

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

***Application to predicting patient response
in cancer clinical trials***

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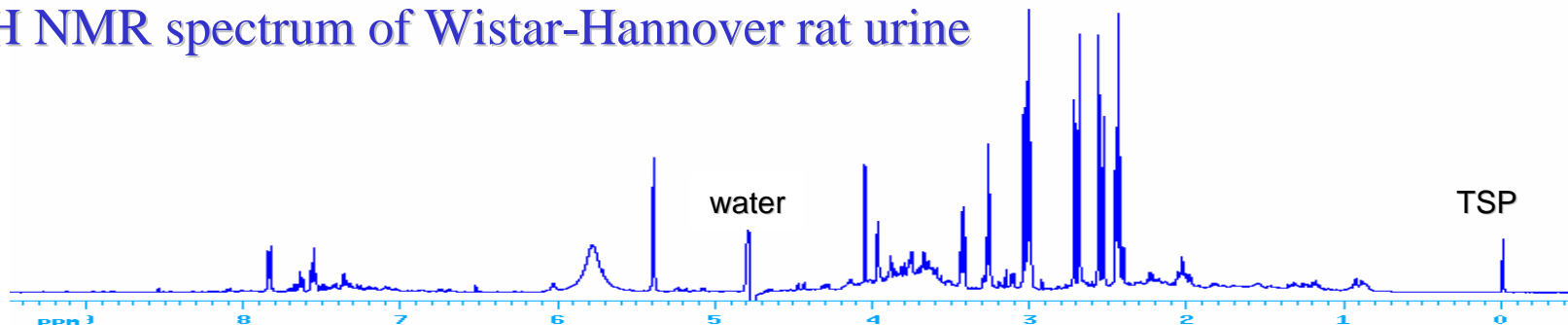


Bayer HealthCare
Pharmaceuticals

NMR-based Metabonomics validated in-house in 2000

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

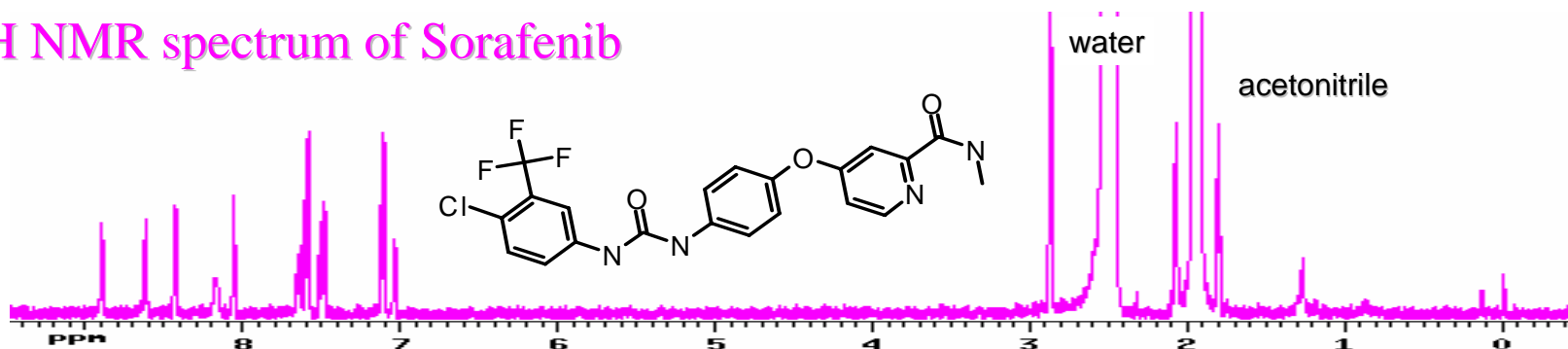
^1H NMR spectrum of Wistar-Hannover rat urine



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

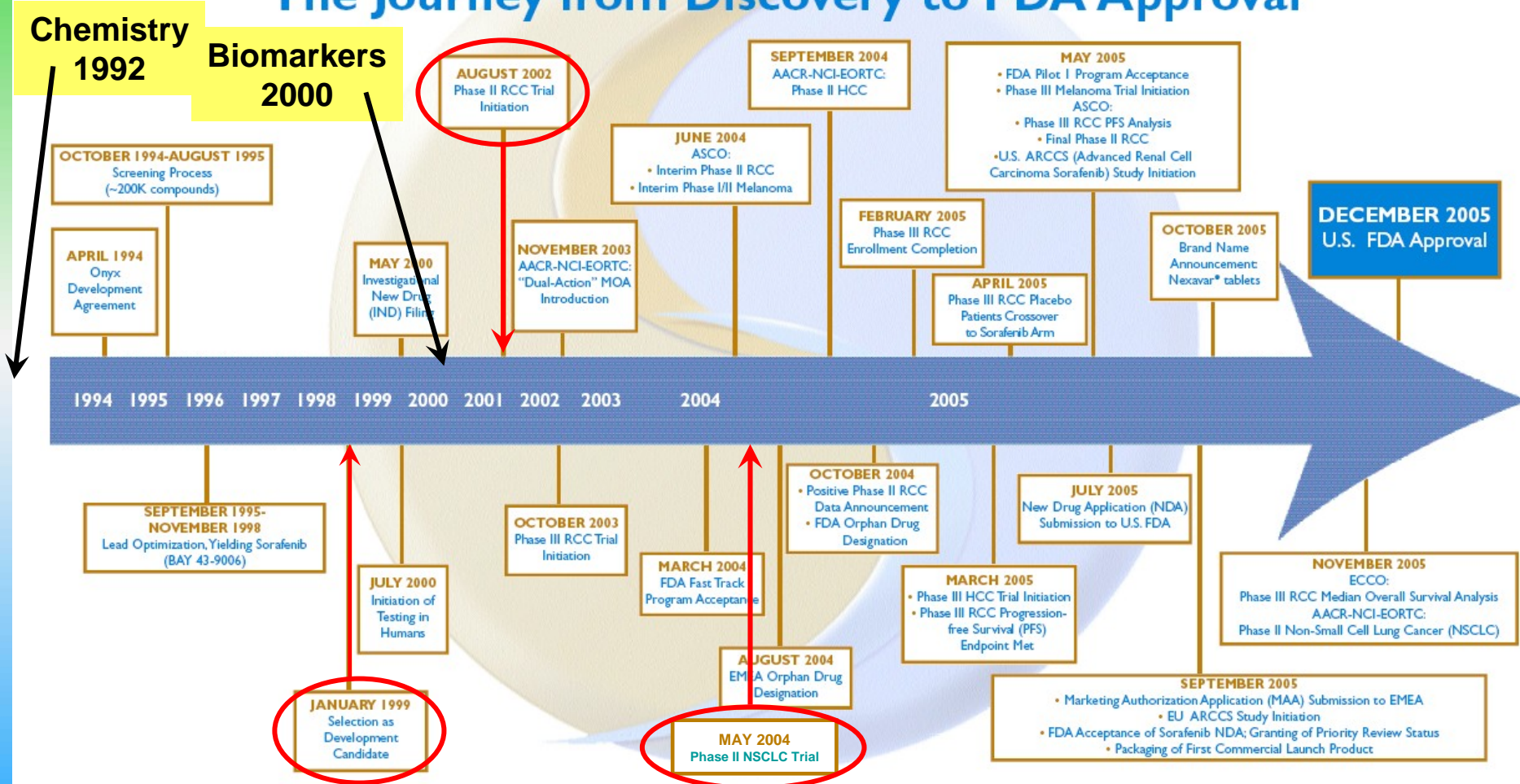
^1H NMR spectrum of Sorafenib





Sorafenib Approved by FDA for RCC

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

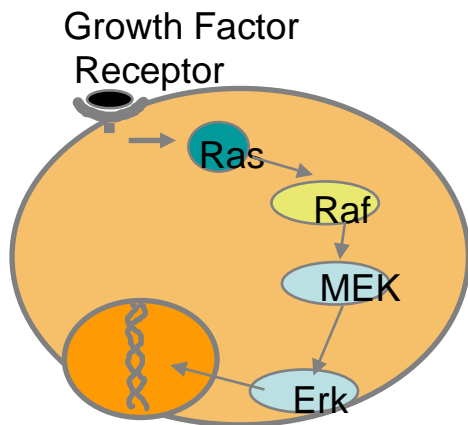




Bayer Biomarker Platform for Oncology

Discovery Strategy - Two Complementary Approaches

Pathway Analysis or Mechanism based

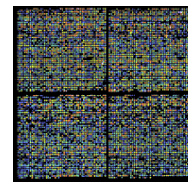


Sample Sources:

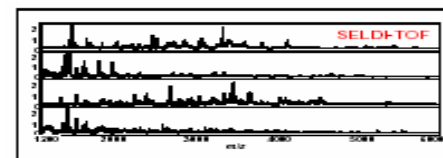
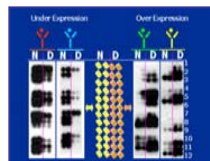
- blood
- plasma / serum
- urine
- historic biopsy

De Novo Biomarker Discovery

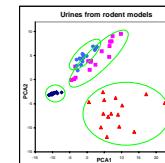
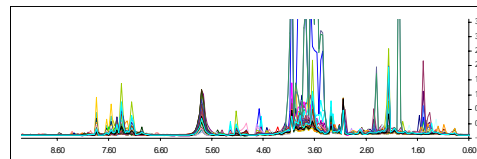
- Gene Expression Profile/Pattern (Affymetrix)



- Plasma Protein Expression Pattern (SELDI-Tof)



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



Apply validated assay - Partnership between Pharma and Diagnostics

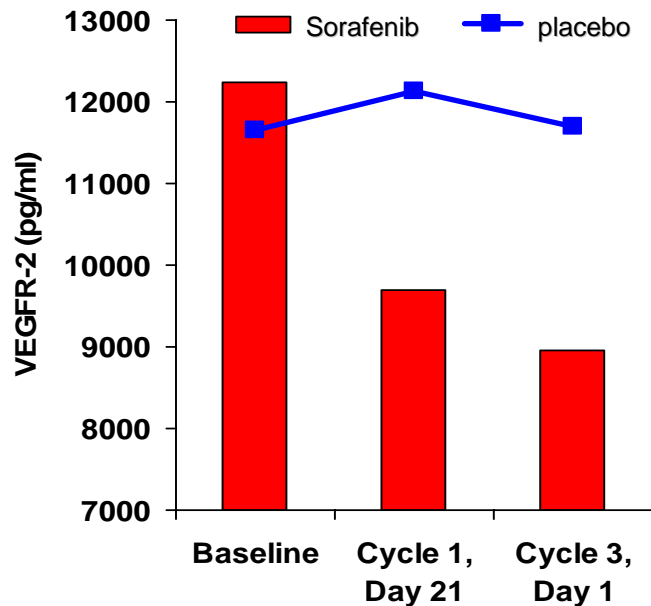


Example of Pharmacodynamic Biomarker*

* Preliminary analysis from Phase III trial for Renal Cell Carcinoma

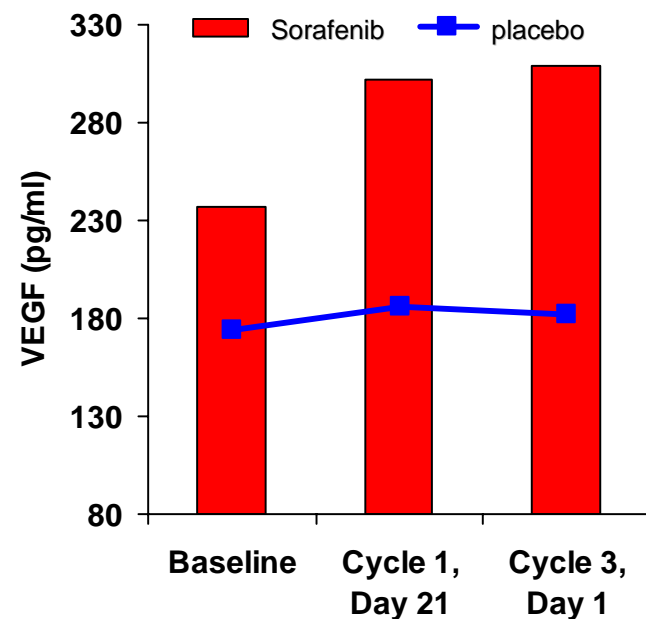
Treatment with Sorafenib Results in Decreased Plasma Levels of VEGFR-2 and Increased Levels of VEGF

VEGFR-2



Sorafenib (n):	149	196	198
Placebo (n):	102	128	132

VEGF



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	<i>prescreen urines out of 202 patients</i>
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

NMR data collection

Data processing

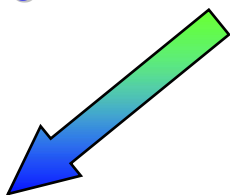
urine + buffer



1D ¹H NOESY

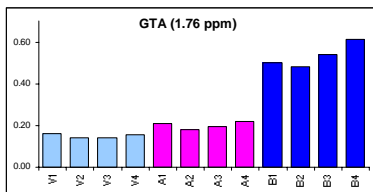


segment spectra into bins



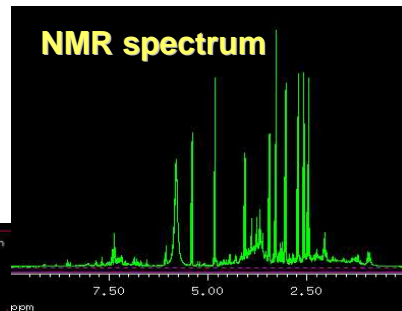
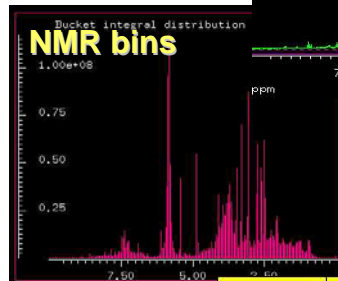
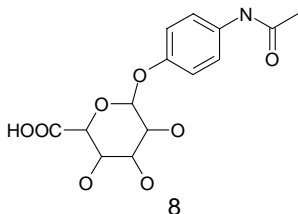
Metabolite levels

Assigned Metabolites



Novel Metabolite ID

HPLC-NMR and MS



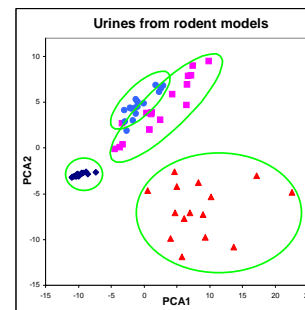
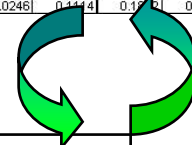
32,000 pts

