ETH Zurich – JST Workshop on Medical Research

15th – 16th of September 2008, ETH Zurich

Session 3

Predictive biomarker for molecular target drugs- proteomic and glycobiological approach

Kazuto Nishio, MD PhD

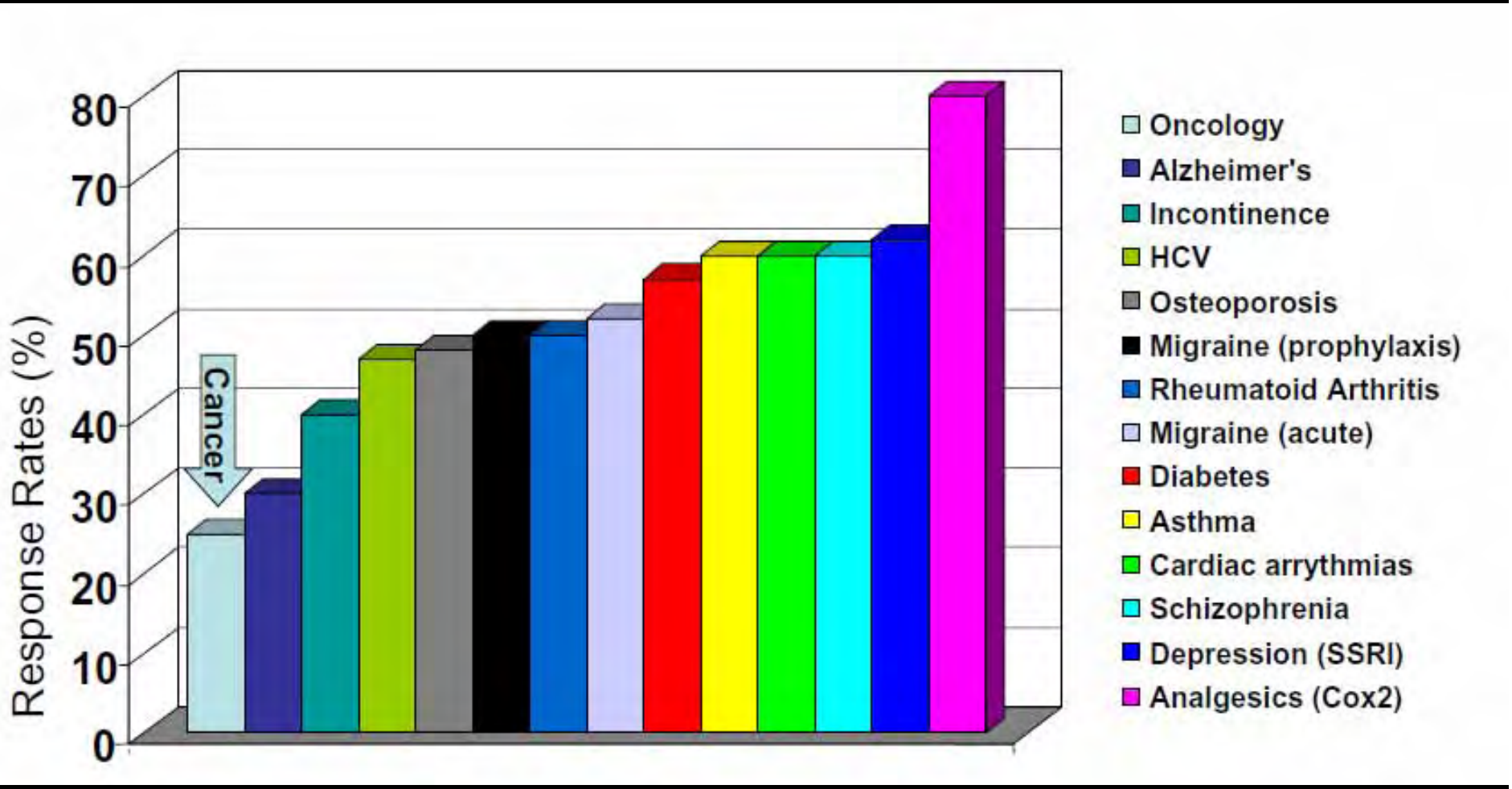
Department of Genome Biology
Kinki University School of Medicine

Biomarkers Definition

Biomarkers are
‘ a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes,
or
pharmacologic responses to a therapeutic intervention’.

