A Data Analysis Method for Identifying Autoantibody Biomarkers in Cancer Patients Following Immunotherapy

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COI Disclosure

- Disclosure: Founder of CytoAnalytics

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Invitrogen/Life Technologies

- John Verburg
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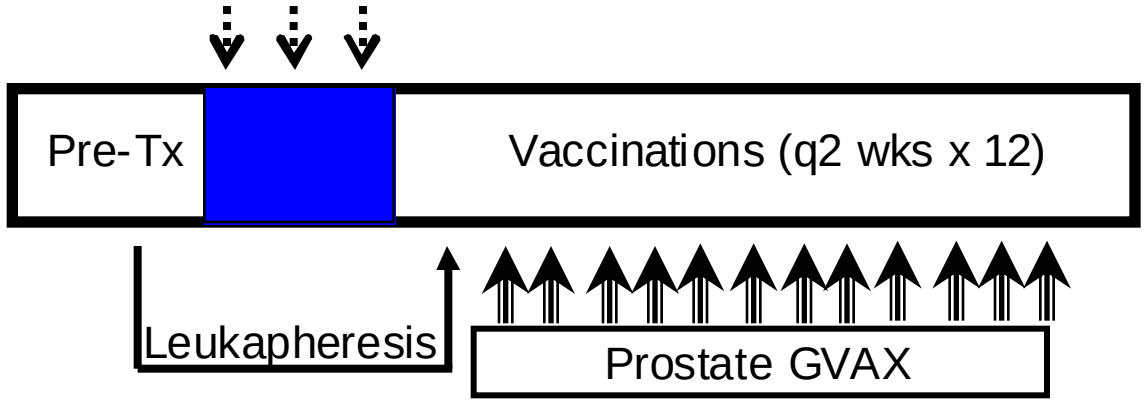
Trial Design

Phase I/II study of allogeneic prostate GVAX™ in advanced prostate cancer patients made lymphopenic by chemotherapy and infused with autologous PBMC - DOD PC020094 / PHS 02-200

A) No chemo
B) Cytoxan 350 mg/m^2 d 1-3
C) Cytoxan + Fludarabine 20 mg/m^2 d 1-3



Vaccine

- Prostate GVAX is composed of two Prostate cancer cell lines, LNCap and PC3
  - Genetically engineered to produce GM-CSF
  - Express a wide range of “common” Prostate cancer-associated antigens

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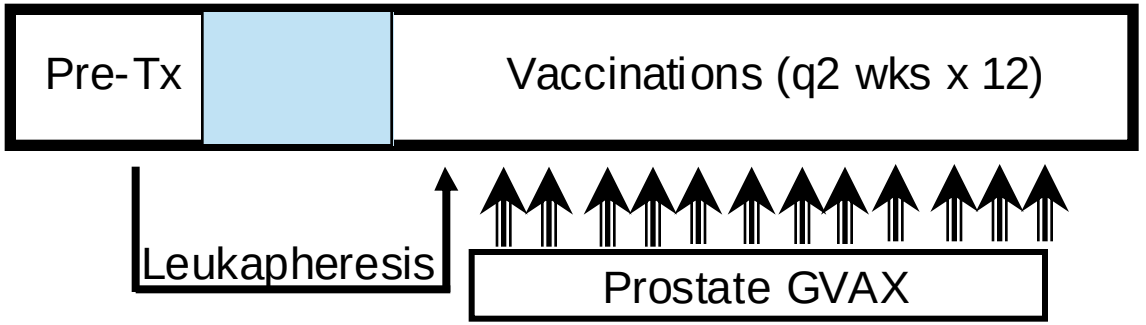
The Biological Questions

- How do we detect a tumor-specific immune response following immunotherapy with a complex cellular product?
  - Complicated by alloresponse to allogeneic cell lines
  - Autologous tumor not available

Rationale / Hypothesis

- Since T cell “Help” is required for immunoglobulin class-switch, can we use the identification of novel antibodies as surrogates for an anti-cancer T cell response?