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	0.1444	0.1171	0.0724

Model Building

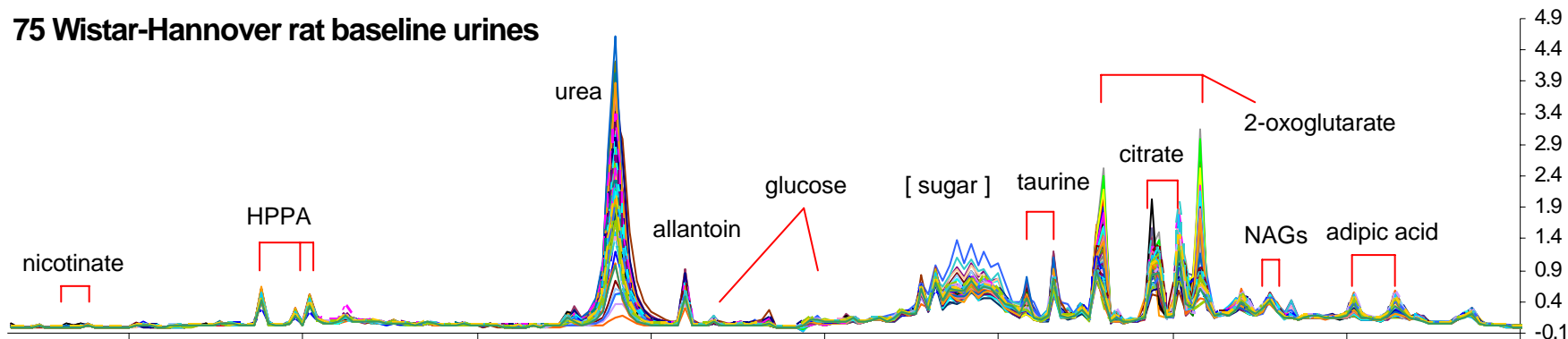
PCA, PLS-DA, OSC



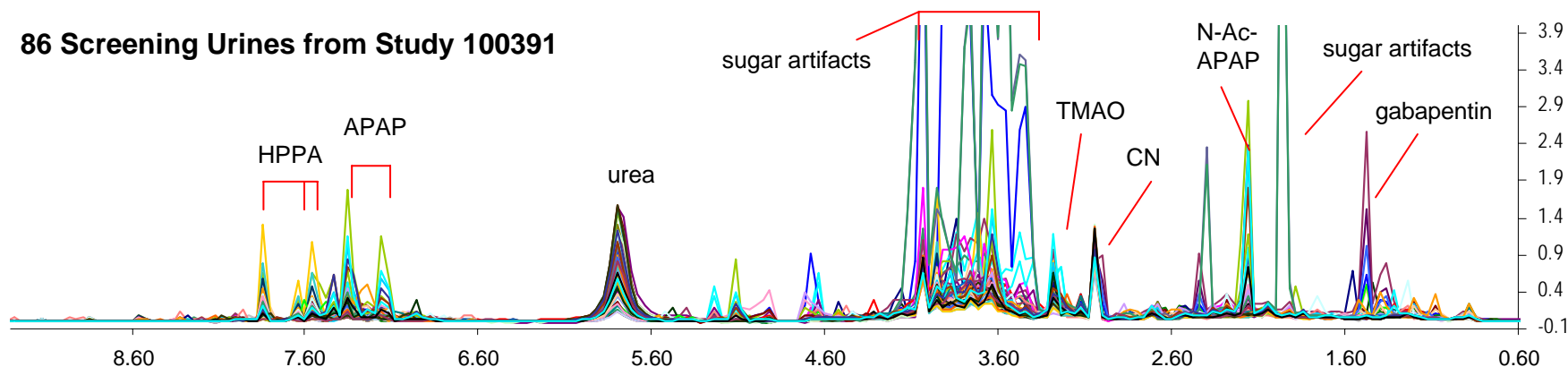


Challenges in NMR Data Interpretation

75 Wistar-Hannover rat baseline urines



86 Screening Urines from Study 100391

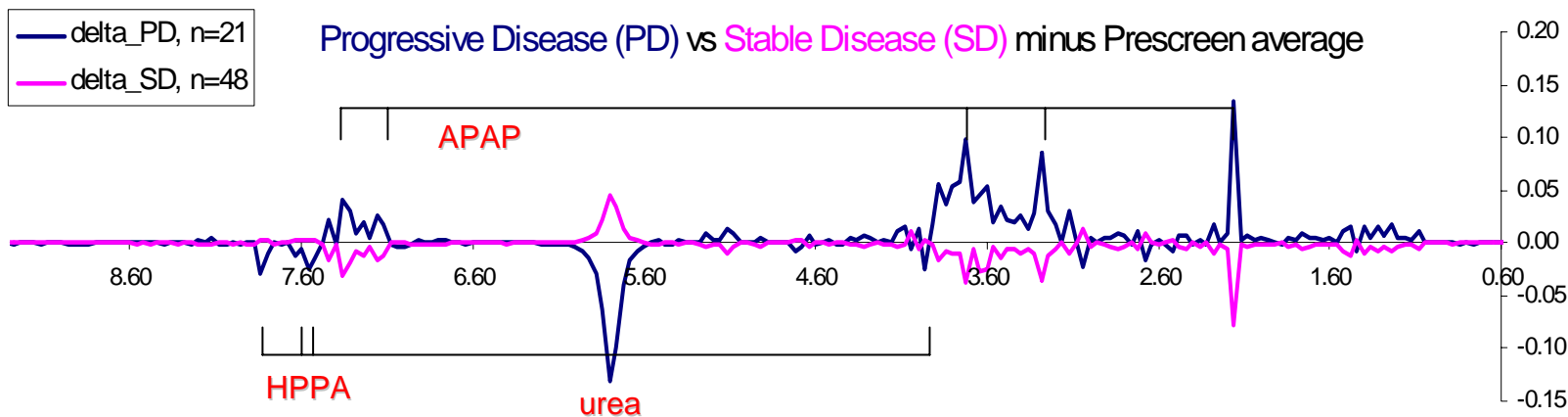


- Rat urines show limited variability, while human urines are highly variable
- Artifacts mask information from endogenous metabolites and can be repaired
- **Less than 10% of the spectra in 100391 needed modification**

