Metabonomics
a.k.a. metabolomics
a.k.a. metabolic profiling

Application to predicting patient response in cancer clinical trials

Sarah L. Heald, Ph.D.

Associate Director, Department of Chemistry Research
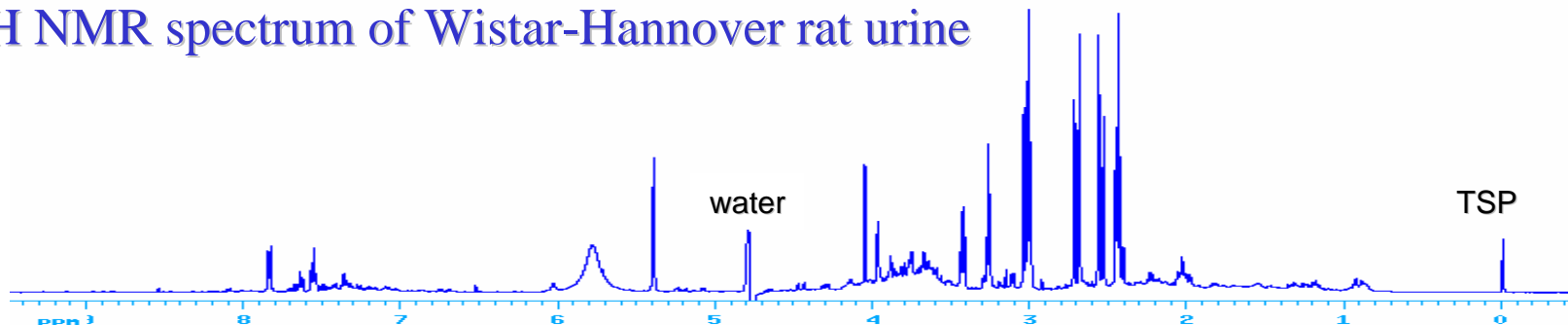
Analytical and Computational Chemistry

January 9, 2006

Bayer HealthCare Pharmaceuticals

NMR-based Metabonomics validated in-house in 2000

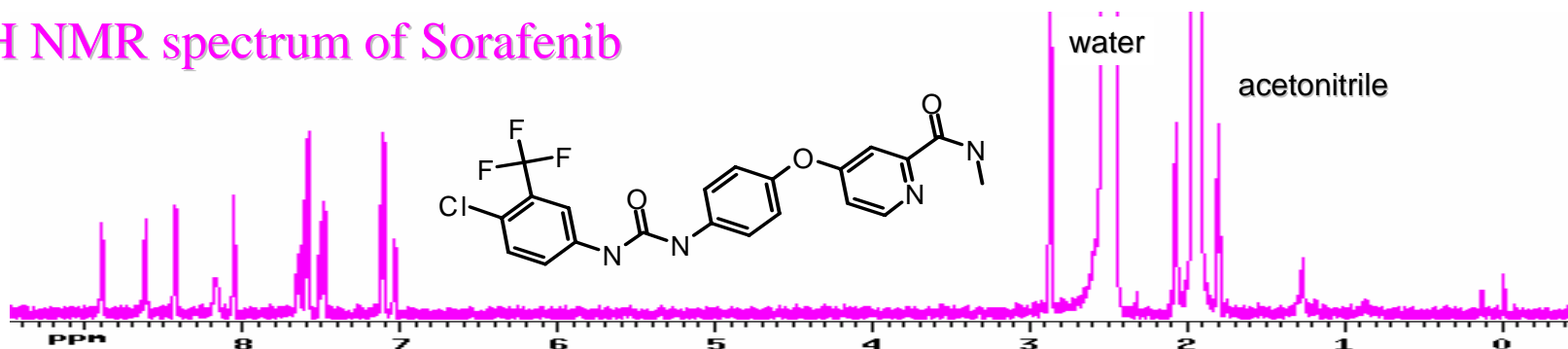- platform for risk assessment in rats – support for efficacy studies

^{1}H NMR spectrum of Wistar-Hannover rat urine

Sorafenib, an anti-cancer agent

- presented by West Haven Research for clinical development in 1999
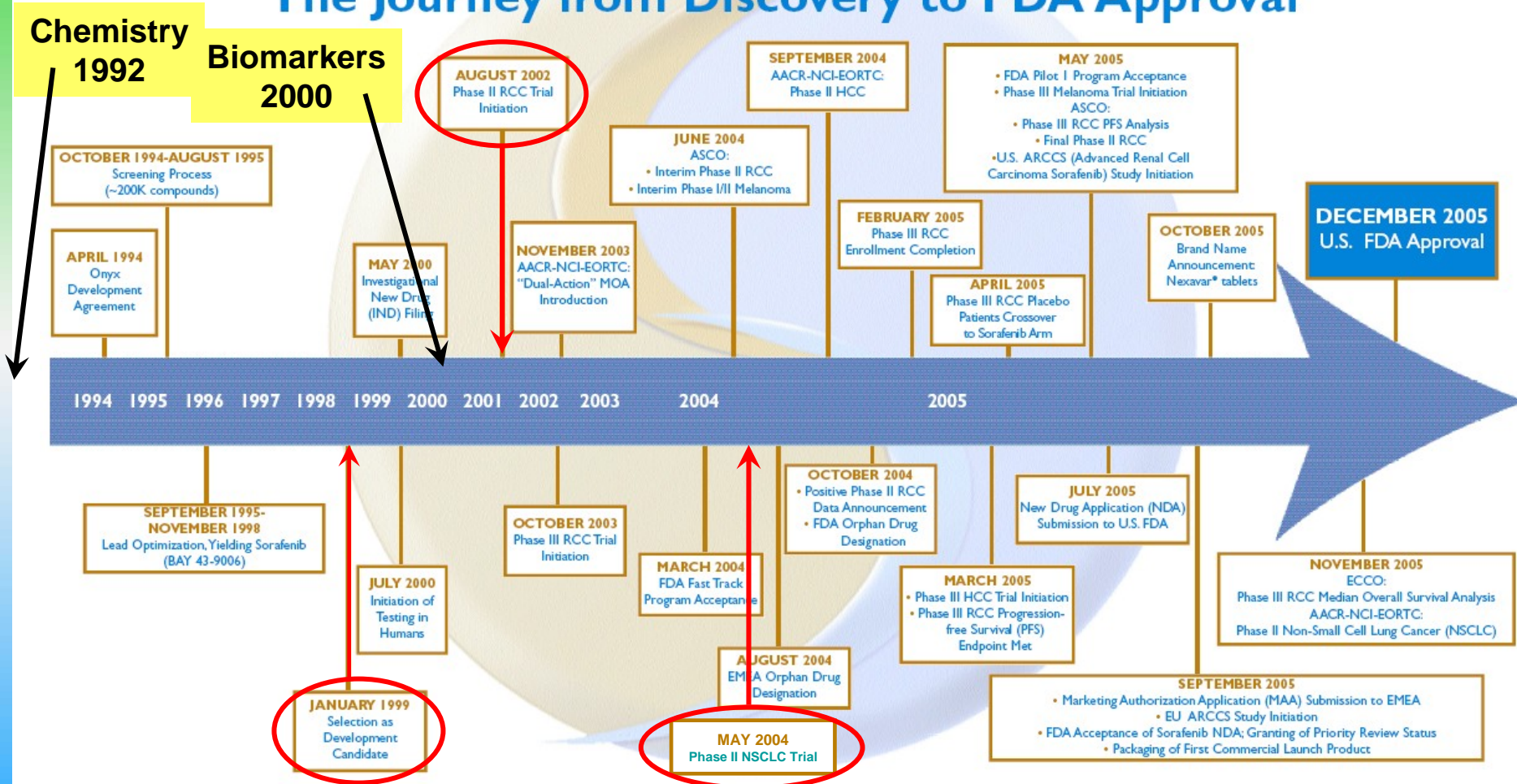- kinase inhibitor with both anti-proliferative and anti-angiogenic activity

^{1}H NMR spectrum of Sorafenib

Sorafenib Approved by FDA for RCC

Nexavar® (sorafenib) tablets:
The Journey from Discovery to FDA Approval
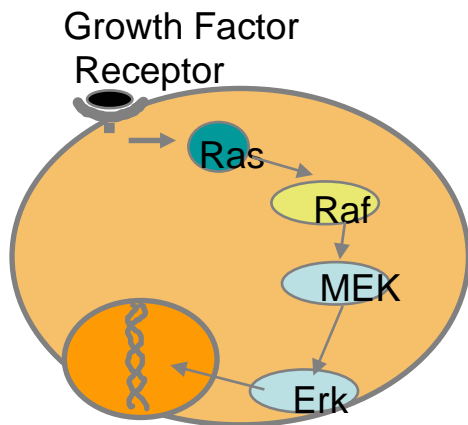
Chemistry 1992