Study Overview



- Pre- and post-treatment (11 weeks after start of immunotherapy) analyzed with Human Protein Microarray

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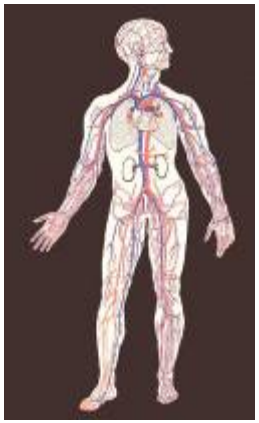
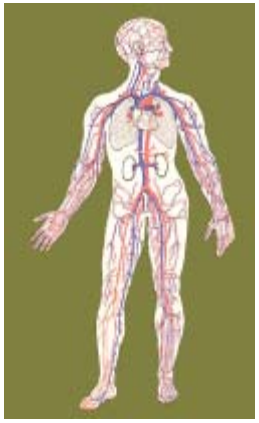
Generic Protocol / Workflow

1. Sample Acquisition

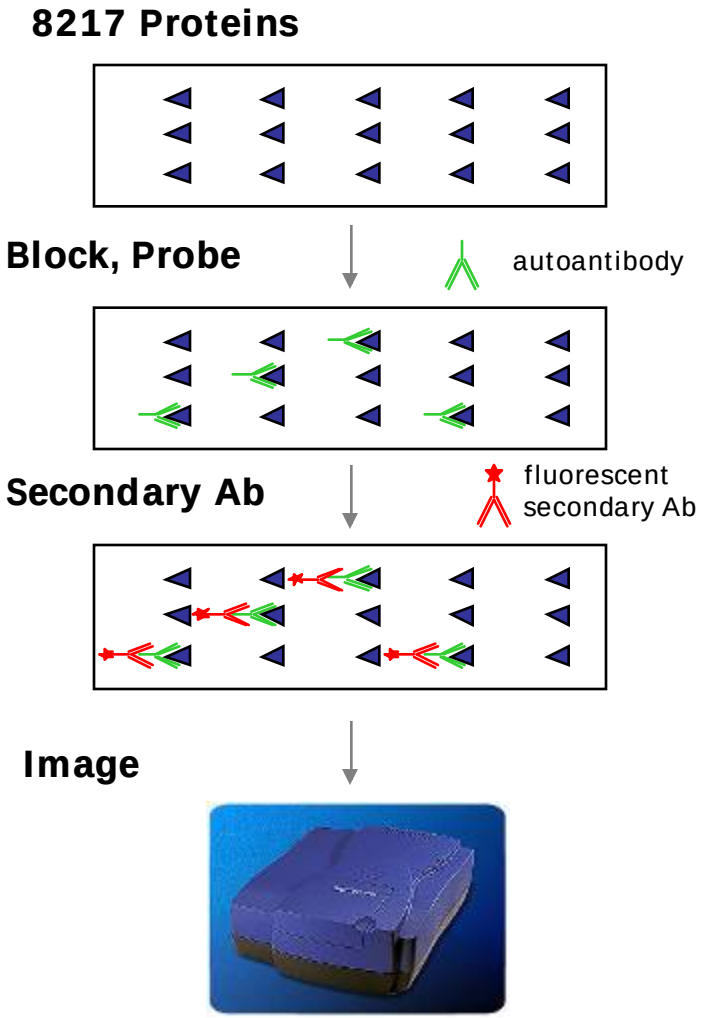
Pre - Treatment





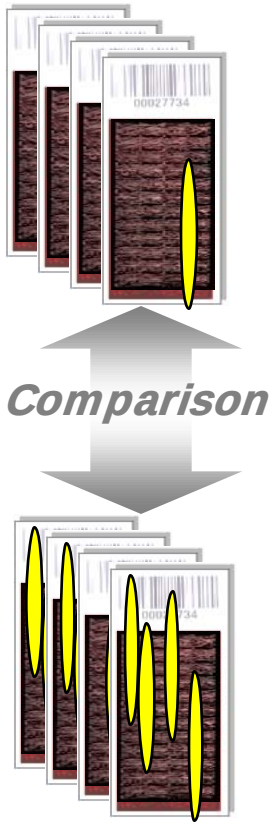