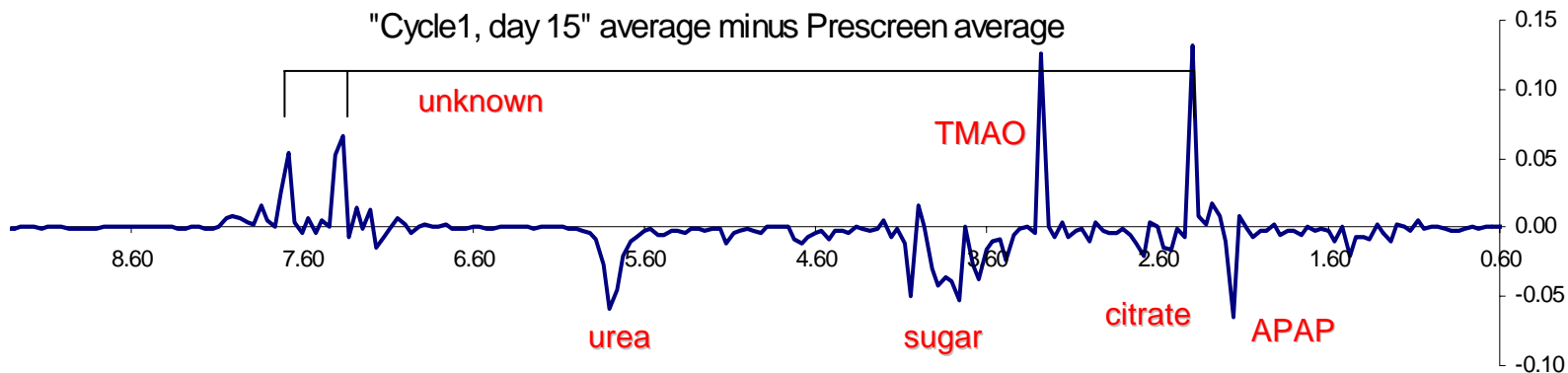


Overviews of Urinary Metabolic Profiles

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

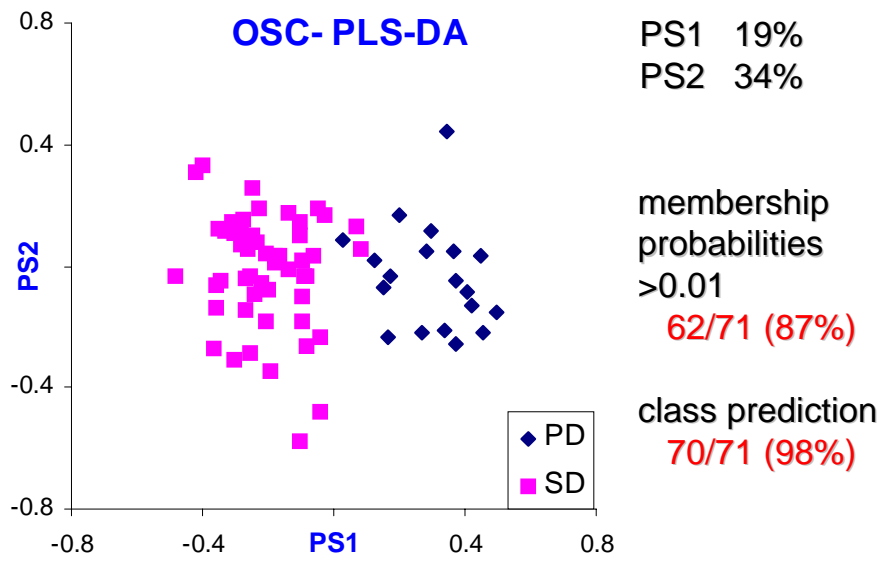
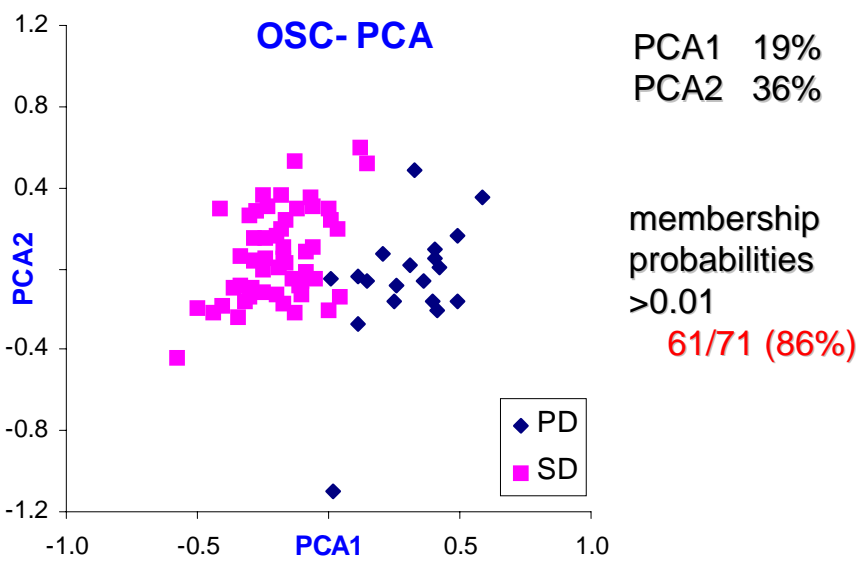
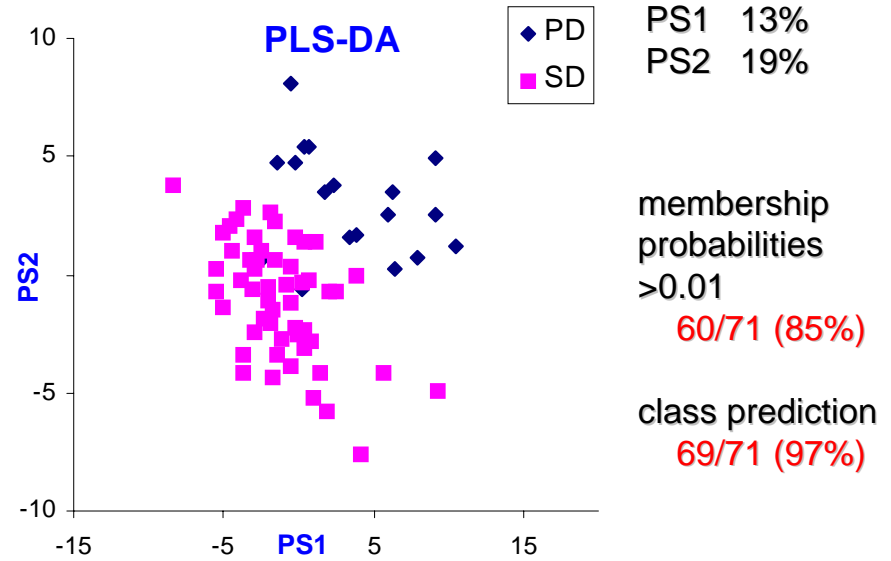
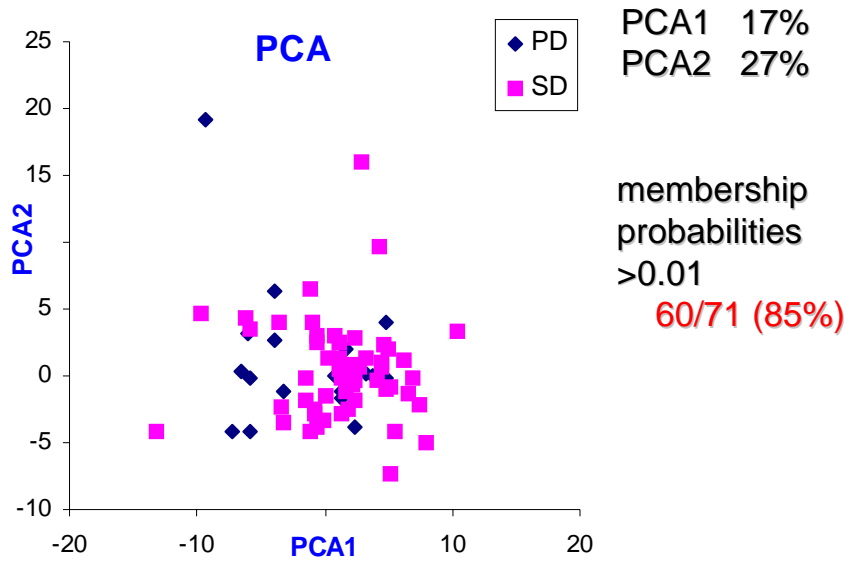


Urines collected post-dose contained an unknown metabolite that was isolated and identified as toluene sulfonic acid





First Pass Modeling* of Prescreen urines

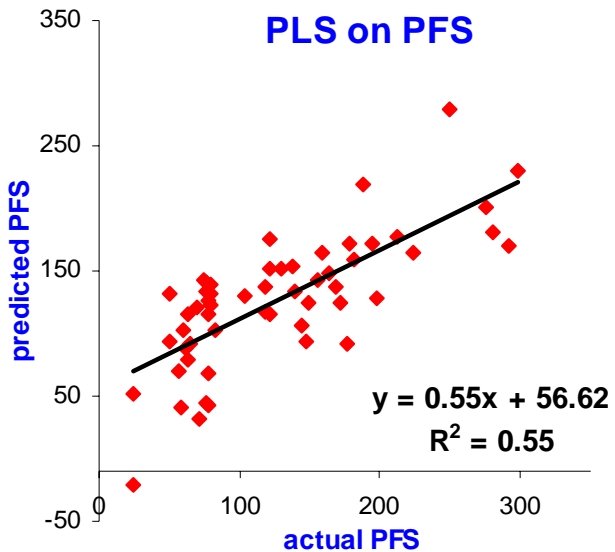


* 190 bins, 9.40 – 0.60 ppm, water and urea excluded



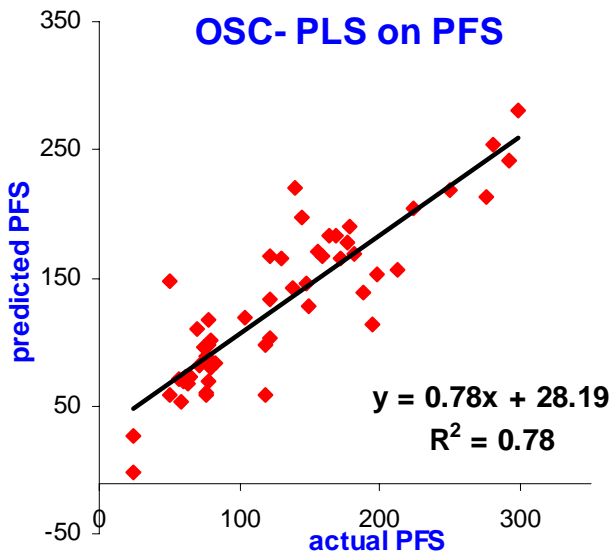
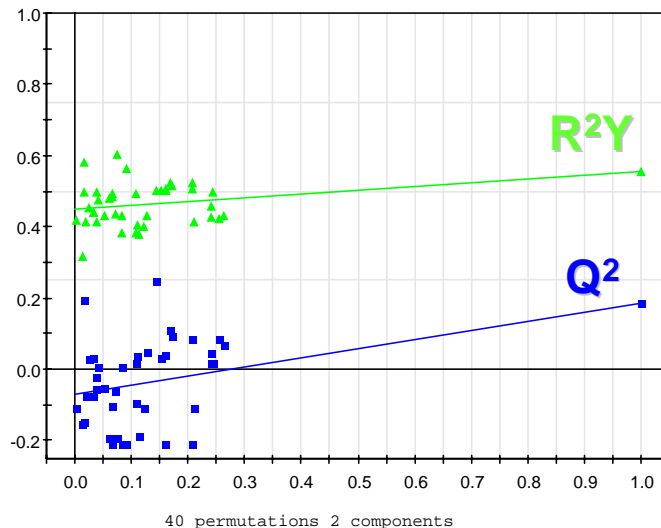
Prediction of PFS from prescreen urine