Biomarkers Definitions Working Group (2001) Clin. Pharmacol. Ther. 69, 89–95

The need for better predictive markers



The average response rate to drug treatment is not acceptable.

Slide: Paul Warning, Genentech (modified)

Molecular target therapy

Oncology New Drug Approvals (FDA) 2001-2006

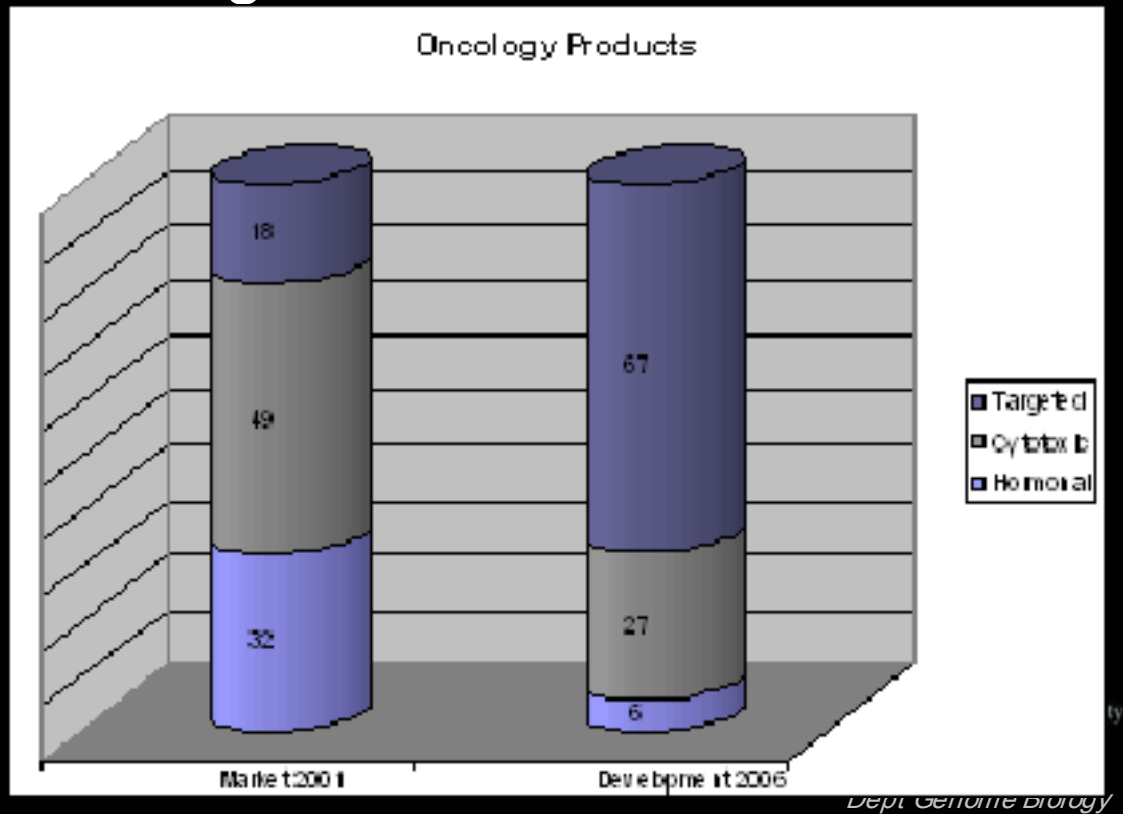
Chabner AACR 2006

- **Molecular Targeted Drug**

  67 % (2015 >75%)

  (27 % Patient Selection)

- **Non-Targeted Drug** 33%



Approved Mol Target Drugs
(small molecules and antibodies)