Post - treatment

2. Sample Processing



3. Data Analysis

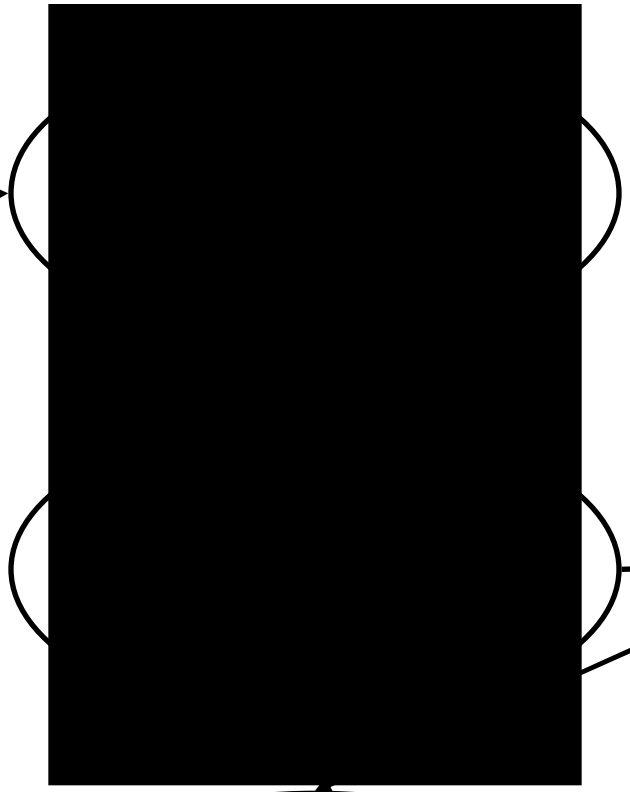
Pre - treatment



Post - treatment

Slide courtesy of John Verburg, Invitrogen

The Secret Life of Data



protein array
data files

select data
and analyze
experiment

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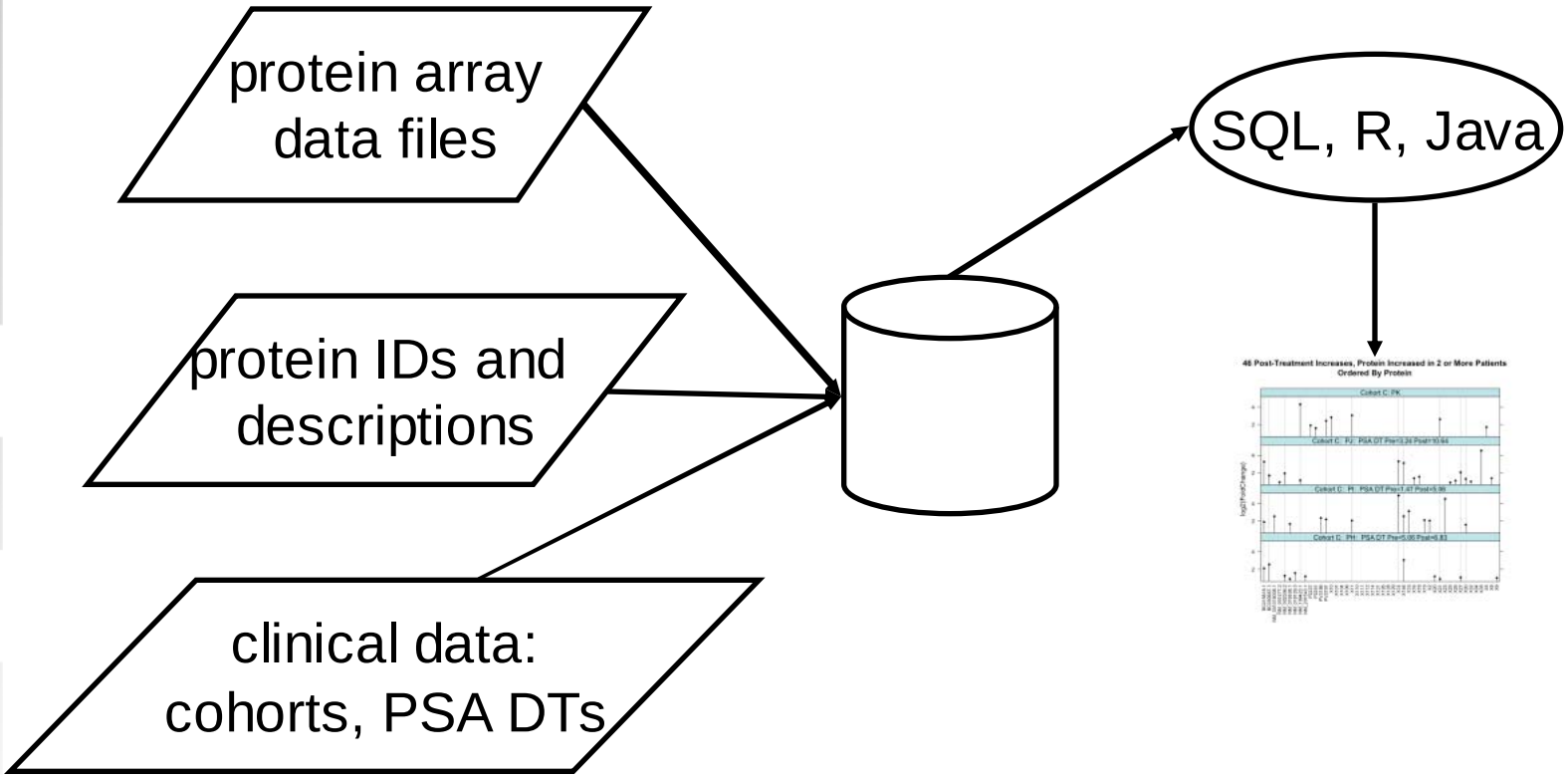
Integrating Heterogeneous Data Sources