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



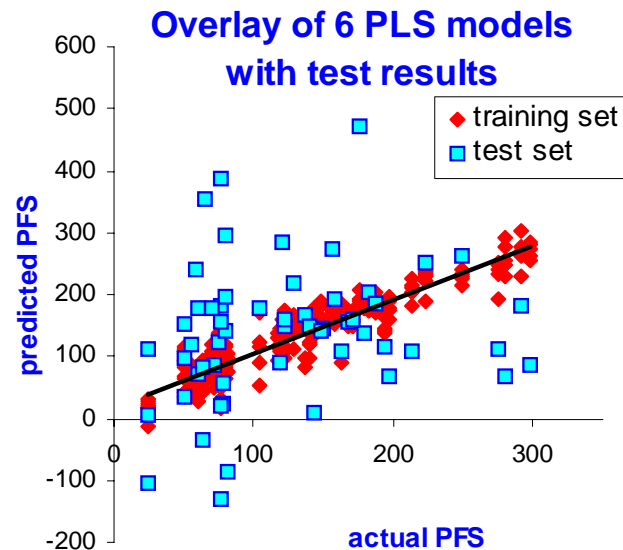
PS1 18%
PS2 26%

membership probabilities >0.01
46/55 (84%)



PS1 14%
PS2 32%

membership probabilities >0.01
48/55 (87%)





Optimization of PLS Models

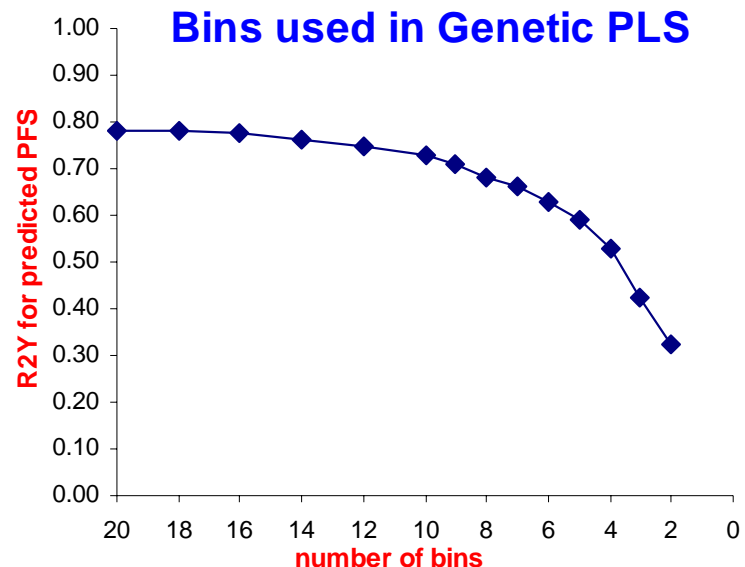
For PLS models, a simple correlation map was used to reduce the number of bins

Correlation matrix Analysis

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

TMAO
malonate
DMA
citrate

Genetic Algorithm approach

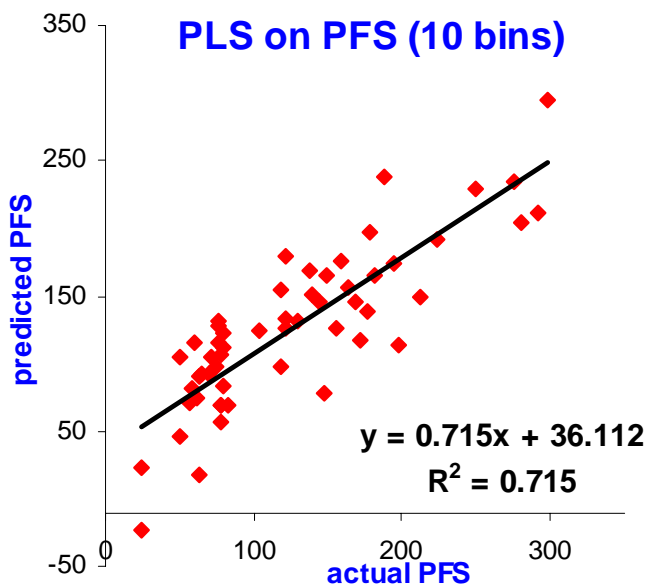
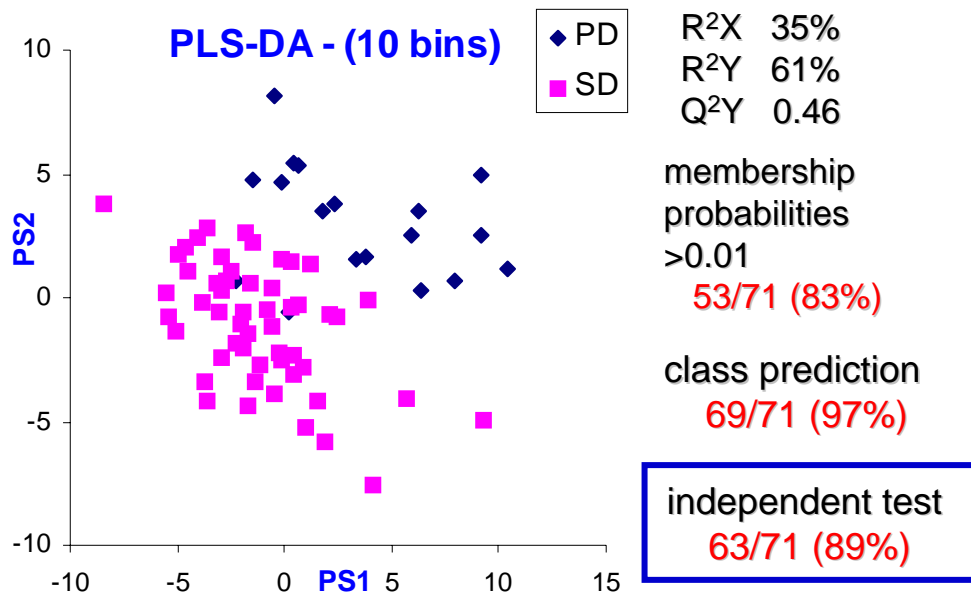


- All bins found in top 20% of the loading factors
- Neither Hippurate or APAP contribute to models
- Genetic PLS then used to further reduce the number of bins



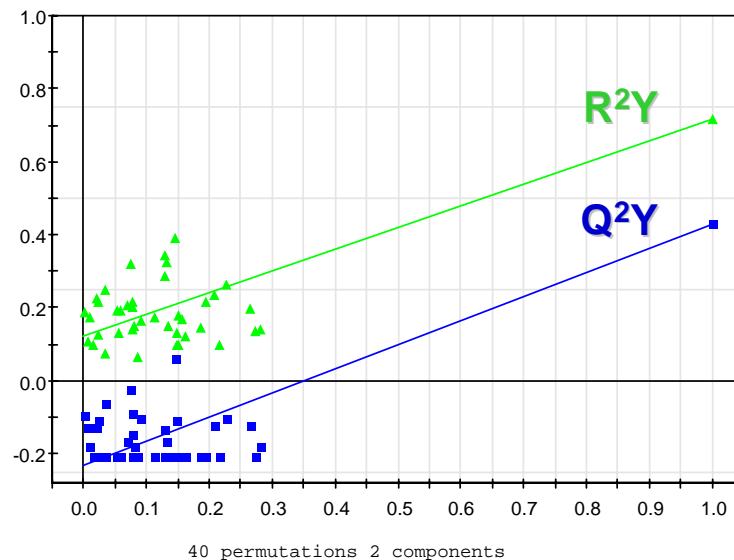
Performance of Optimized Models

- **PLS-DA used correlation matrix and 'leave-one-out' approach**
- **Parameters R^2X and Q^2Y significantly improve**
- **Undesirable noise and food metabolites avoided**



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

membership probabilities
 >0.01
 53/55 (96%)