Drugs	Target	Application**	FDA	Japan
Rituxan	CD20	BCLL	1997	2001
Herceptin	*Her2* *	breast ca	1998	2001
Gleevec	*Bcr-Abl/Kit* *	CML, GIST	2001	2001
Iressa	*EGFR* *	NSCLC	2003	2002
Velcade	Proteasome	MM　2003年	2007	2007
Avastin	VEGF	CRC	2004	2008
Erbitux	*EGFR* *	CRC	2004	applied
Tarceva	*EGFR* *	NSCLC, panc ca	2005	2007
Nexavar	*Multi-kinases* *	RCC	2005	2007
Sutent	*Multi-kinases* *	GIST, RCC	2006	applied
Sprycel	*Bcr-Abl/Src* *	CML**, Ph+ALL	2006	applied

* *kinase inhibitor*　** Gleevec-resistance

Kinki University School of Medicine
Dept Genome Biology

Biomarkers for patient selection

Compounds	Target	Tumors	Diagnosis
Herceptin	Humanized anti HER2 Ab	Overexpression of HER2	IH (Hercep test) FISH
Rituxisan	Chimeric anti CD30 m-Ab	CD20 (+) B-cell non Hodking lymphoma	IH FCM
Gleevec	bcr-abl / c-kit-TKI	1.CML 2. GIST with KIT (CD117) +g	Chromosomal test Gene analysis IH
Irinotecan	Topo I inhibitor	NSCLC or ovarian ca. e.g.	Invader assay (UGT1A1gene polymorphism)

Draft
Preliminary Concept Paper — Not for Implementation

Drug-Diagnostic Co-Development Concept Paper

Draft — Not for Implementation

New, more informative trial designs

Approach: Pair diagnostic with therapeutic

- Identify responders and non-responders
- Prevent toxicity
- Monitor response

Answer series of questions, e.g.,

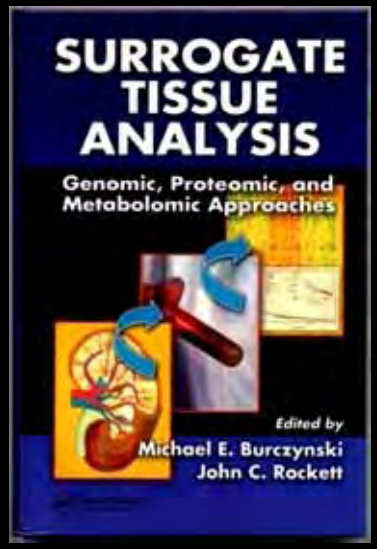
- Which dose is correct for which sub-population?
- Which sub-population should be treated?

Biomarker study for target based drugs

- **Feasibility**
- Power for prediction
- Sensitivity
- Accuracy : false positive e.g.

We focused on

Operative
Biopsy
Cytology
PBMC (Blood)
Serum (Blood)
Plasma (Blood)
Pleural effusion



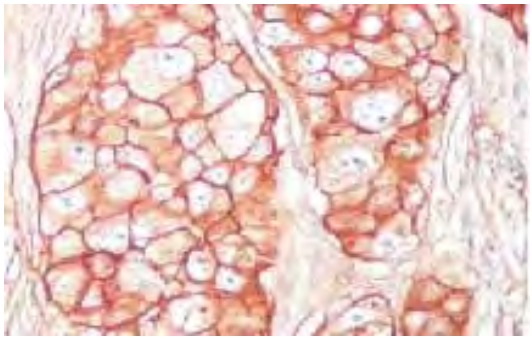
Search for biomarkers in surrogate tissue (circulating samples)

1. EGFR somatic mutation
   in circulating tumor cells in lung cancer
2. Gene expression profile in PBMC
3. Serum proteomics
4. Multiplex ELISA using bio beads
5. Glycoprofiling