Selecting the Data

- Filter results, global level: Remove antibodies that are a “hit” in a negative control sample of only buffer
  (plus secondary fluoursecent antibody)
- Filter results, per-patient, per-antibody:
  - Duplicate/replicate readouts reasonably close
  - Magnitude of readout reasonably greater than negative control features on array
- Collapse duplicate/replicate readouts for each sample into 1, based on minimum readout

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Analyzing the Data

- For each patient for each antibody, compute fold change:

$$\frac{post - treatment}{pre - treatment}$$

- For each patient, identify 50 greatest increases where fold change > 1.1
- For each patient, identify 50 greatest decreases where fold change < .9
- Combine patient-level lists to “Top 50” master lists

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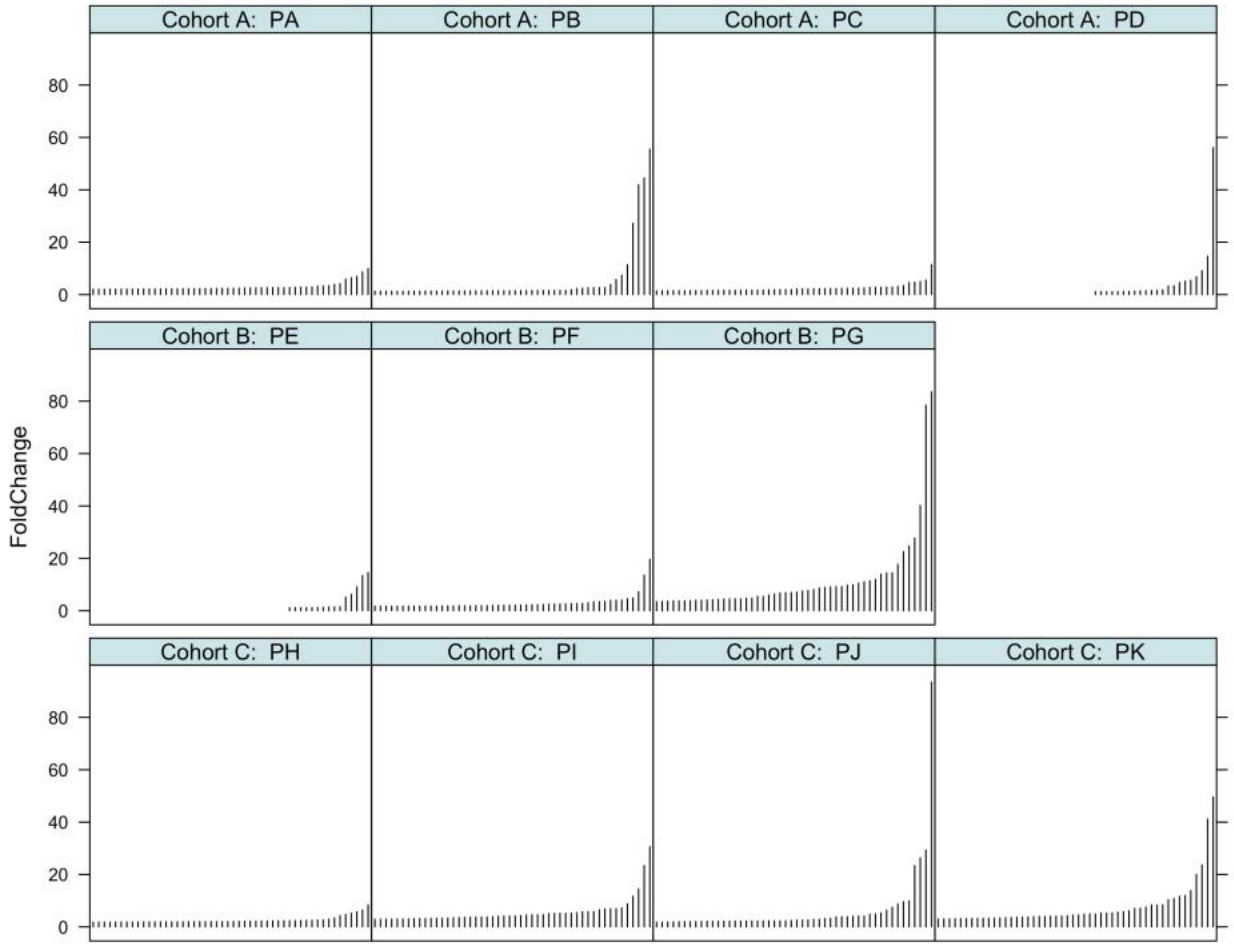
Some High Level Metrics

- 8,217 antibody readouts per sample
- 37 to 7,585 credible antibody readouts per patient; avg=2,133
  - Duplicates reasonably close; greater than negative controls
- Increased antibody responses to 418 distinct antibodies in "Top 50" increase list (fold change > 1.1)
  - 46 antibodies with increased recognition by 2 or more patients
  - 13 antibodies with increased recognition by 3 or more patients
- Decreased antibody responses to 393 distinct antibodies in "Top 50" decrease list (fold change < .9)
  - 44 antibodies with decreased recognition by 2 or more patients
  - 14 antibodies with decreased recognition by 3 or more patients