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

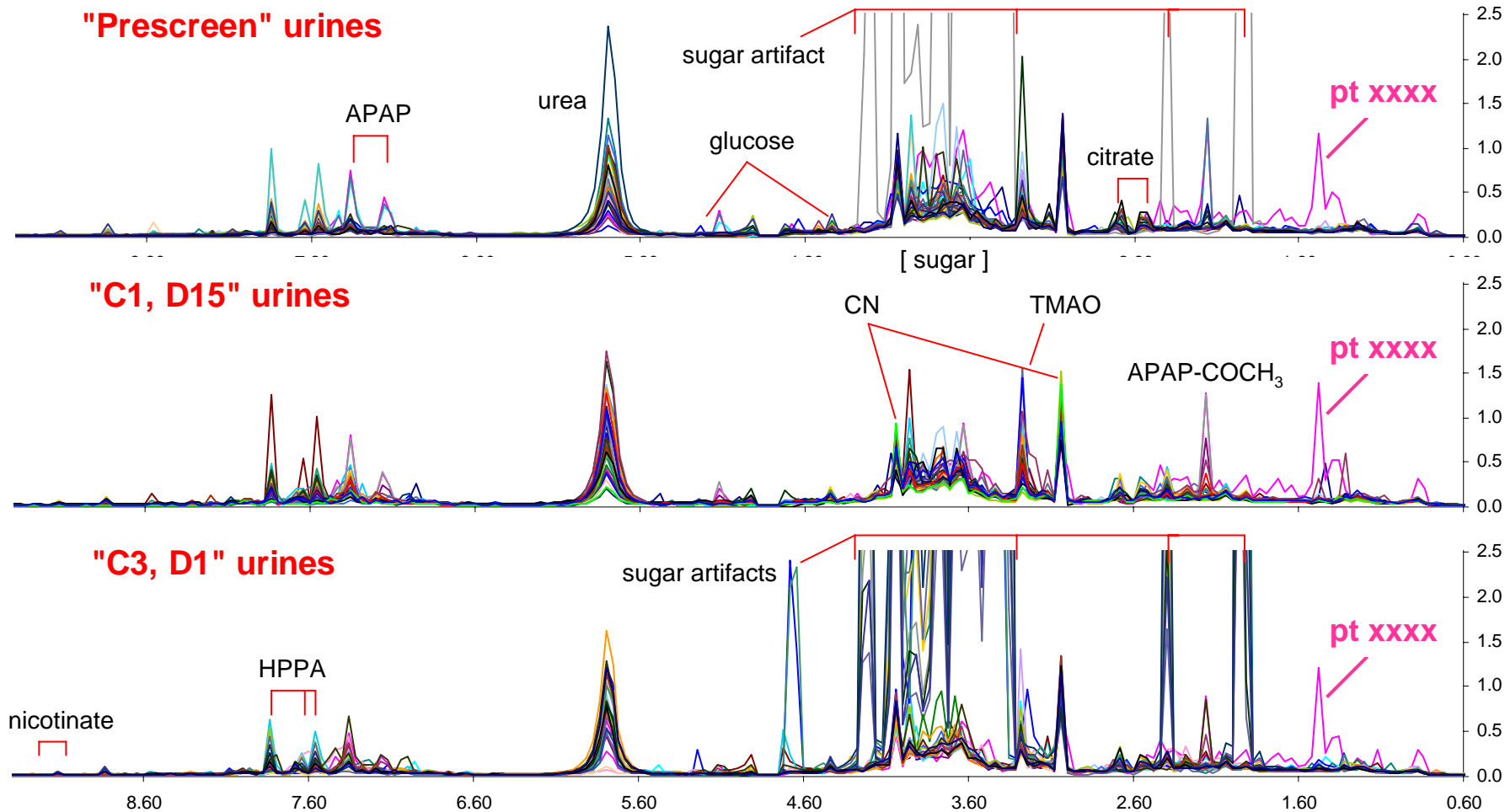
155 urine samples received and analyzed by ¹H NMR

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

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

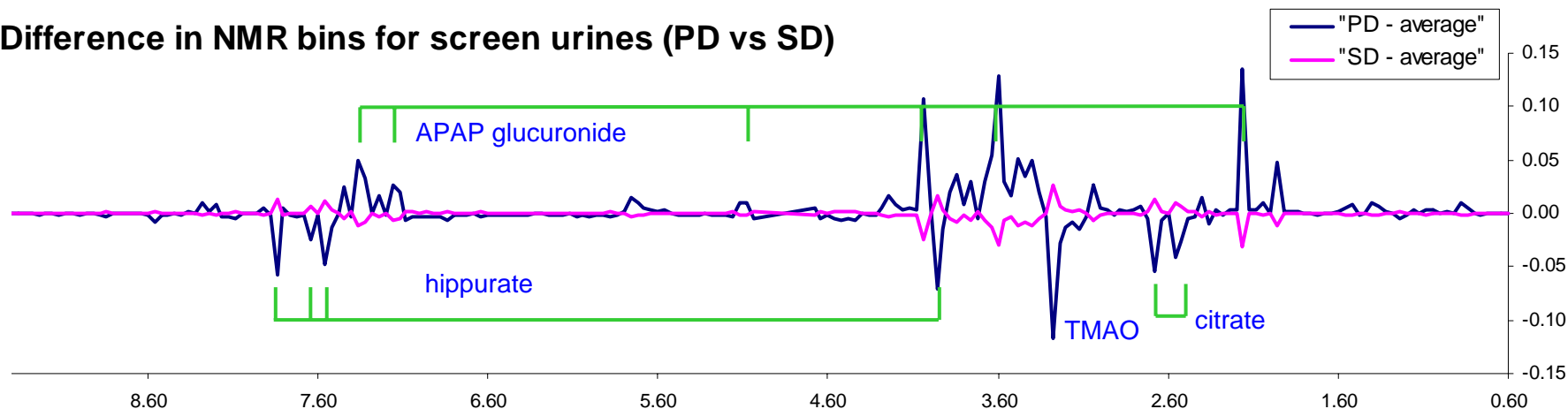


Overview of 100557 Metabolic Profiles

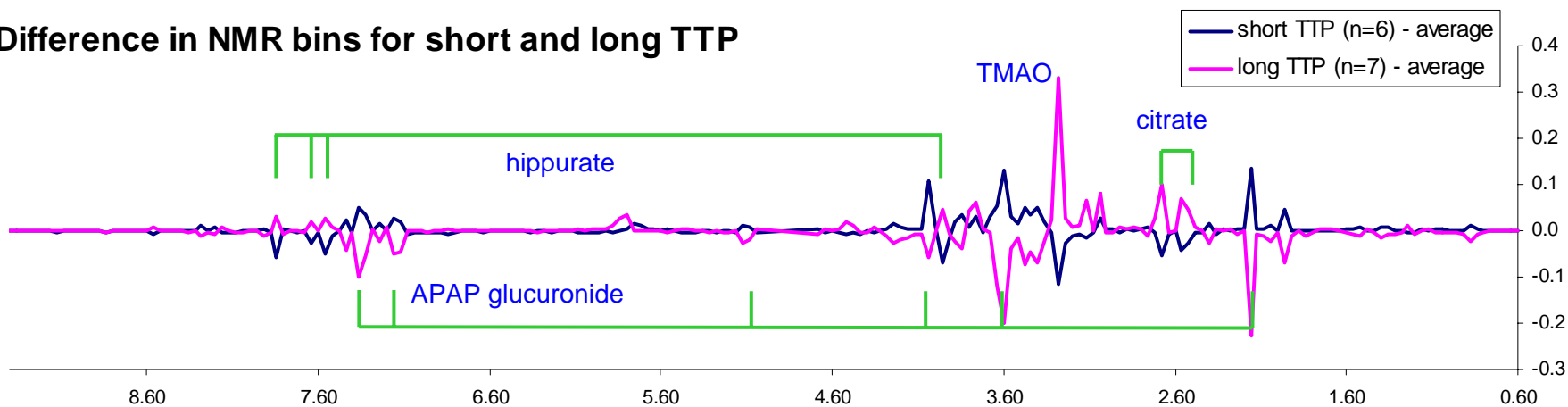
Non-Responders and patients with short TTP have similar profiles