Biomarkers 2000

AUGUST 2002
Phase II RCC Trial Initiation

SEPTEMBER 2004
AACR-NCI-EORTC:
Phase II HCC

MAY 2005
• FDA Pilot I Program Acceptance
• Phase III Melanoma Trial Initiation
ASCO:
• Phase III RCC PFS Analysis
• Final Phase II RCC
• U.S. ARCCS (Advanced Renal Cell Carcinoma Sorafenib) Study Initiation

OCTOBER 1994-AUGUST 1995
Screening Process
(~200K compounds)

JUNE 2004
ASCO:
• Interim Phase II RCC
• Interim Phase I/II Melanoma

FEBRUARY 2005
Phase III RCC
Enrollment Completion

OCTOBER 2005
Brand Name
Announcement:
Nexavar® tablets

DECEMBER 2005
U.S. FDA Approval

APRIL 1994
Onyx
Development
Agreement

MAY 2000
Investigational
New Drug
(IND) Filing

NOVEMBER 2003
AACR-NCI-EORTC:
"Dual-Action" MOA
Introduction

APRIL 2005
Phase III RCC Placebo
Patients Crossover
to Sorafenib Arm

1994 1995 1996 1997 1998 1999 2000 2001 2002 2003   2004   2005

SEPTEMBER 1995-NOVEMBER 1998
Lead Optimization, Yielding Sorafenib
(BAY 43-9006)

OCTOBER 2003
Phase III RCC Trial
Initiation

OCTOBER 2004
• Positive Phase II RCC
Data Announcement
• FDA Orphan Drug
Designation

JULY 2005
New Drug Application (NDA)
Submission to U.S. FDA

NOVEMBER 2005
ECCO:
Phase III RCC Median Overall Survival Analysis
AACR-NCI-EORTC:
Phase II Non-Small Cell Lung Cancer (NSCLC)

JULY 2000
Initiation of
Testing in
Humans

MARCH 2004
FDA Fast Track
Program Acceptance

MARCH 2005
• Phase III HCC Trial Initiation
• Phase III RCC Progression-
free Survival (PFS)
Endpoint Met

JANUARY 1999
Selection as
Development
Candidate

AUGUST 2004
EMEA Orphan Drug
Designation

MAY 2004
Phase II NSCLC Trial

SEPTEMBER 2005
• Marketing Authorization Application (MAA) Submission to EMEA
• EU ARCCS Study Initiation
• FDA Acceptance of Sorafenib NDA; Granting of Priority Review Status
• Packaging of First Commercial Launch Product

Discovery Strategy - Two Complementary Approaches

Pathway Analysis or Mechanism based
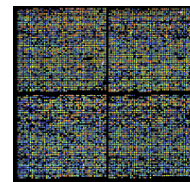
Growth Factor Receptor

Ras

Raf

MEK

Erk

Sample Sources:
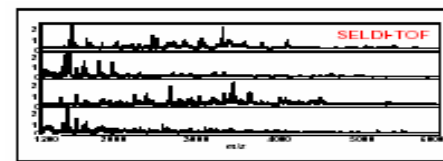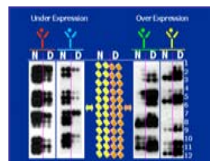
blood

plasma / serum

urine

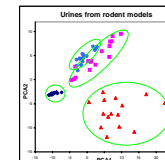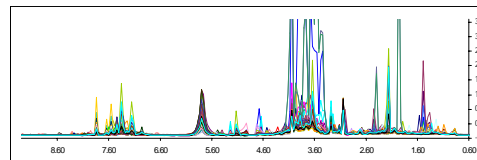historic biopsy

De Novo Biomarker Discovery

• Gene Expression Profile/Pattern (Affymetrix)

• Plasma Protein Expression Pattern (SELDI-Tof)

• Metabolite Profiles (Metabonomics; NMR/LC-MS)

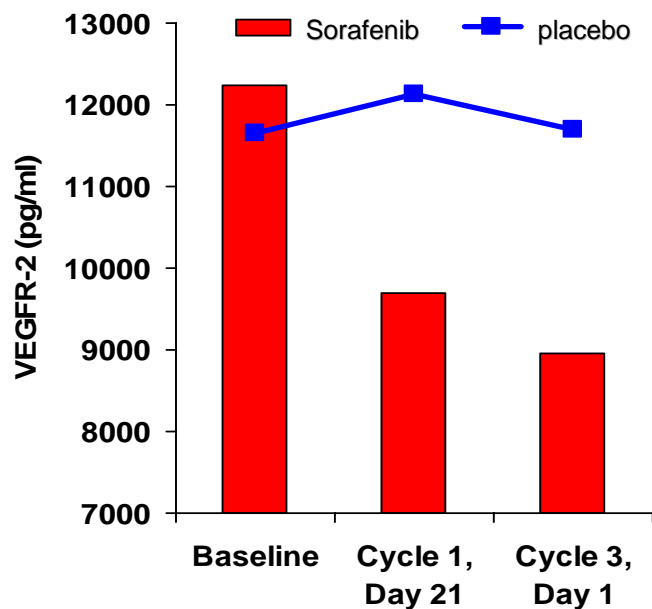## Apply validated assay - Partnership between Pharma and Diagnostics

*Example of Pharmacodynamic Biomarker**

* Preliminary analysis from Phase III trial for Renal Cell Carcinoma

Treatment with Sorafenib Results in Decreased Plasma Levels of VEGFR-2 and Increased Levels of VEGF

VEGFR-2

	Baseline	Cycle 1, Day 21	Cycle 3, Day 1
Sorafenib (n):	149	196	198
Placebo (n):	102	128	132

VEGF
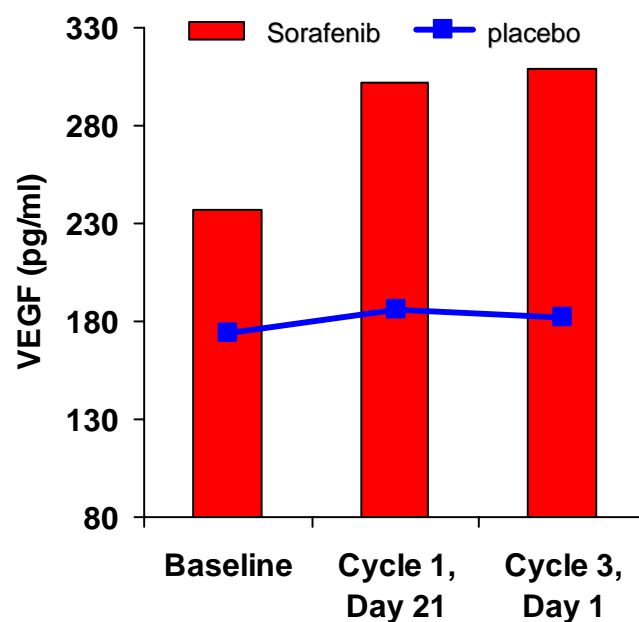
	Baseline	Cycle 1, Day 21	Cycle 3, Day 1
Sorafenib (n):	149	196	197
Placebo (n):	102	128	132

Oncology Phase II Trials

Sorafenib phase II clinical trial(s)

- 100391 – randomized discontinuation trial (501 patients)
- 100557 – focus on non-small cell lung cancer (52 patients)

Sample Collection

- Urine collected at prescreen and fixed time points during treatment cycles – random collection, no diet restrictions

Surrogate vs Clinical Endpoint

- Clinical endpoint for cancer is death (change in survival rate)
- For cytotoxic agents – a surrogate marker is tumor shrinkage
- Progression-free survival (PFS) may be a more meaningful and relevant surrogate for survival benefit than tumor shrinkage

Application in Clinical Study 100391

GOAL: To investigate the possible predictive relationship of urinary metabolic profiles with patient response

Phase II study 100391: **670 urines received**

Urines collected at prescreen (prior to study), cycle 1-day 15, cycle 4-day 1, etc

RENAL:	95 *prescreen urines out of*	202 *patients*
COLORECTAL:	46	139
OTHER:	27	70
MALIG. MELANOMA:	11	37
OVARIAN, PANCREATIC,		
BREAST, NSCLC, THYROID:	18	53

Clinical data* included Best Response and Progression Free Survival

* ~ 25% of patients have no clinical assessment and 30-40% have censored PFS data

In-House Metabonomic Protocol for Urine

Sample Handling

urine + buffer

NMR data collection

1D ^{1}H NOESY

Data processing
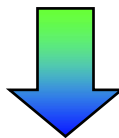
segment spectra into bins