Top 50 Antibody Increases, Ordered by Rank



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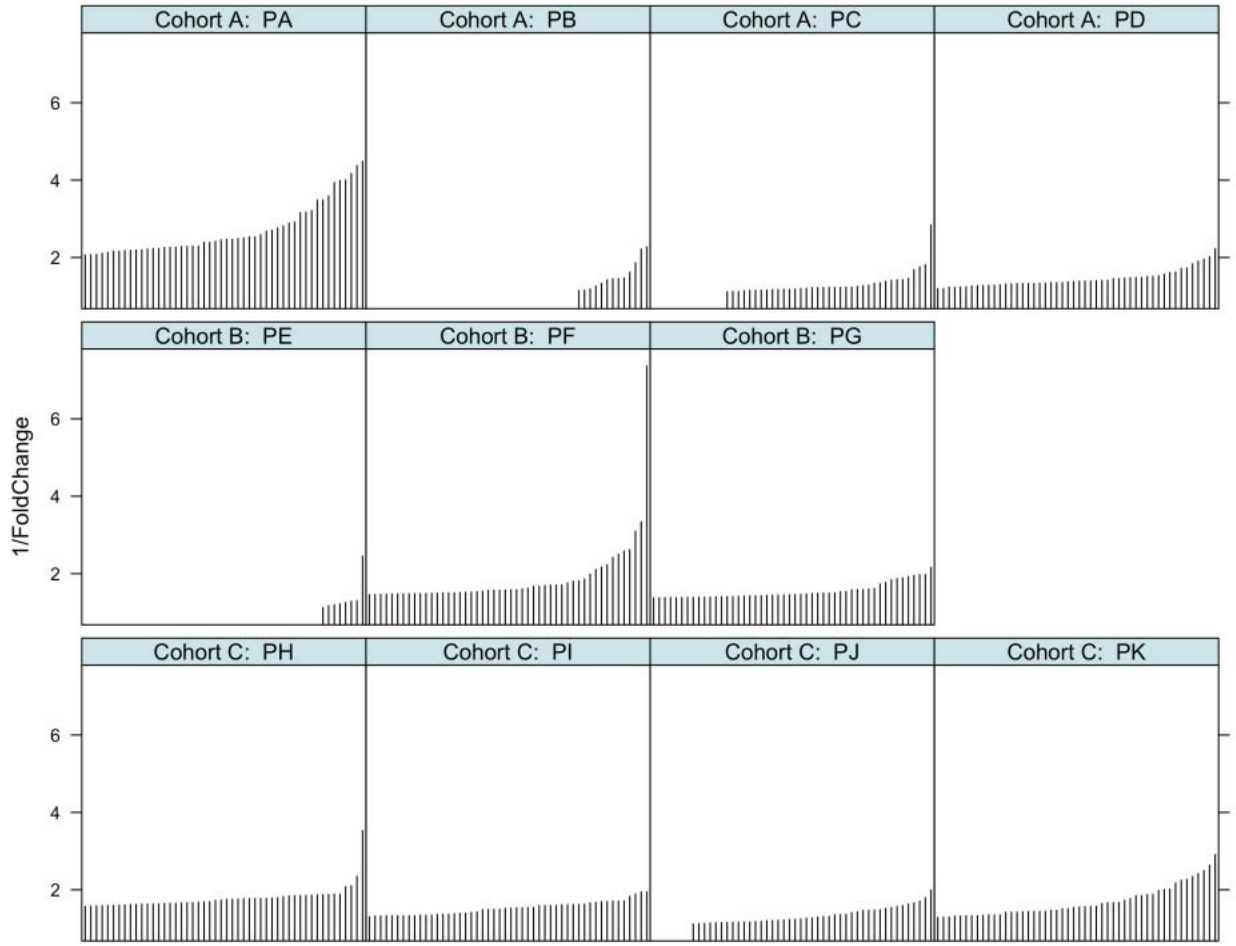
Top 50 Antibody Decreases, Ordered by Rank