- 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

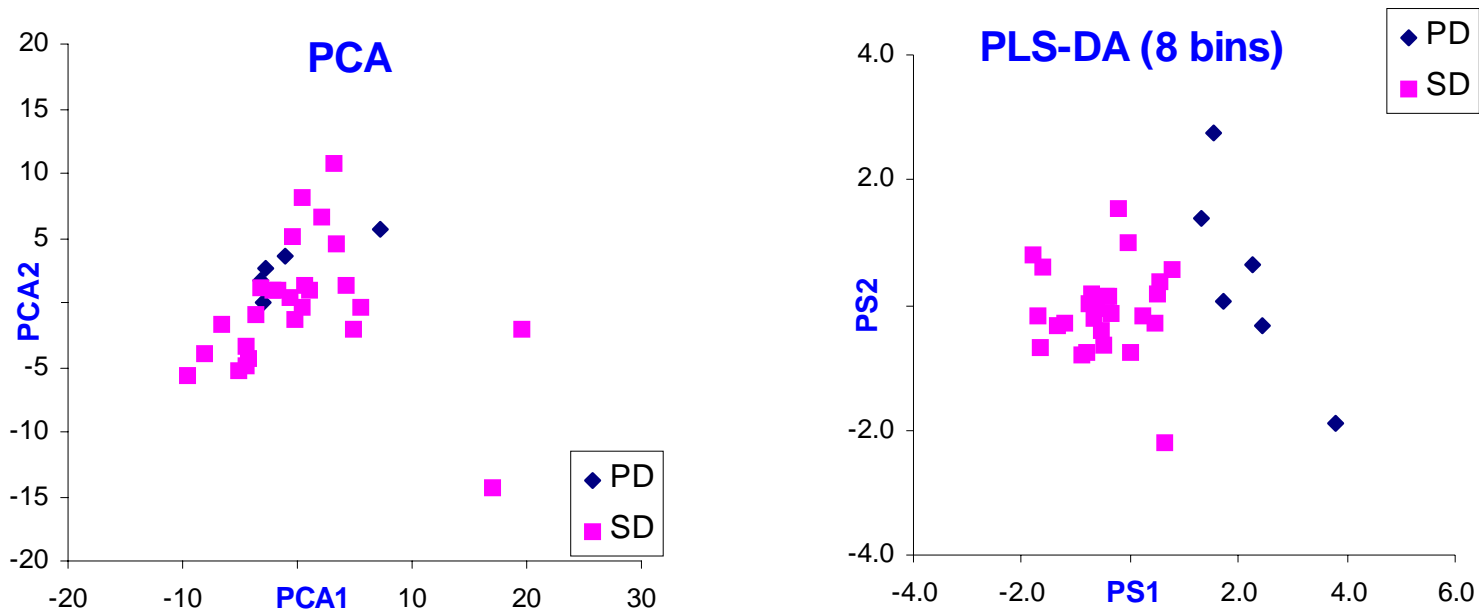


TTP = time to regression



Prediction of Best Response from Prescreen

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



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

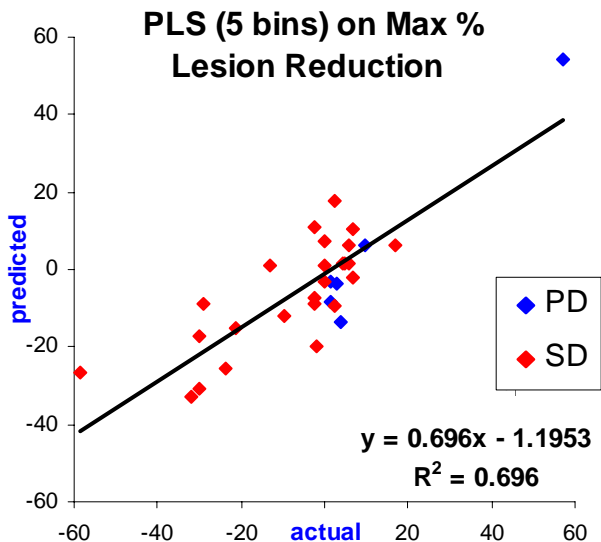
R ² X	35%	membership	class prediction
R ² Y	70%	probabilities >0.05	32/32 (100%)
Q ² Y	0.31	32/32 (100%)	

Validation testing of the PLS-DA model via independent test sets resulted in 30/32 (94%) classified correctly



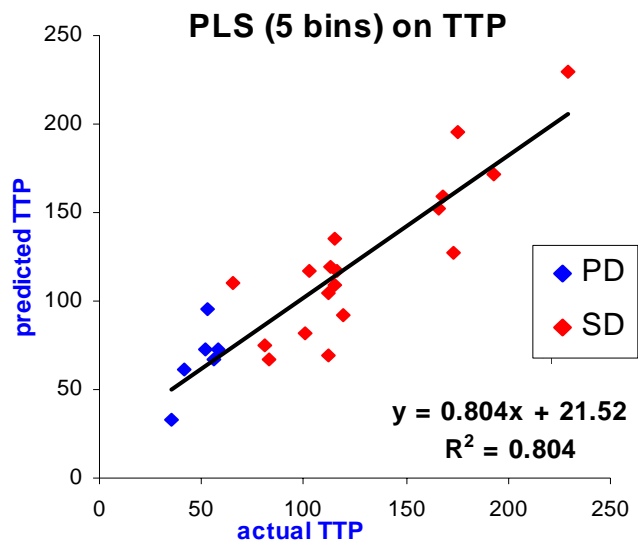
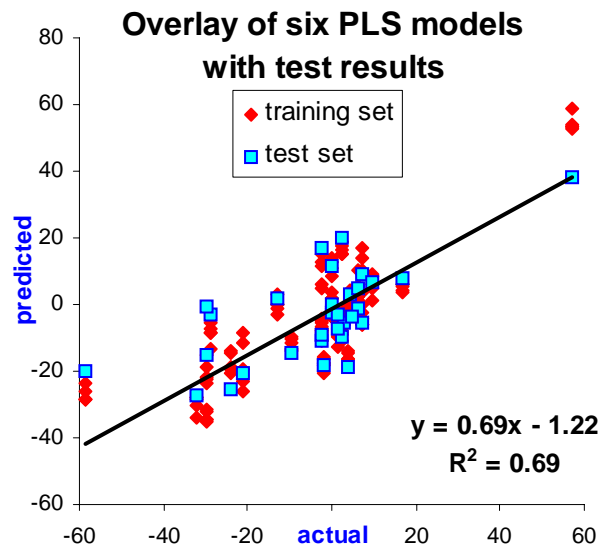
Lesion Reduction and Time to Progression

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



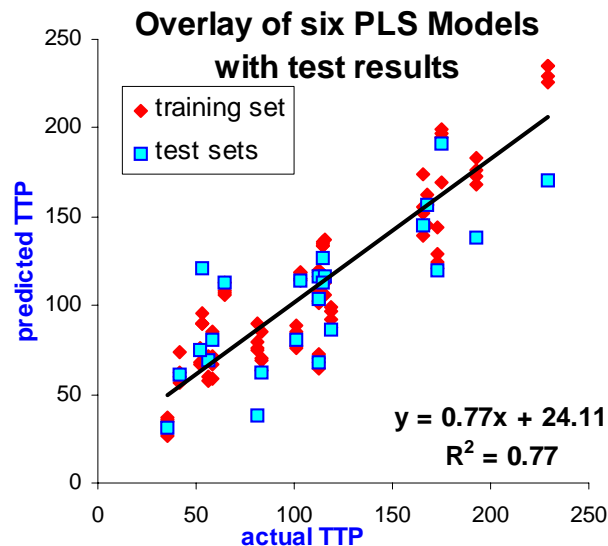
R^2X 39%
 R^2Y 69%
 Q^2Y 0.46

membership probabilities >0.05
32/32 (100%)