NMR spectrum

NMR bins

32,000 pts

256 pts

	4.7600	4.7200	4.6800	4.6400	4.6000
metab_urin_1	0.2003	0.0248	0.0640	0.0530	0.0471
metab_urin_2	0.0198	0.0099	0.0457	0.0364	0.0284
metab_urin_5	0.2533	0.0300	0.0637	0.0727	0.0408
metab_urin_6	0.2187	0.0235	0.0683	0.0602	0.0468
metab_urin_7	0.1944	0.0231	0.0719	0.0471	0.0441
metab_urin_37	0.1212	0.0247	0.0438	0.0445	0.0385
metab_urin_38	0.0015	0.0093	0.0310	0.0321	0.0268
metab_urin_41	0.0573	0.0608	0.0805	0.1059	0.0597
metab_urin_42	0.0949	0.0573	0.0756	0.0952	0.0592
metab_urin_43	0.0545	0.0569	0.0755	0.0940	0.0650
metab_urin_73	0.1267	0.0153	0.1023	0.1579	0.0597
metab_urin_74	0.0838	0.0150	0.1049	0.0981	0.0443
metab_urin_77	0.0971	0.0246	0.1414	0.1	0.0724

Metabolite levels

Assigned Metabolites

Novel Metabolite ID

HPLC-NMR and MS

Model Building

PCA, PLS-DA, OSC
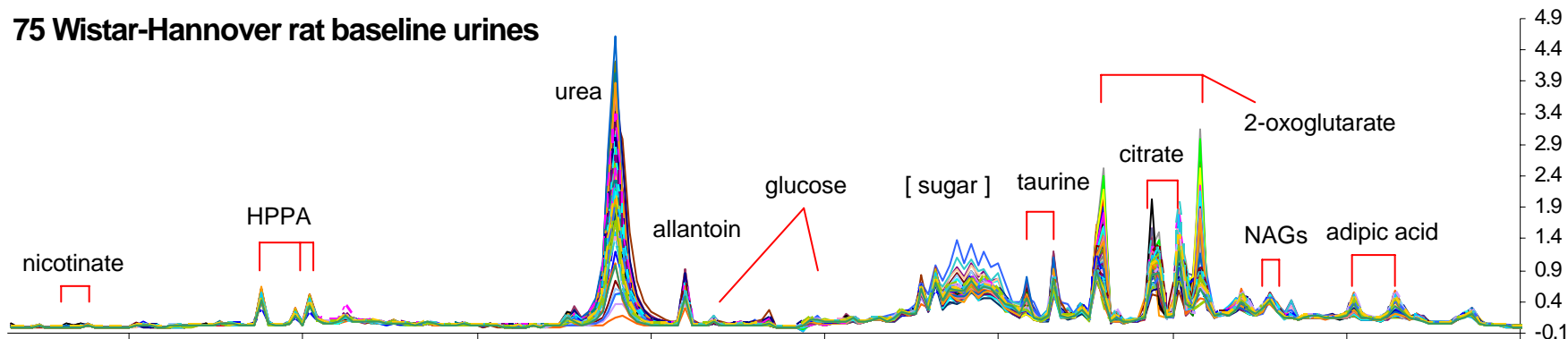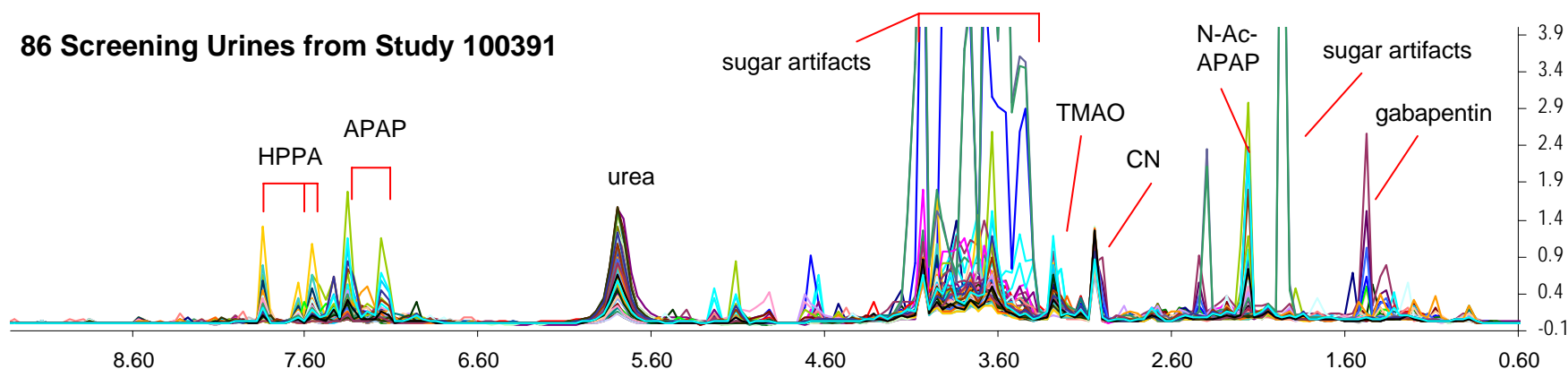
GTA (1.76 ppm)

Urines from rodent models
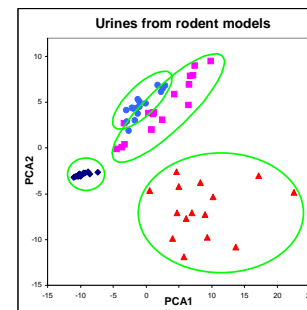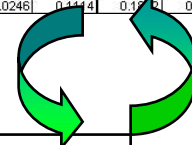
Challenges in NMR Data Interpretation

75 Wistar-Hannover rat baseline urines

**86 Screening Urines from Study 100391**

- Rat urines show limited variability, while human urines are highly variable
- Artifacts mask information from endogenous metabolites and can be repaired

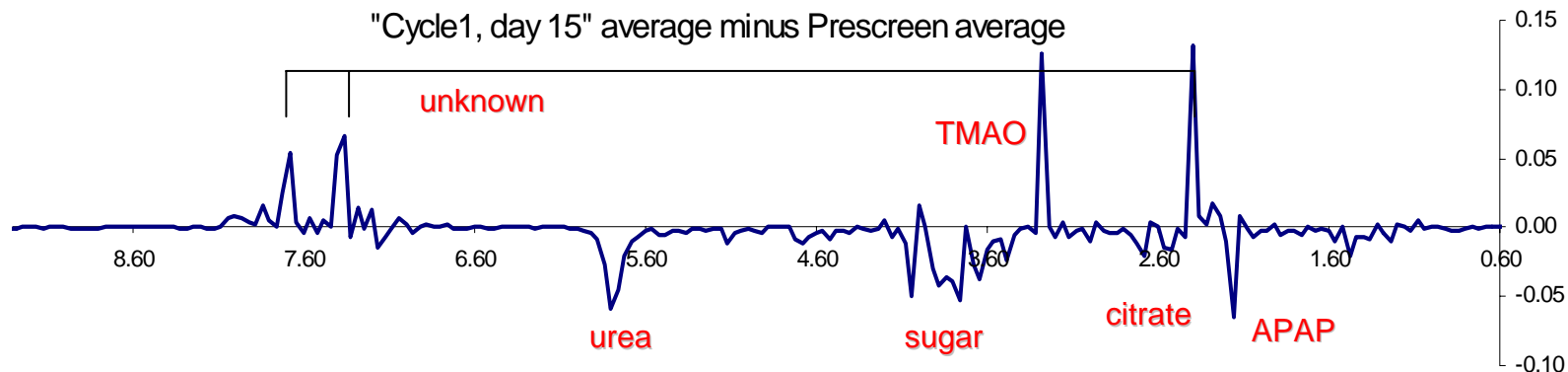- Less than 10% of the spectra in 100391 needed modification