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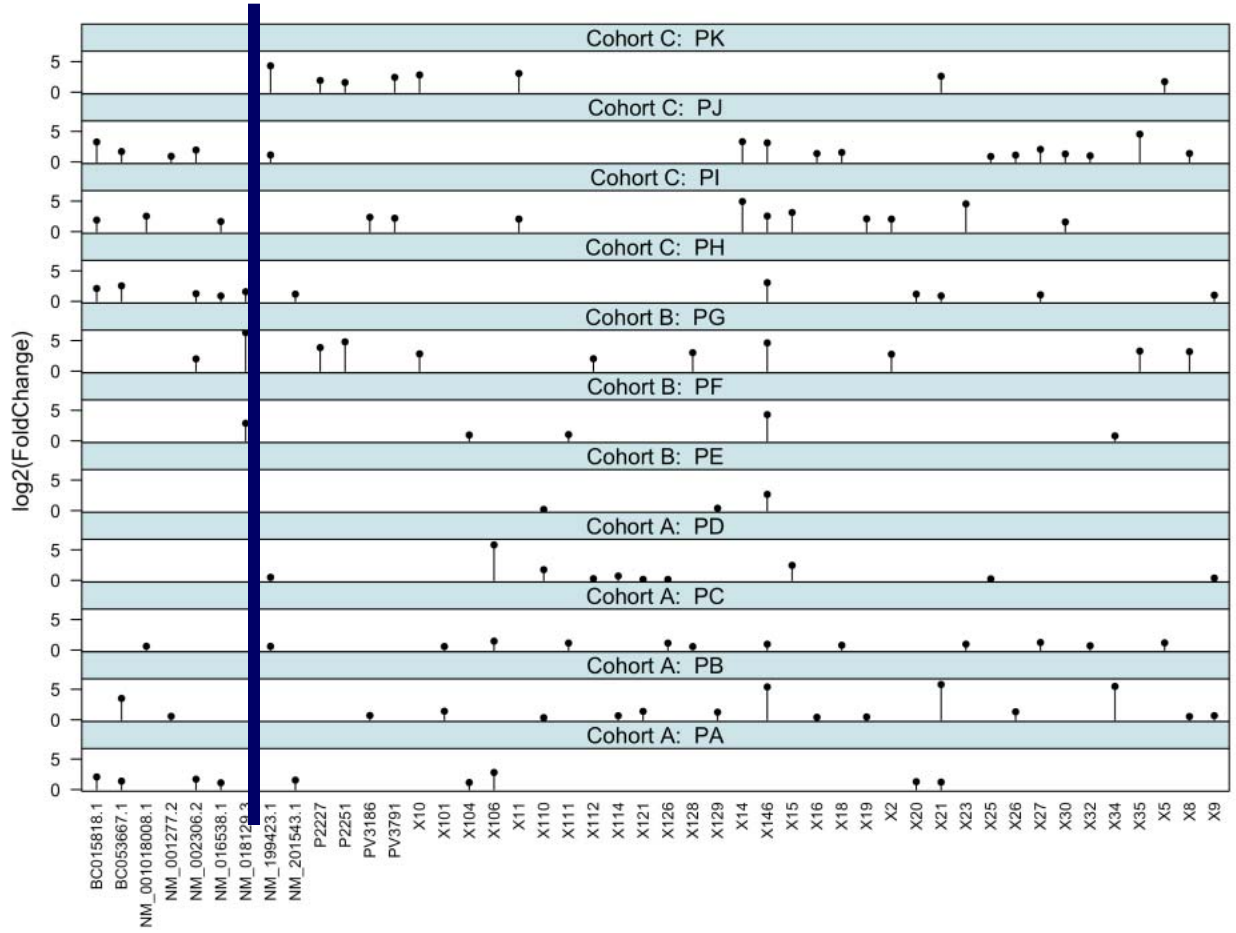
46 “Top 50” Increases, Hits in 2 or More Patients