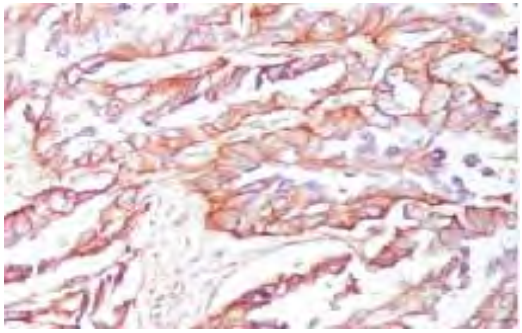
EGFR tyrosine kinase inhibitors (EGFR-TKIs)

EGFR expression in NSCLC

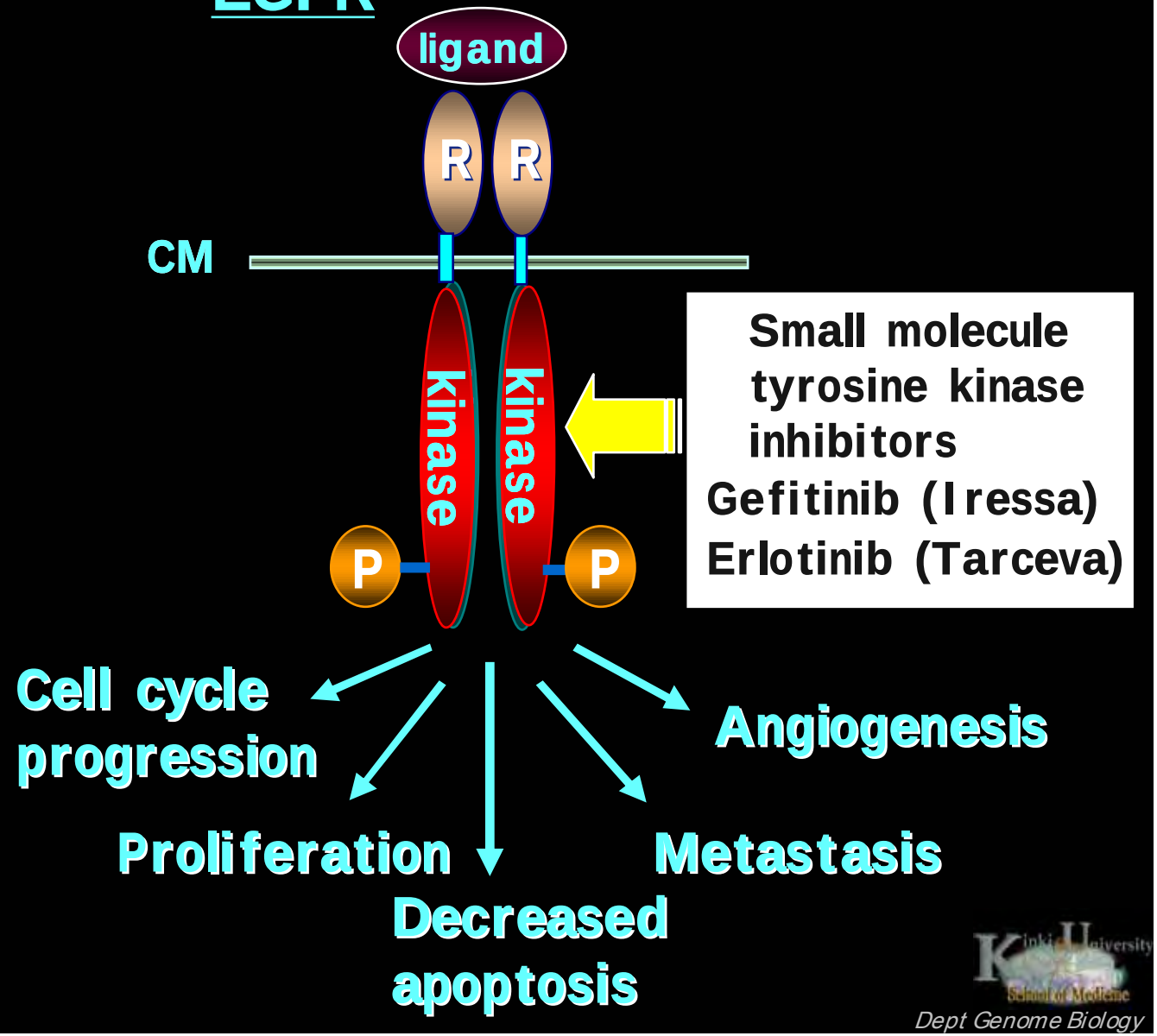
Squamous cell ca.