At prescreen, non-responders had lower levels of hippurate and urea and higher levels of acetominophen metabolites in their urine
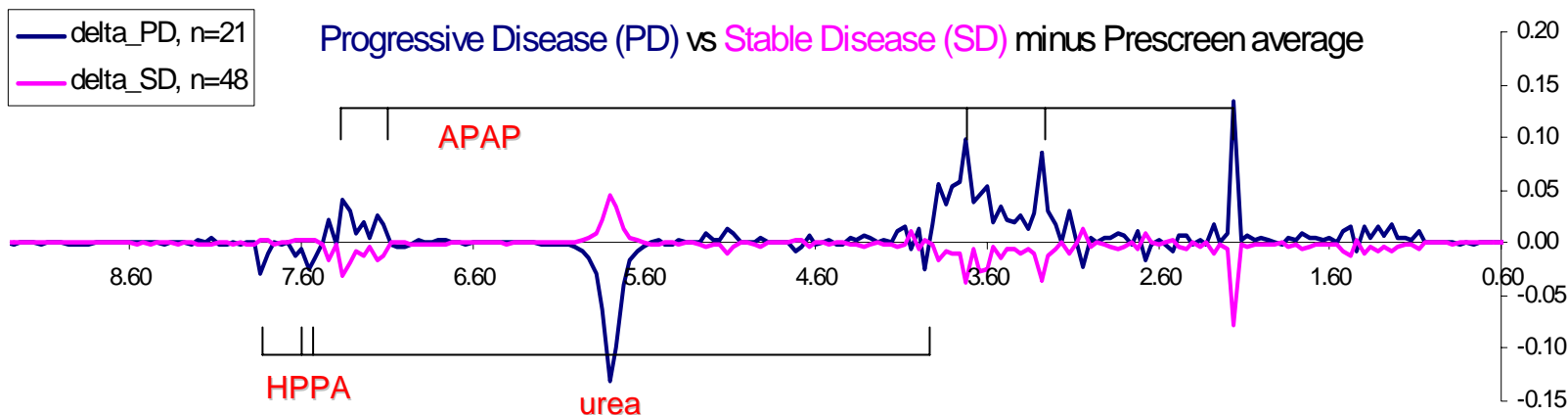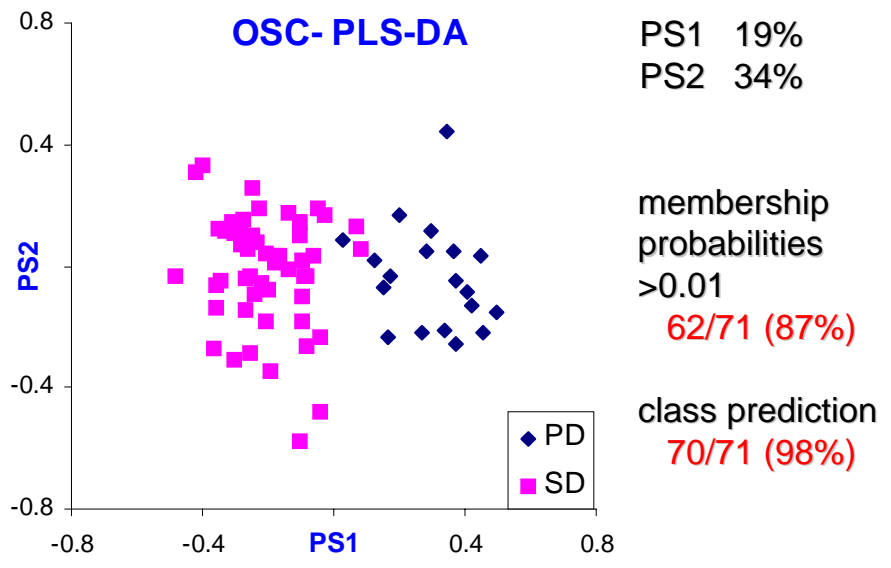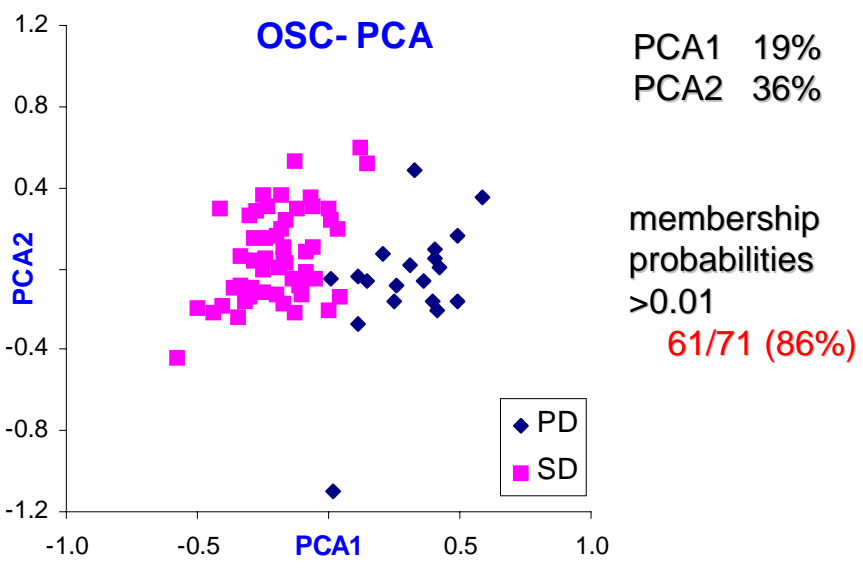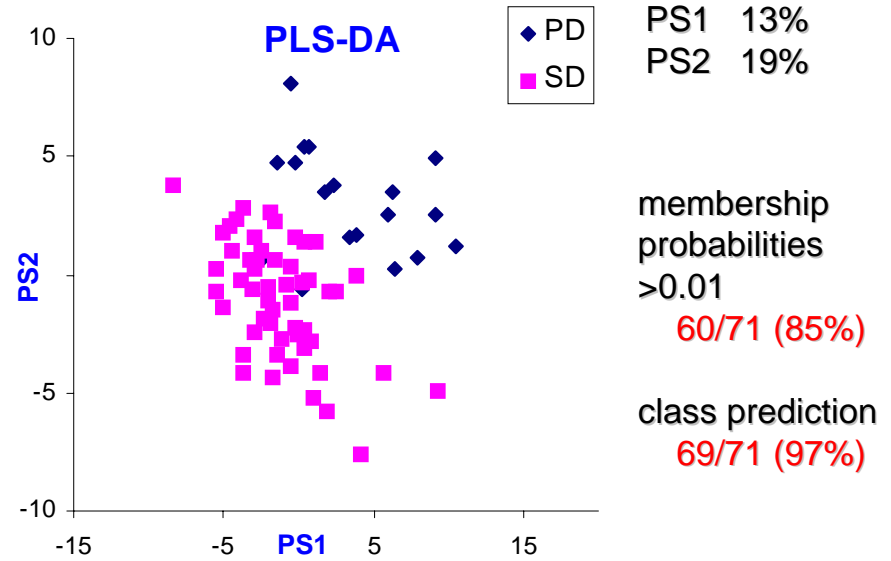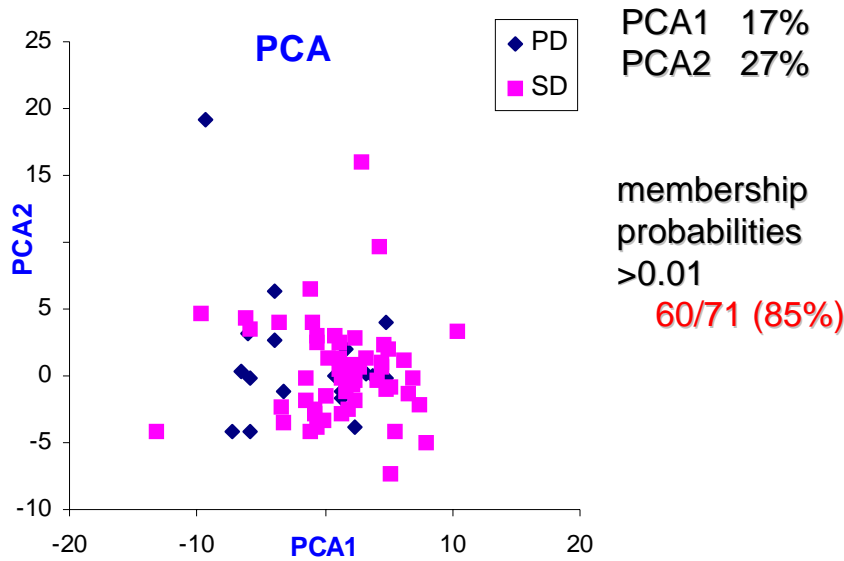

Urines collected post-dose contained an unknown metabolite that was isolated and identified as toluene sulfonic acid

First Pass Modeling of Prescreen urines*

PCA

PCA1 17%
PCA2 27%

membership
probabilities
>0.01
60/71 (85%)

PLS-DA

PS1 13%
PS2 19%

membership
probabilities
>0.01
60/71 (85%)

class prediction
69/71 (97%)

OSC- PCA

PCA1 19%
PCA2 36%

membership
probabilities
>0.01
61/71 (86%)

OSC- PLS-DA

PS1 19%
PS2 34%

membership
probabilities
>0.01
62/71 (87%)