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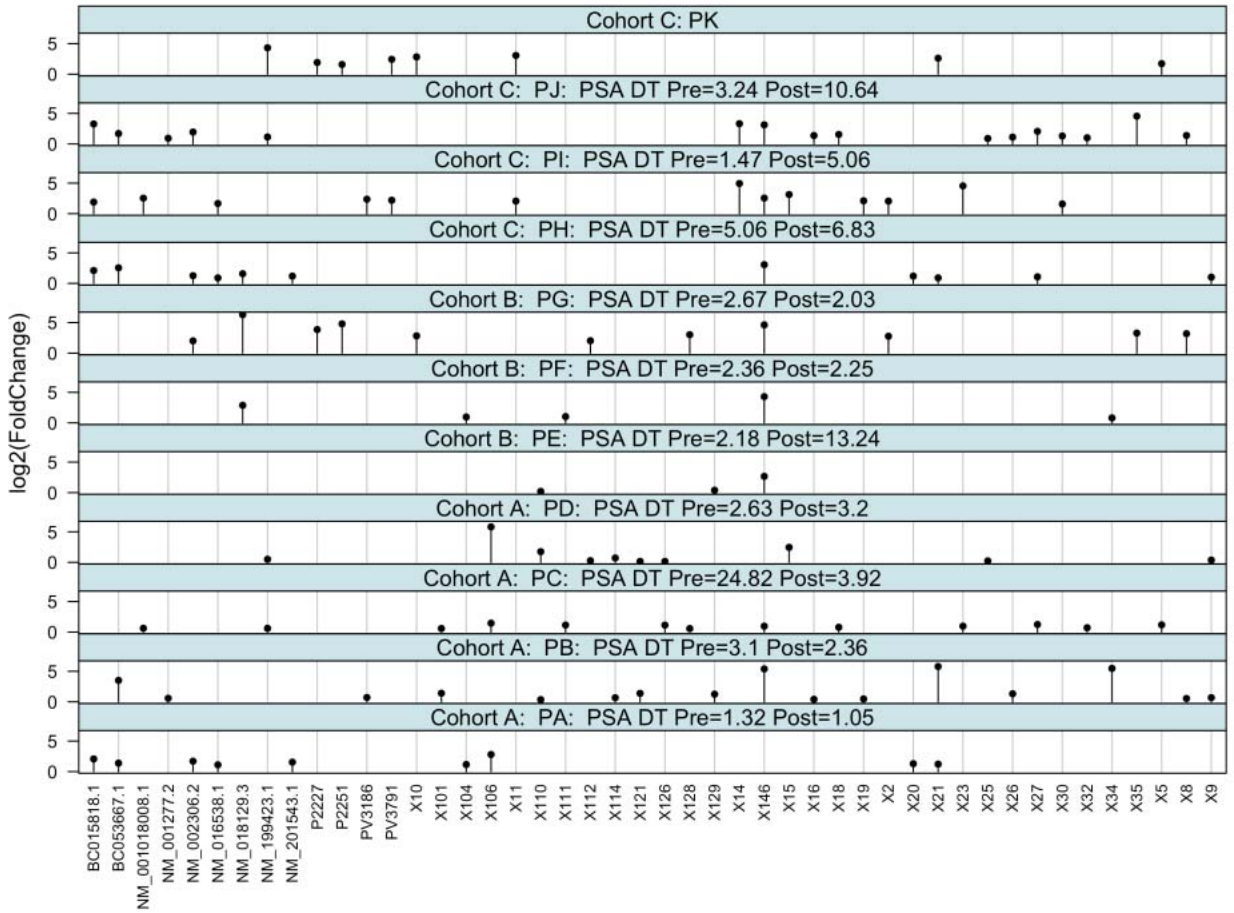
46 “Top 50” Increases, Hits in 2 or More Patients (Verbose)



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Antibody Responses Increased in 3 or More Patients

Protein ID	Number of Patients	Avg Fold Change	Log2 (Avg Fold Change)	Description
NM_018129.3	3	29.6	4.9	Homo sapiens pyridoxine-5'-phosphate oxidase (PNPO), mRNA,
X106	3	21.9	4.5	
X21	4	16.5	4.0	
X146	8	14.7	3.9	
NM_199423.1	4	6.3	2.7	BC000108 Homo sapiens, Similar to Nedd-4-like ubiquitin-protein ligase, clone MGC:2079 IMAGE:3508225, mRNA, complete cds,,
BC053667.1	4	5.8	2.5	Homo sapiens lectin, galactoside-binding, soluble, 3 (galectin 3), mRNA (cDNA clone MGC:61529 IMAGE:6148401), complete cds
BC015818.1	4	5.5	2.5	Homo sapiens lectin, galactoside-binding, soluble, 8 (galectin 8), transcript variant 1, mRNA (cDNA clone MGC:19307 IMAGE:4080313), complete cds
X8	3	4.4	2.1	
NM_002306.2	4	3.4	1.8	BC001120 Homo sapiens, lectin, galactoside-binding, soluble, 3 (galectin 3), clone MGC:2058 IMAGE:3050135, mRNA, complete cds
X27	3	2.9	1.5	
NM_016538.1	3	2.4	1.3	BC017305 Homo sapiens, sirtuin (silent mating type information regulation 2, S.cerevisiae, homolog) 7, clone MGC:29505 IMAGE:5087554, mRNA, complete cds
X110	3	1.9	1.0	
X9	3	1.6	0.7	

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Summary

- Protein array data requires multiple pre-processing steps
- Custom informatics provides power and flexibility to fully interrogate the data
- We can detect treatment effects in serum antibody expression
  - Both dramatic differences across individuals and some consolidation/similarities
  - 13 “Top 50” antibodies common to 3 or more patients
- To learn more, visit
  - Poster No. 92 (Sachin Puri et al)
  - Friday, 12:00pm - 1:00pm & 5:30pm - 6:30pm

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Acknowledgments

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Kristen Hege

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John Verburg
Greg Korbel

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