Adenocarcinoma

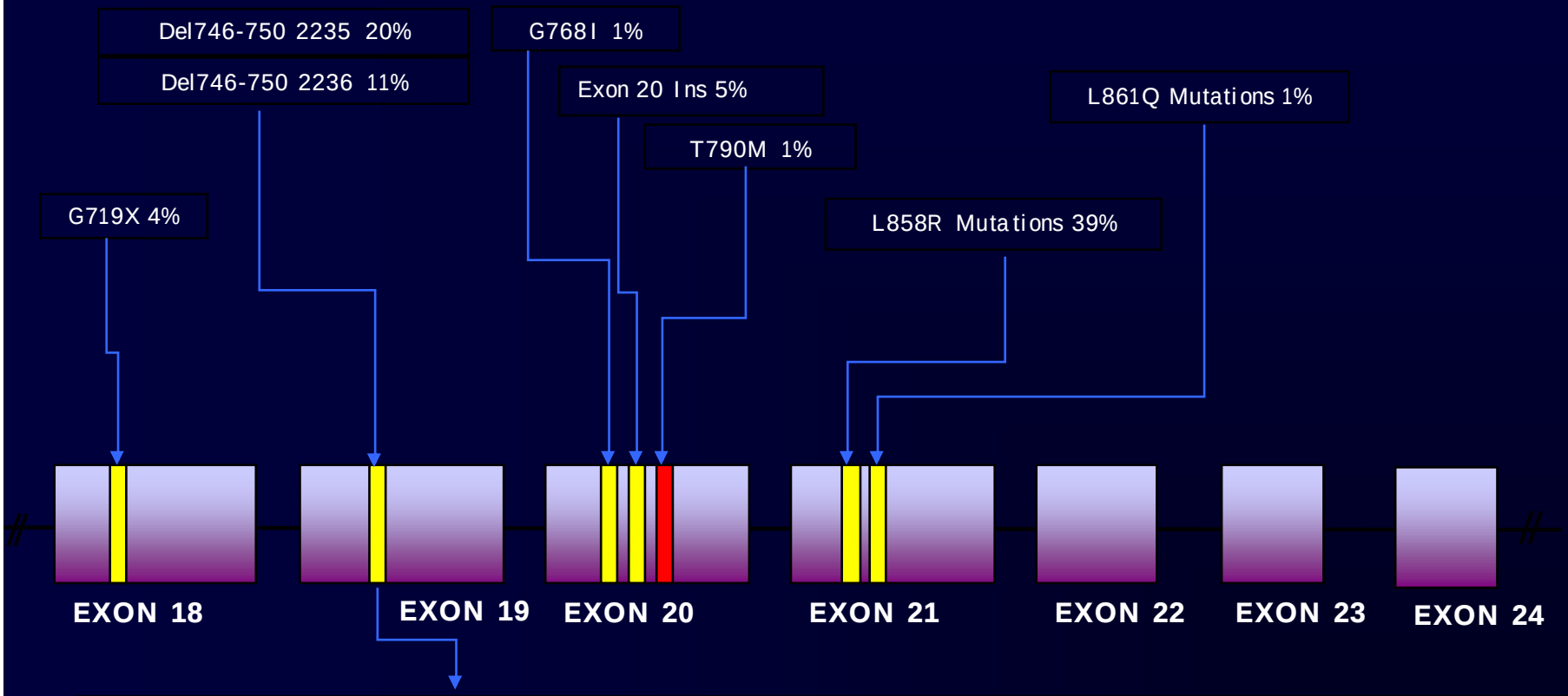


EGFR



Kinki University School of Medicine
Dept Genome Biology

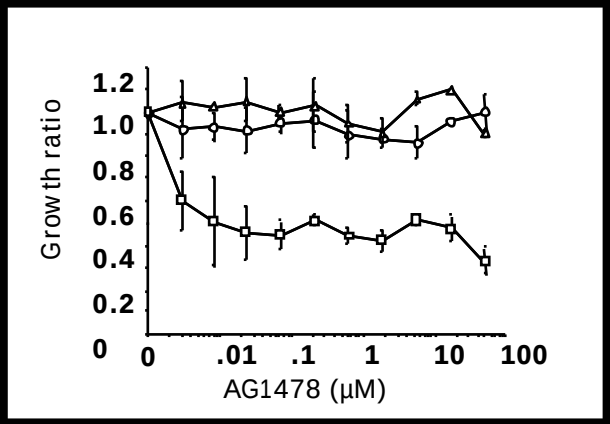
EGFR mutation



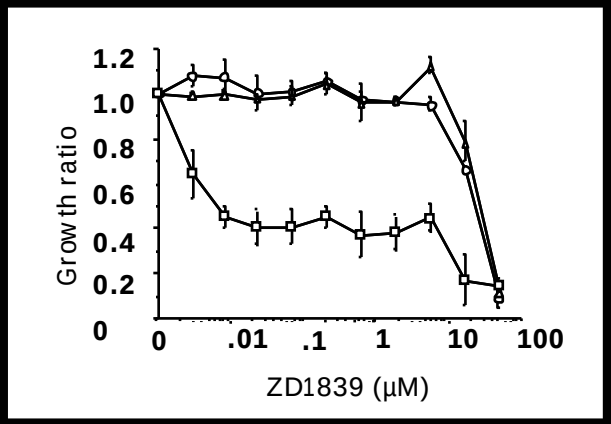
Other Exon 19 del 13%.
2237_2251del15; 2237_2254del18; 2237_2255>T (complex); 2236_2250del15; 2238_2255del18; 2238_2248>GC (complex);
2238_2252>GCA (complex); 2239_2247del9; 2239_2253del15; 2239_2256del18; 2239_2248TTAAGAGAAG>C (complex)
2239_2258>CA (complex); 2240_2251del12; 2240_2257del18; 2240_2254del15; 2239_2251>C (complex); 2235_2252>AAT (complex)