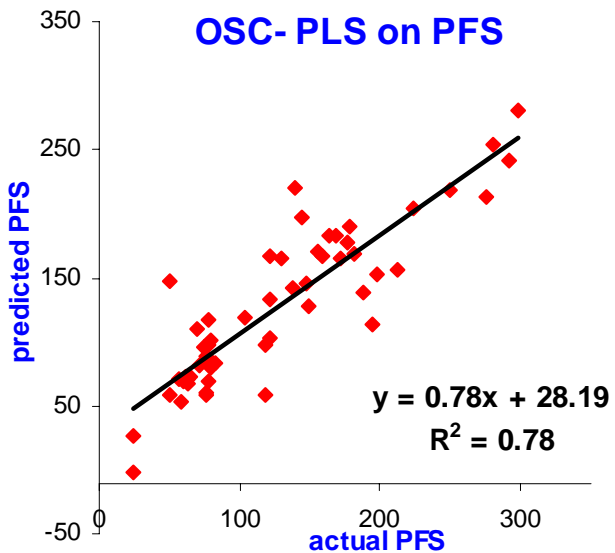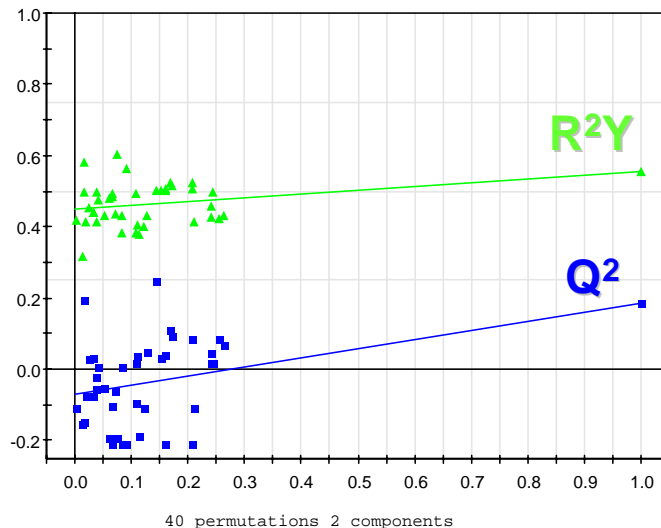class prediction
70/71 (98%)

* 190 bins, 9.40 – 0.60 ppm, water and urea excluded

Prediction of PFS from prescreen urine

BUT fail to perform well under validation testing – models are over fit
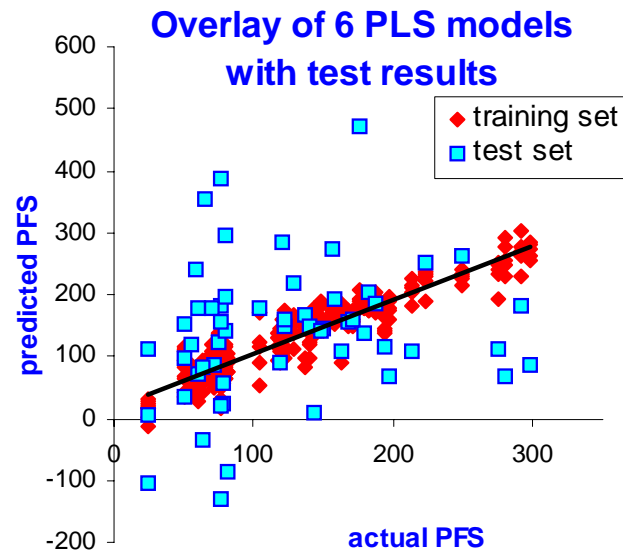
PLS on PFS

$y = 0.55x + 56.62$
$R^2 = 0.55$
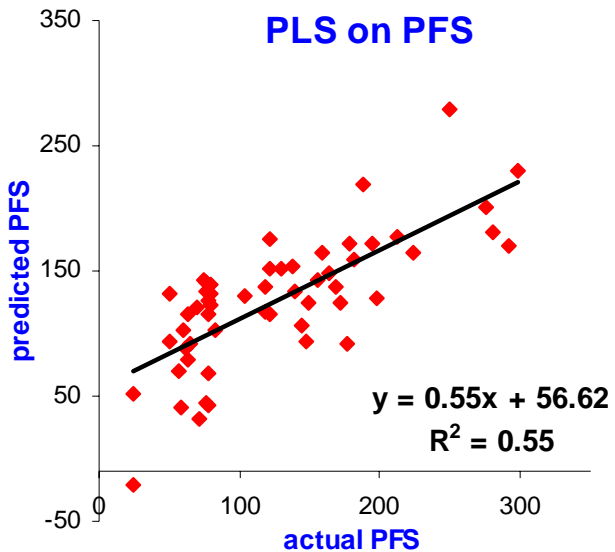
PS1 18%
PS2 26%

membership
probabilities
>0.01
46/55 (84%)

R^2Y

Q^2

40 permutations 2 components

OSC- PLS on PFS

$y = 0.78x + 28.19$
$R^2 = 0.78$

PS1 14%
PS2 32%

membership
probabilities
>0.01
48/55 (87%)

Overlay of 6 PLS models with test results

- training set
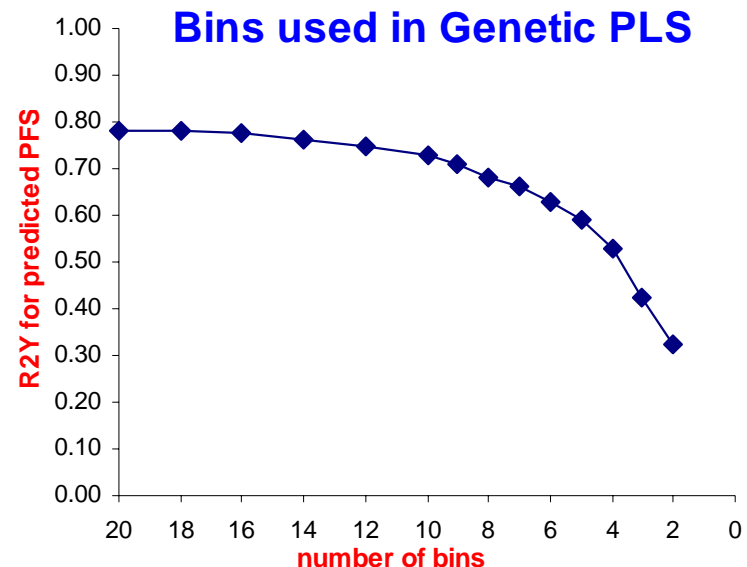- test set

Optimization of PLS Models

For PLS models, a simple correlation map was used to reduce the number of bins

Correlation matrix Analysis

NMR bin	correlation	used in model	
3.28	-0.20		TMAO
3.12	0.24	***	malonate
2.72	-0.22	***	DMA
2.68	0.31	***	
2.56	0.29		citrate
2.52	0.26		

Genetic Algorithm approach
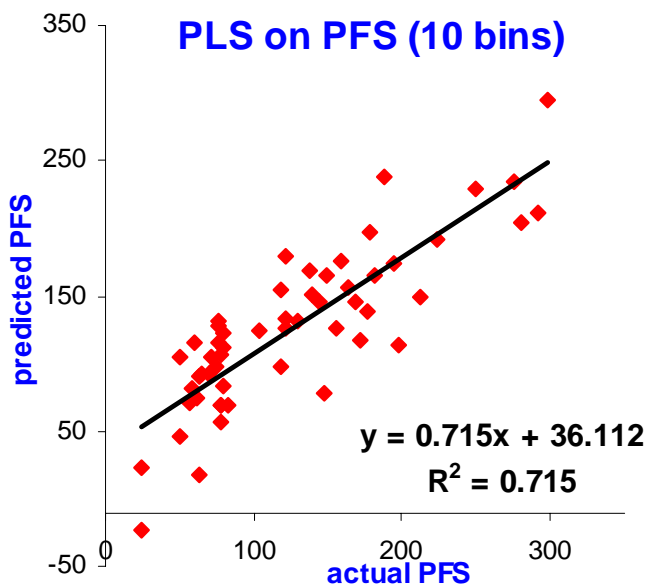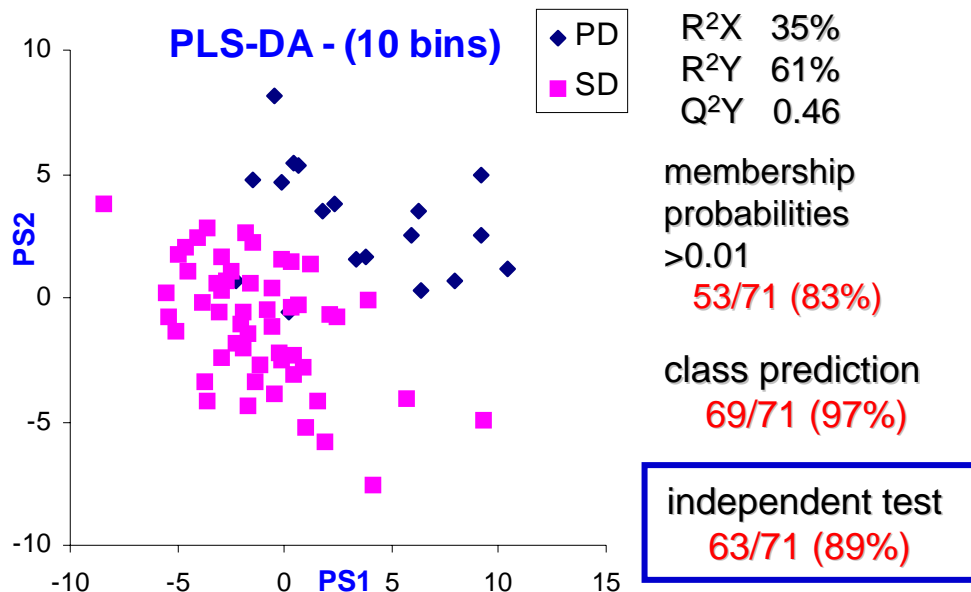
Bins used in Genetic PLS