R^2X 59%
 R^2Y 80%
 Q^2Y 0.60

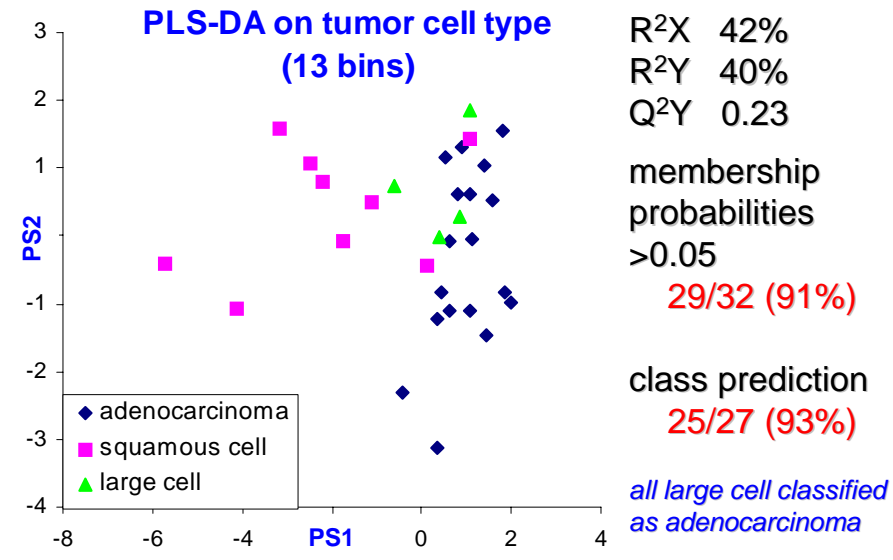
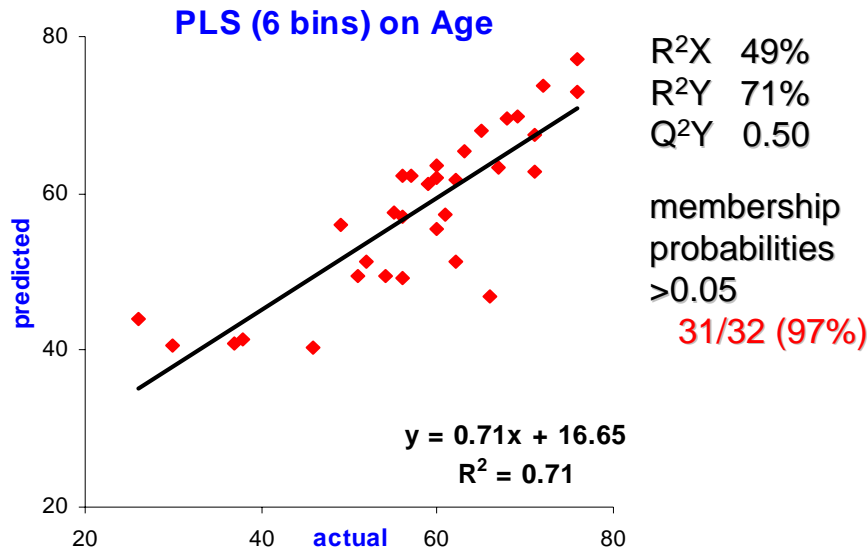
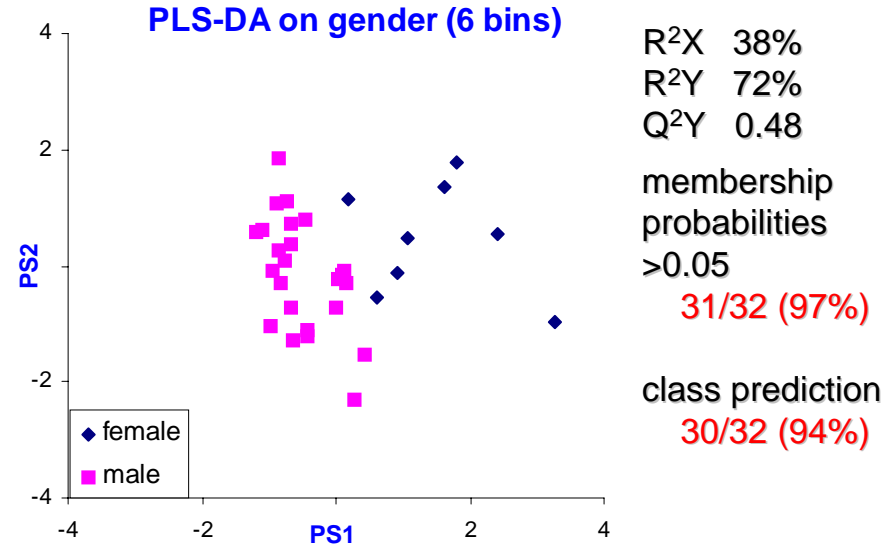
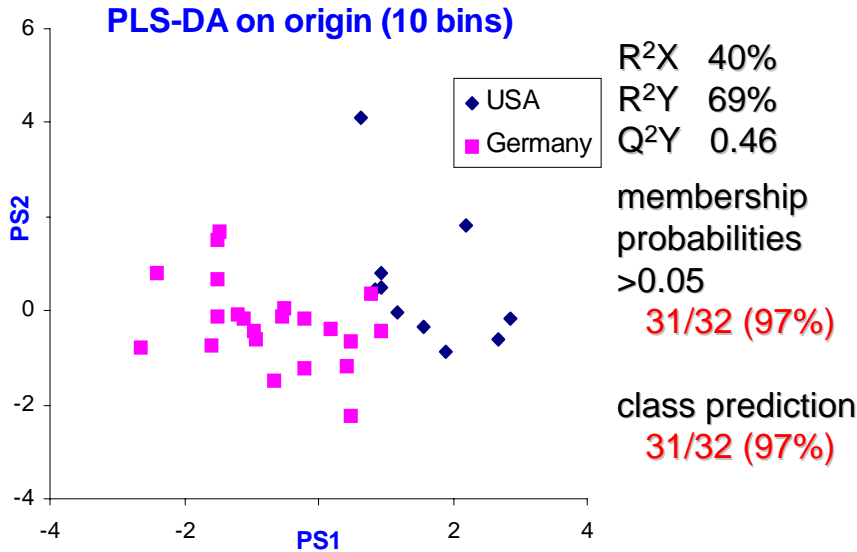
membership probabilities >0.05
31/32 (96%)





Demographic Analysis of Screening Urines

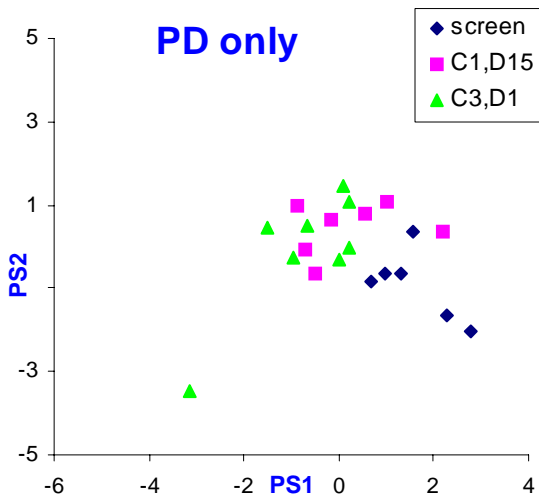
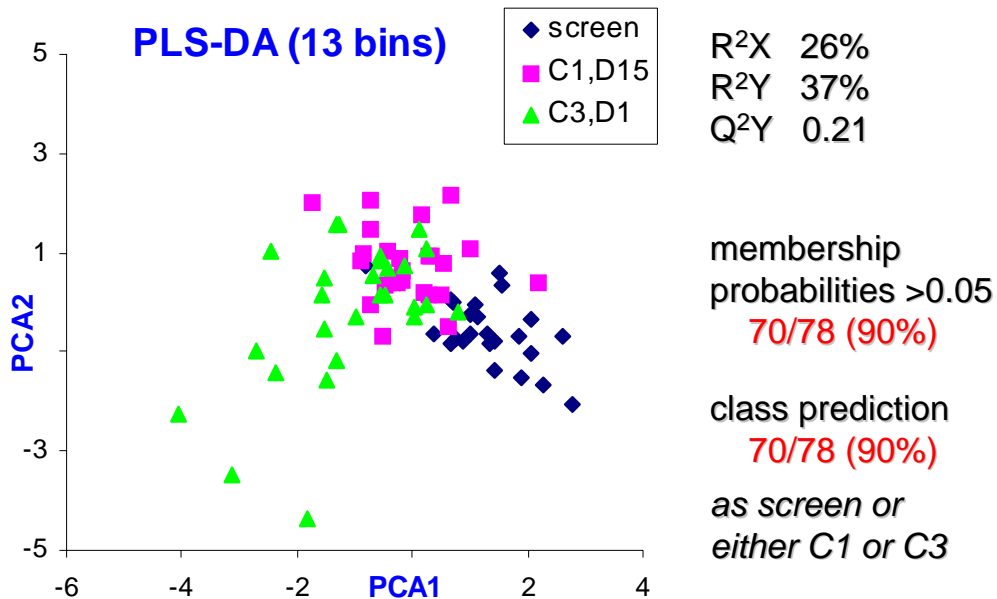
Metabolite profiling of urine samples also sensitive to demographics



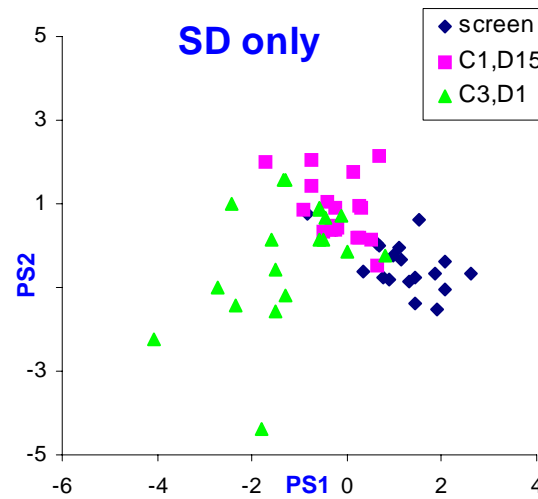


Prediction of Pharmacodynamic Response

Model suggests that responders progress away from starting disease state



Sub-sets from model above





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

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