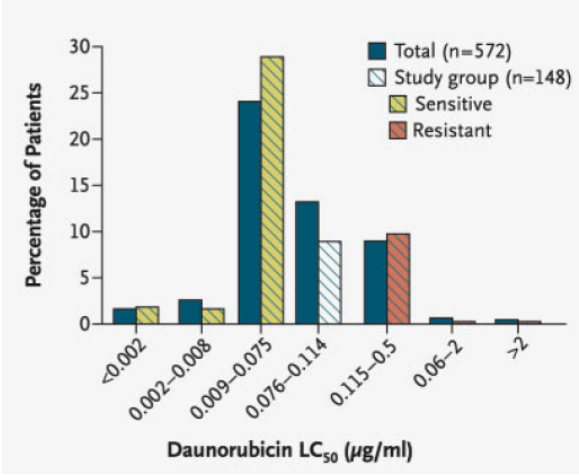


Chang et al, Lancet 2003, 362:362-9, Fig 2 top

Predicting Response: Adriamycin

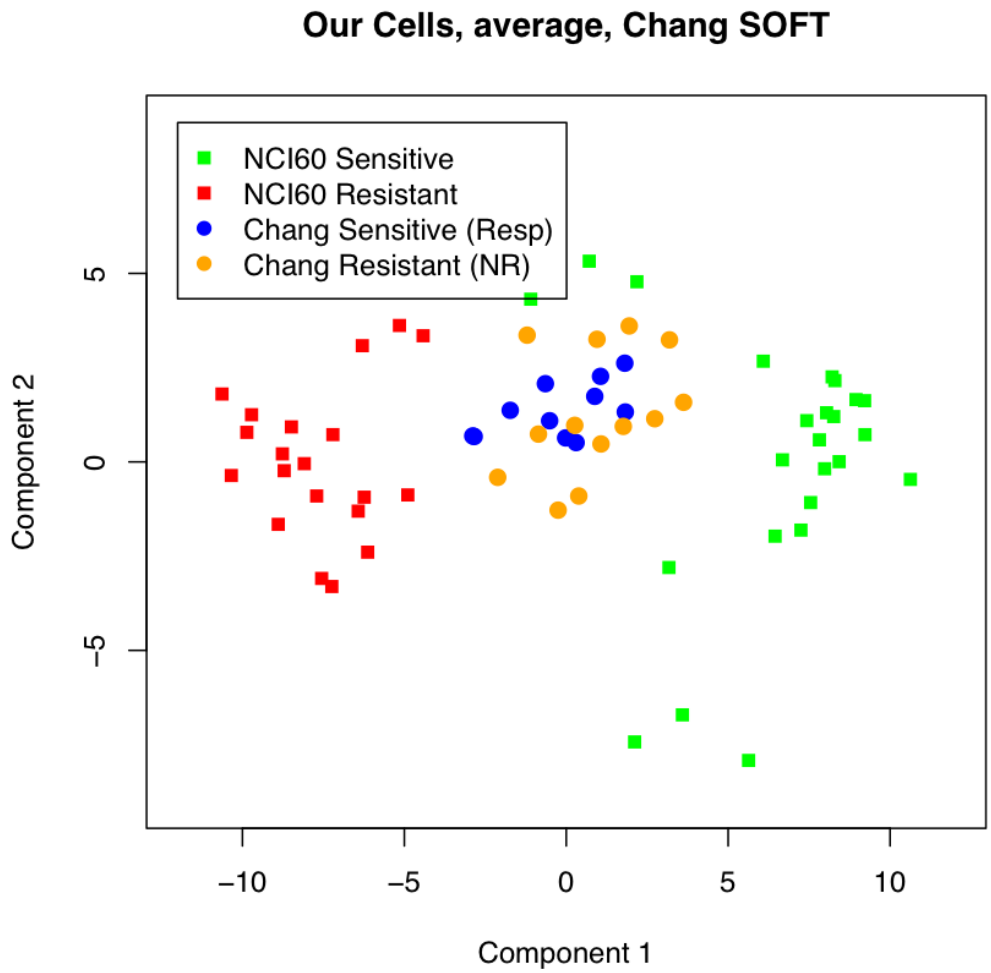


Potti et al, Nat Med 2006, 12:1294-300, Fig 2c



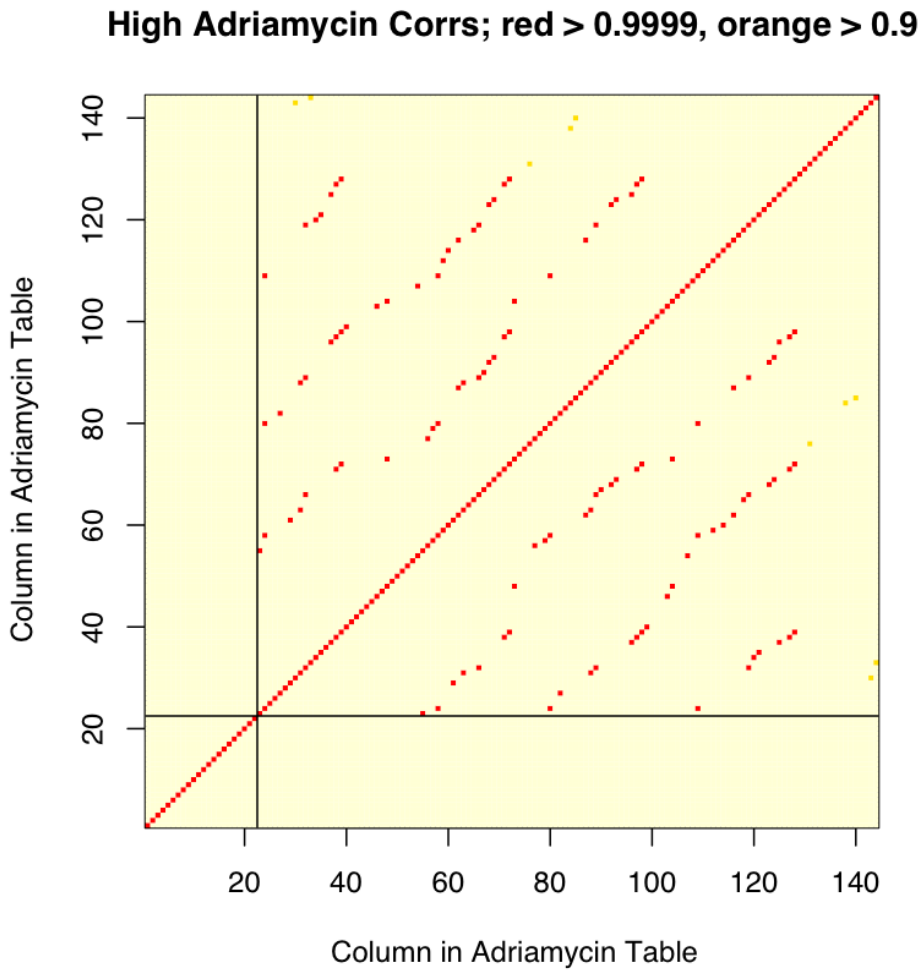
Holleman et al, NEJM 2004, 351:533-42, Fig 1

Trying it Ourselves



When we try it, *it doesn't work.*

Adriamycin 0.9999+ Correlations (Reply)



Redone Aug 08, “using ... 95 unique samples”.

The Reason We Really Care

Jun 2009: we learn clinical trials had begun.
2007: pemetrexed vs cisplatin, pem vs vinorelbine.
2008: docetaxel vs doxorubicin, topotecan vs dox (Moffitt).

Sep 1, 2009: We submit a paper describing case studies to the *Annals of Applied Statistics*.

Sep 14, 2009: Paper accepted and available online at the *Annals of Applied Statistics*.