Sensitivity of transfected cells with deletional mutation to EGFR-TKI

AG1478



gefitinib



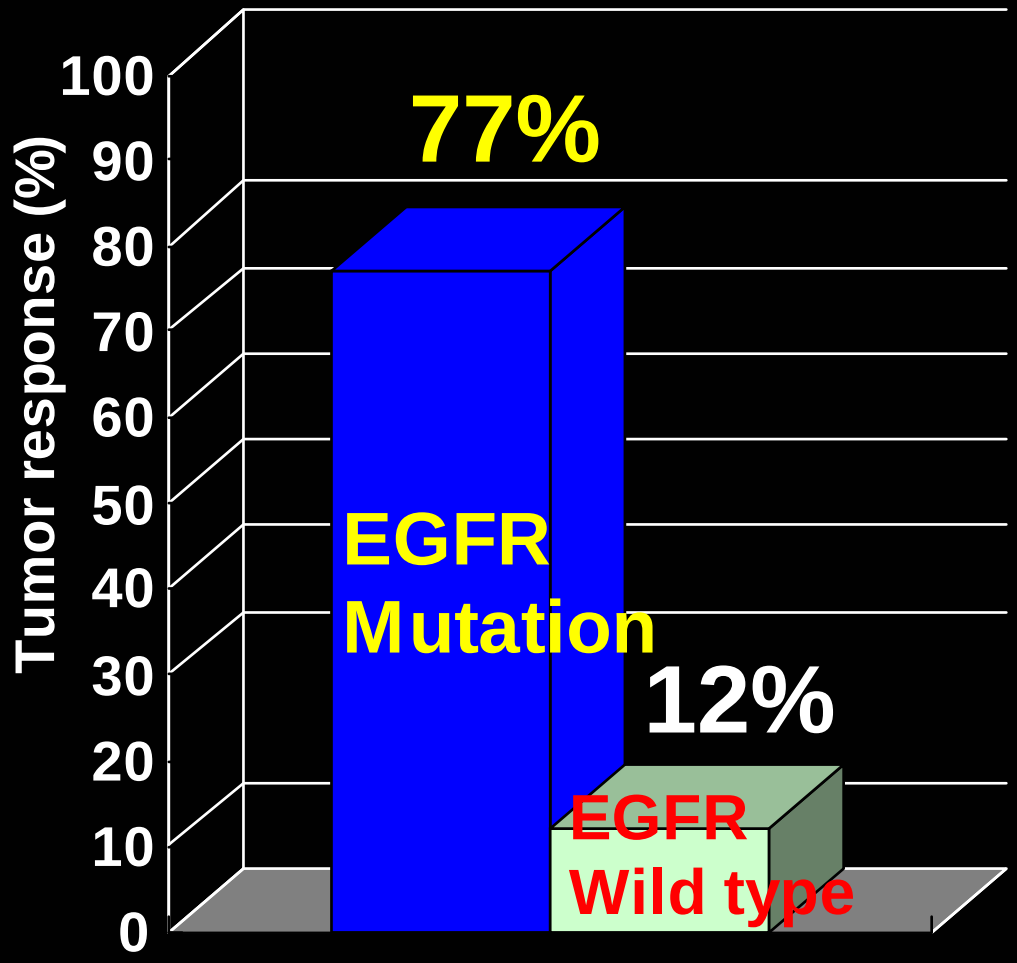
Detection of pEGFR (1068)

ZD1839 uM for 3h

M:293_pcDNA3.1/Zeo
W:293_pcDNA3.1/EGFR wt
D:293_pcDNA3.1/EGFR15d

Arao et al. Cancer Res '05

Impact of EGFR mutation on the response to EGFR-TKI (N=616)



Paez et al., Science 2004
Lynch et al.,NEJM 2004
Pao et al.,PNAS 2004
Huang et al., CCR 2004
Tokumo et al., CCR 2005
Mitsudomi et al., JCO 2005
Han et al., JCO 2005
Kim et al., CCR 2005
Cotes-Funes et al., Ann Oncol 2005
Cappuzzo et al, JNCI 2005
Chou et al., CCR 2005
Taron et al., CCR 2005
Takano et al., JCO 2005

Analysis of circulating DNA

Detection of Epidermal Growth Factor Receptor Mutations in Serum as a Predictor of the Response to Gefitinib in Patients with Non–Small-Cell Lung Cancer

Hideharu Kimura,[1,4,5] Kazuo Kasahara,[5] Makoto Kawaishi,[1,2] Hideo Kunitoh,[2] Tomohide Tamura,[2] Brian Holloway,[6] and Kazuto Nishio[1,3,4]