- ➤ All bins found in top 20% of the loading factors
- ➤ Neither Hippurate or APAP contribute to models
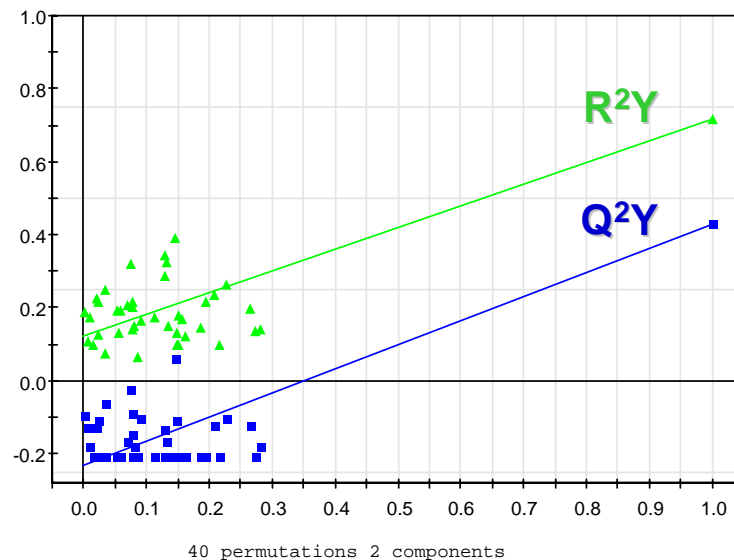- ➤ Genetic PLS then used to further reduce the number of bins

Performance of Optimized Models

- **PLS-DA used correlation matrix and 'leave-one-out' approach**

- **Parameters R^2X and Q^2Y significantly improve**

- **Undesirable noise and food metabolites avoided**

PLS-DA - (10 bins)

Legend: ◆ PD ■ SD

R^2X 35%
R^2Y 61%
Q^2Y 0.46

membership probabilities >0.01
53/71 (83%)

class prediction
69/71 (97%)

independent test
63/71 (89%)

PLS on PFS (10 bins)

y = 0.715x + 36.112
$R^2 = 0.715$

R^2X 30%
R^2Y 72%
Q^2Y 0.45

membership probabilities >0.01
53/55 (96%)

R^2Y

Q^2Y

40 permutations 2 components

Application to NSCLC Clinical Study 100557

GOAL: To investigate the possible predictive relationship of urinary metabolic profiles with patient response

Phase II study 1000557:   **52 NSCLC patients**

Urines collected at prescreen (prior to study), cycle 1-day 15, cycle 3-day 1, etc
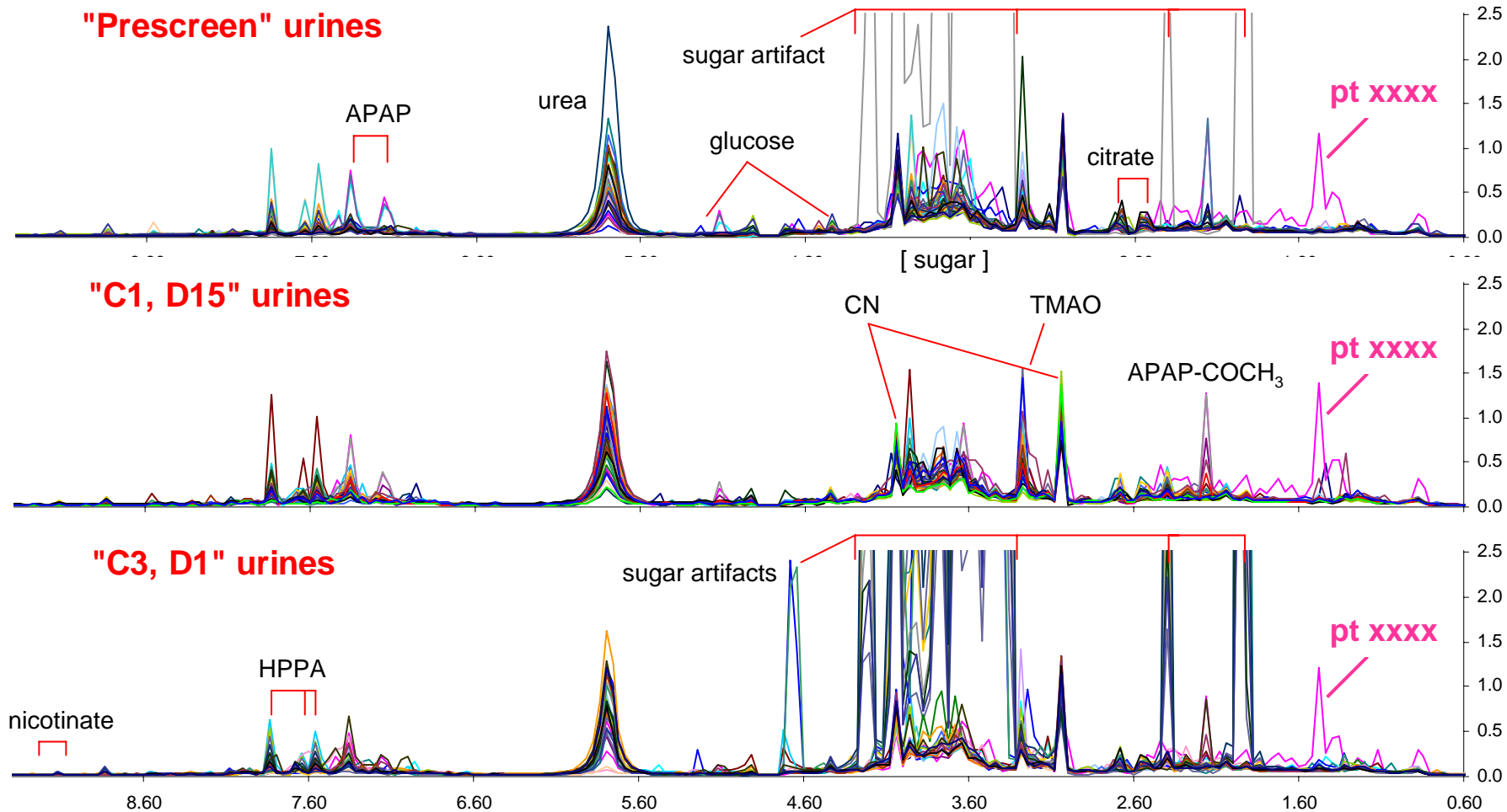
155 urine samples received and analyzed by ^{1}H NMR

Prescreen:	35	
cycle 1, day 1:	4	*not used*
cycle 1, day 15:	36	
cycle 3, day 1 :	34	
cycle 5, day 1 :	21	*not used – responder only*
beyond :	25	*not used – responder only*

Clinical data included Best Response, Maximum % Lesion Reduction (M%LR), Time to Progression (TTP) and demographic data