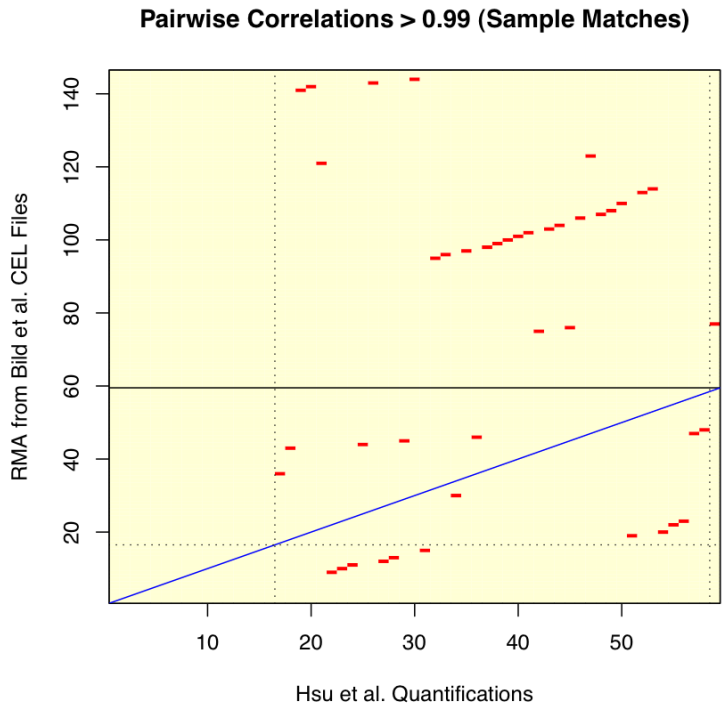
Sep-Oct 2009: Story covered by *The Cancer Letter*.
NCI raises concerns with Duke's IRB behind the scenes.
Duke starts internal investigation, suspends trials.

New Data

Early-Nov ’09 (mid-investigation), the Duke team posted new data for cisplatin and pemetrexed (in lung trials since ’07).

These included quantifications for the 59 ovarian cancer test samples (from GSE3149, which has 153 samples) they used to validate their predictor.

We Tried Matching The Samples



43 samples are mislabeled.

16 samples don't match because the genes are mislabeled.

All of the validation data are wrong.

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

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."*

We Asked for the Data

"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.

This did give us one more option...

In May 2010, we obtained a copy of the reviewers' report from the NCI under FOIA.

In our assessment, it did not justify restarting trials.

There was no mention of our Nov 2009 report.

A Catalyzing Event: 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

Jul 19/20: Letter to Varmus; Duke resuspends trials.

Oct 22/9: First call for paper retraction.

Nov 9: Duke terminates trials.

Nov 19: call for Nat Med retraction, Potti resigns

Other Developments

117 patients were enrolled in the trials.
Sep, 2011: Patient lawsuits filed (11+ settlements).

Misconduct investigation (ongoing).
10 retractions, 6 corrections/partial retractions to date.

Jul 8, 2011: Front Page, NY Times.
Feb 12, 2012: 60 Minutes.
http://www.cbsnews.com/8301-18560_
162-57376073/deception-at-duke/

Mar 23, 2012: IOM Report Released.
http://www.iom.edu/Reports/2012/
Evolution-of-Translational-Omics.aspx

Recent Links

Science, March 6, 2013 http://www.aaas.org/news/releases/2013/0311_alberts.shtml

Nature, April 24, 2013 http://www.nature.com/news/announcement-reducing-our-irreproducibility-1.12852

Colbert report, April 23, 2013 http://www.colbertnation.com/the-colbert-report-videos/425749/april-23-2013/austerity-s-spreadsheet-error---thomas-herndon

Nature, BMC Medicine, Oct 17, 2013 http://www.nature.com/nature/journal/v502/n7471/full/nature12564.html, http://www.biomedcentral.com/1741-7015/11/220

Is This an Isolated Problem?

Ioannidis et al. (2009), *Nat. Gen.*, **41**:149-55. Tested reproducibility of microarray papers. Could reproduce 2/18.

Begley and Ellis (2012), *Nature*, **483**:531-3. Amgen attempted validation of clinical “breakthroughs” prior to further study. Validated 6/53.

Some Cautions/Observations

These cases are pathological.

But we've seen similar problems before.

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

Reasons for Hope

1. Our Own (Evolving!) Experience
2. Better tools (knitr, Markdown, GenePattern/Firehose)
3. Journals, Code and Data
4. The IOM, the FDA, and IDEs*
5. The NCI and Trials it Funds
6. OSTP, Congress, Science, Nature
7. The Power of Ridicule

Acknowledgments

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M.D. Anderson Ovarian, Lung and Breast SPOREs

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

For updates: `http://bioinformatics.mdanderson.org/Supplements/ReproRsch-All/Modified`.