Metabolite variability in NSCLC urines

- Screening and "C1, D15" urines have only one sample with a high food artifact
- Starting with "C3, D1", ~30% of the patients received refreshment prior to collection
- All urines from patient xxxx were abnormal and excluded from the modeling efforts
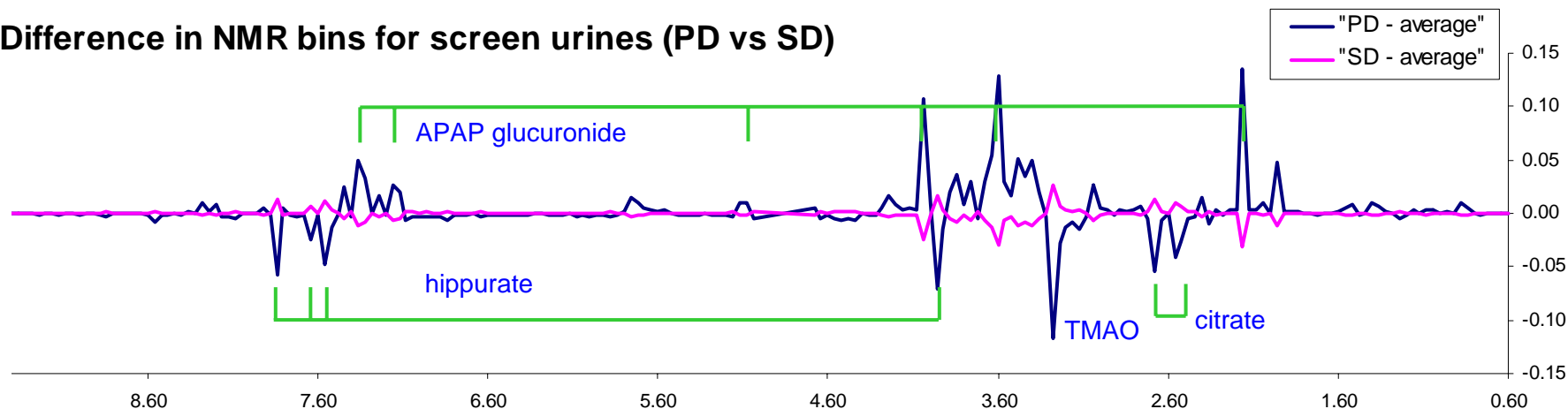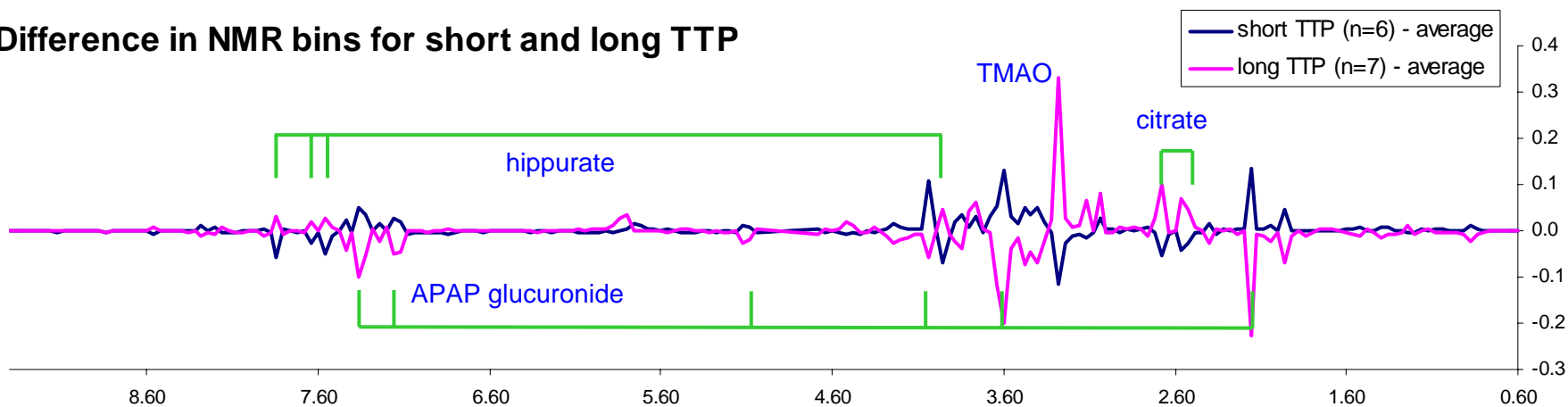
Overview of 100557 Metabolic Profiles

Non-Responders and patients with short TTP have similar profiles

- lower levels of hippurate, citrate and TMAO and higher acetaminophen

Difference in NMR bins for screen urines (PD vs SD)
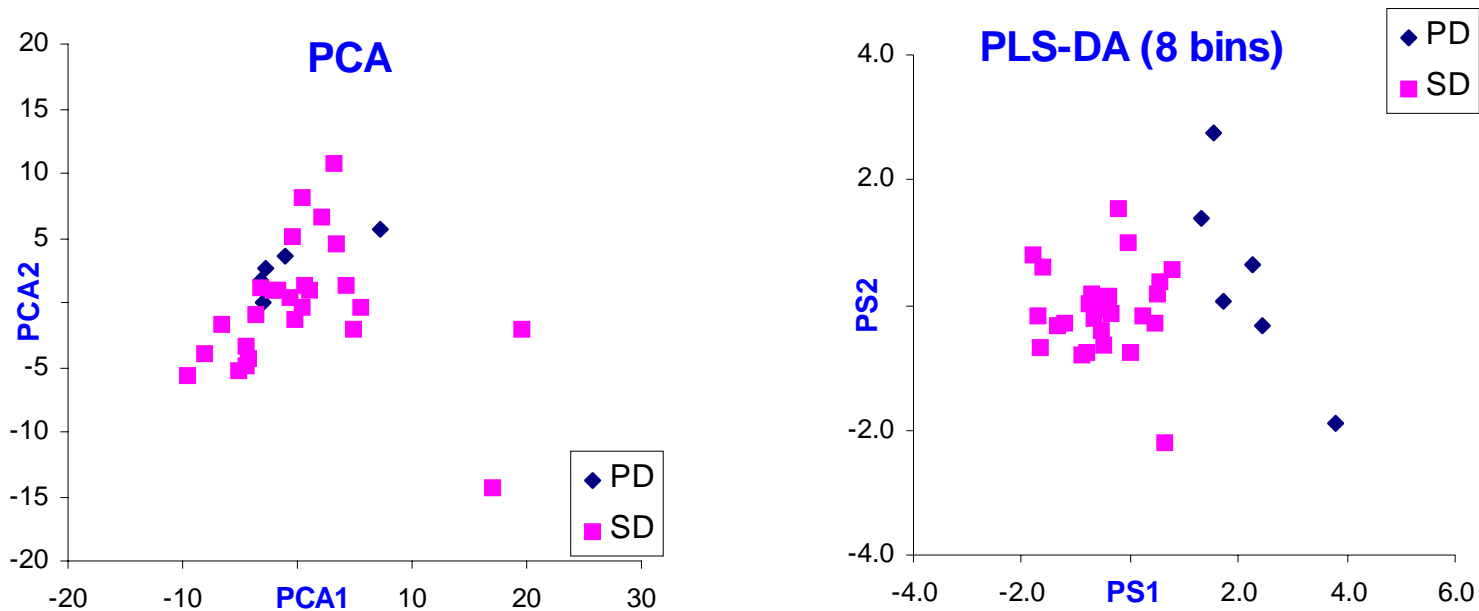
Difference in NMR bins for short and long TTP

TTP = time to regression

Separation of responders and non-responders achieved in PLS-DA with 8 of 190 bins

PCA

PLS-DA (8 bins)

PCA1	20%	membership	class prediction
PCA2	32%	probabilities >0.05	
PCA3	40%	28/32 (88%)	

R^2X	35%	membership	class prediction
R^2Y	70%	probabilities >0.05	32/32 (100%)
Q^2Y	0.31	32/32 (100%)	

Validation testing of the PLS-DA model via independent test sets resulted in 30/32 (94%) classified correctly
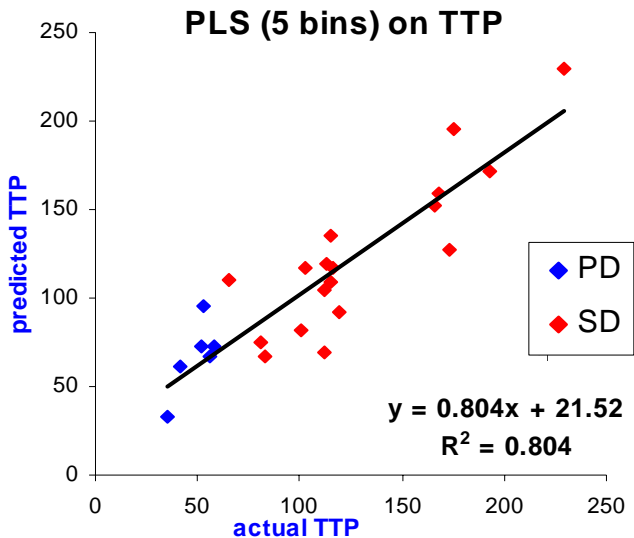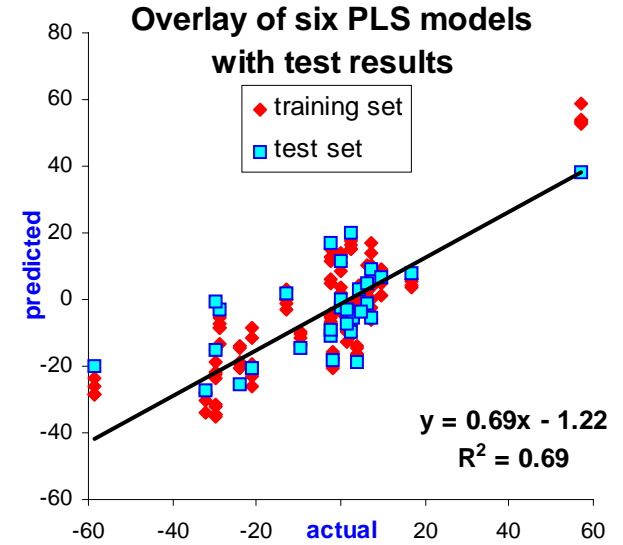
Lesion Reduction and Time to Progression

PLS models for Maximum % Lesion Reduction (M%LR) and Time to Progression (TTP) based on 5 bins selected via a genetic algorithm from the top loading factors
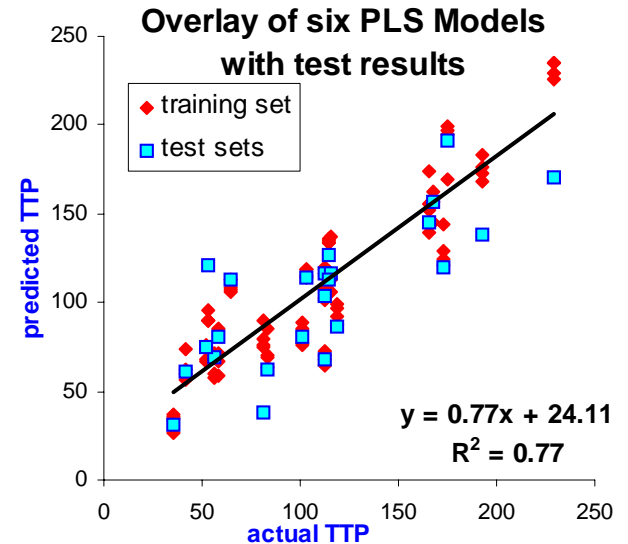

PLS (5 bins) on Max % Lesion Reduction

R^2X 39%
R^2Y 69%
Q^2Y 0.46

membership probabilities >0.05
32/32 (100%)

y = 0.696x - 1.1953
R^2 = 0.696

PD
SD

Overlay of six PLS models with test results

training set
test set

y = 0.69x - 1.22
R^2 = 0.69

PLS (5 bins) on TTP
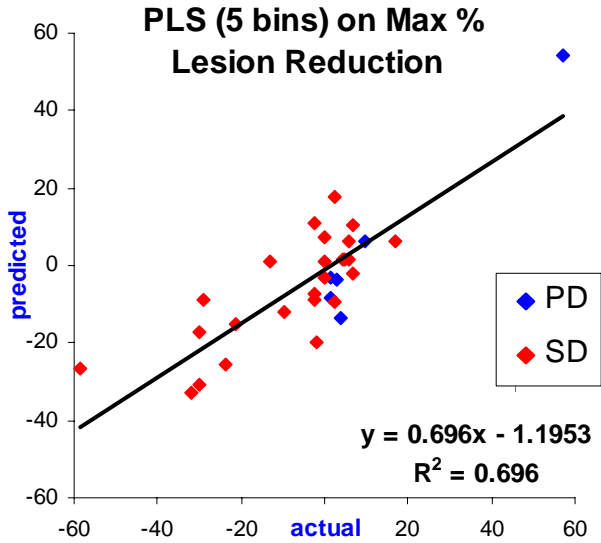
R^2X 59%
R^2Y 80%
Q^2Y 0.60

membership probabilities >0.05
31/32 (96%)

PD
SD

y = 0.804x + 21.52
R^2 = 0.804

Overlay of six PLS Models with test results

training set
test sets

y = 0.77x + 24.11
R^2 = 0.77

Demographic Analysis of Screening Urines

Metabolite profiling of urine samples also sensitive to demographics

PLS-DA on origin (10 bins)

Legend: ◆ USA, ■ Germany

R^2X 40%
R^2Y 69%
Q^2Y 0.46

membership probabilities >0.05
31/32 (97%)

class prediction
31/32 (97%)

PLS-DA on gender (6 bins)

Legend: ◆ female, ■ male

R^2X 38%
R^2Y 72%
Q^2Y 0.48

membership probabilities >0.05
31/32 (97%)

class prediction
30/32 (94%)

PLS (6 bins) on Age

y = 0.71x + 16.65
R^2 = 0.71

R^2X 49%
R^2Y 71%
Q^2Y 0.50

membership probabilities >0.05
31/32 (97%)

PLS-DA on tumor cell type (13 bins)

Legend: ◆ adenocarcinoma, ■ squamous cell, ▲ large cell

R^2X 42%
R^2Y 40%
Q^2Y 0.23

membership probabilities >0.05
29/32 (91%)

class prediction
25/27 (93%)

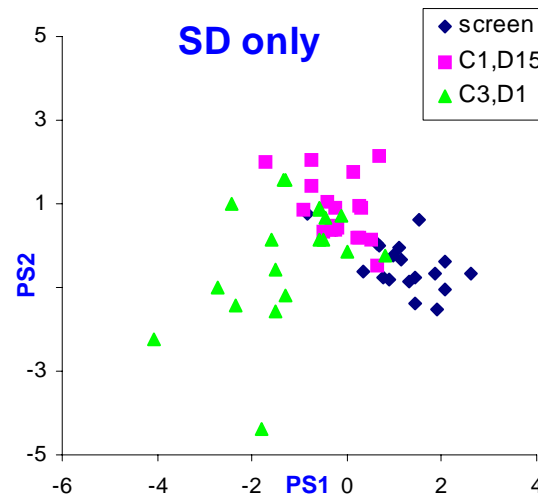*all large cell classified as adenocarcinoma*

Prediction of Pharmacodynamic Response

Model suggests that responders progress away from starting disease state
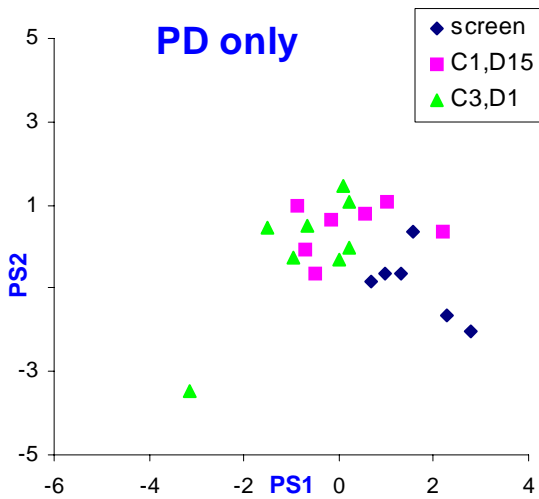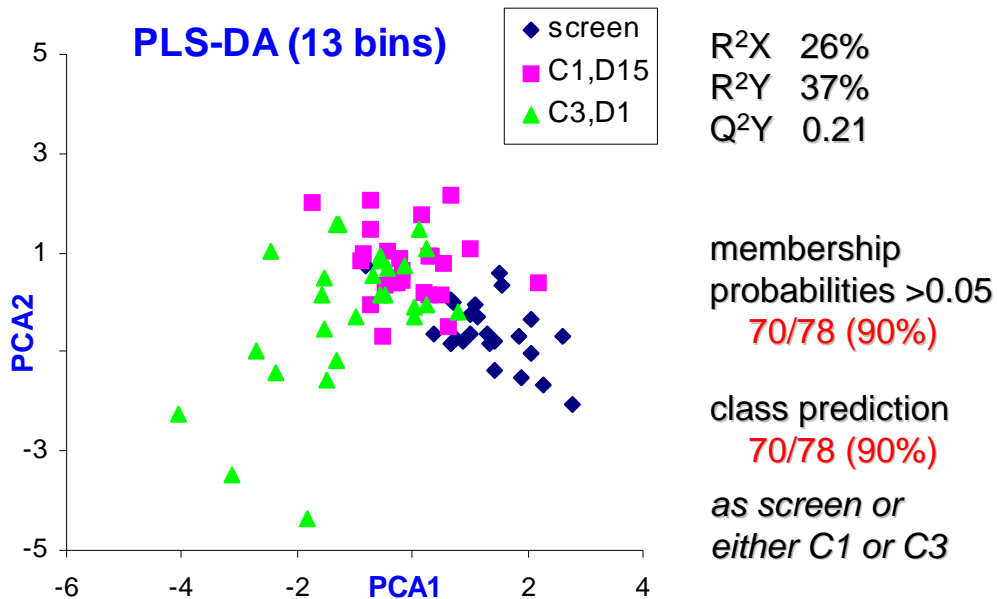
Sorafenib achieved FDA approval without needing a biomarker

Metabonomic data obtained from prescreen urines was shown to be capable of predicting patient outcome in cancer phase II trials

- PLS Models – need to minimize the number of bins used in each model

- Use of <5% of the NMR spectrum in the model reduces issues over sample variability and food metabolites in urine

- Demographic data helped prioritize metabolites, flagged as potentially important predictors of patient response, for further prosecution

- Method to overcome over fitting caused the OSC-filter was not identified

- Success in part due to small number of patient samples

  ➢ Analysis of RCC phase III trial (3,215 urine samples) in progress

Acknowledgements

Jim Elting
Ian Taylor
Brian Dixon
Chetan Lathia

Informatics
Douglas Bigwood

Clinical Development
Brian Schwartz
Edward Huguenel

BHC-Diagnostics
Walter Carney
Sheryl Brown-Shimer

Sample Logistics
Maura Broggi

Immunohistochemistry
Sean Trombley
Angela McNabola
Kate Georgelos
Eileen Novicki
David Williams
Dean Wilkie

Metabonomics
Jeff Chin
Tony Paiva
Joanne Salageanu
Sally Heald

Proteomics
Gary Davis
Timothy Nicholas
Christine Silverstrim
Walter Pickett

Pharmacogenomics
Timothy Sarr
Deborah Webb
Nicole Pauloski
Gwenda Ligon