Clin Cancer Res 2006;12(13) July 1, 2006

Editorial

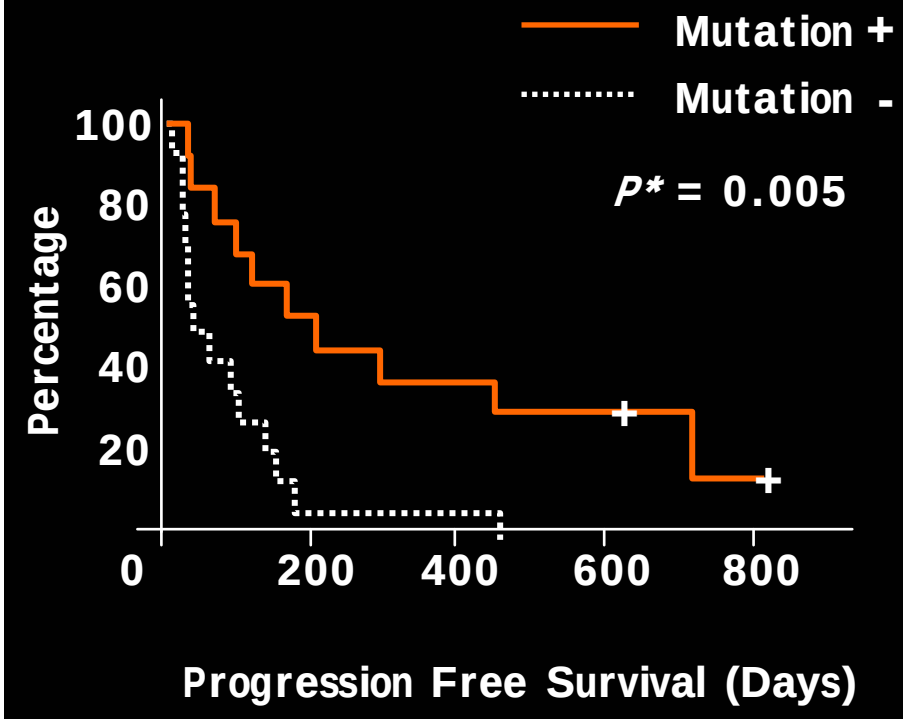
A Blood-Based Test for *Epidermal Growth Factor Receptor* Mutations in Lung Cancer

□□*Commentary on Kimura et al., p. 3915*

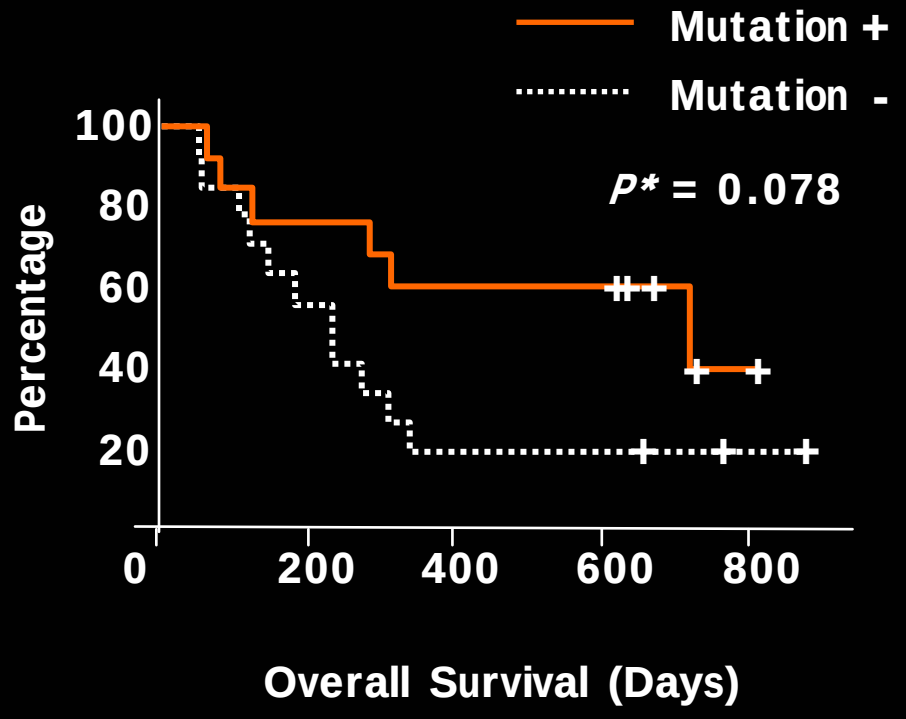
Daphne W. Bell and Daniel A. Haber

Detection of EGFR mutation by Scorpion-ARMS in serum samples

PFS



OS



Kinki University School of Medicine
Dept Genome Biology

Search for biomarkers in surrogate tissue (circulating samples)

1. EGFR somatic mutation
   in circulating tumor cells in lung cancer
2. Gene expression profile in PBMC
3. Serum proteomics
4. Multiplex ELISA using bio beads
5. Glycoprofiling

proteomics using LC-MS/MS

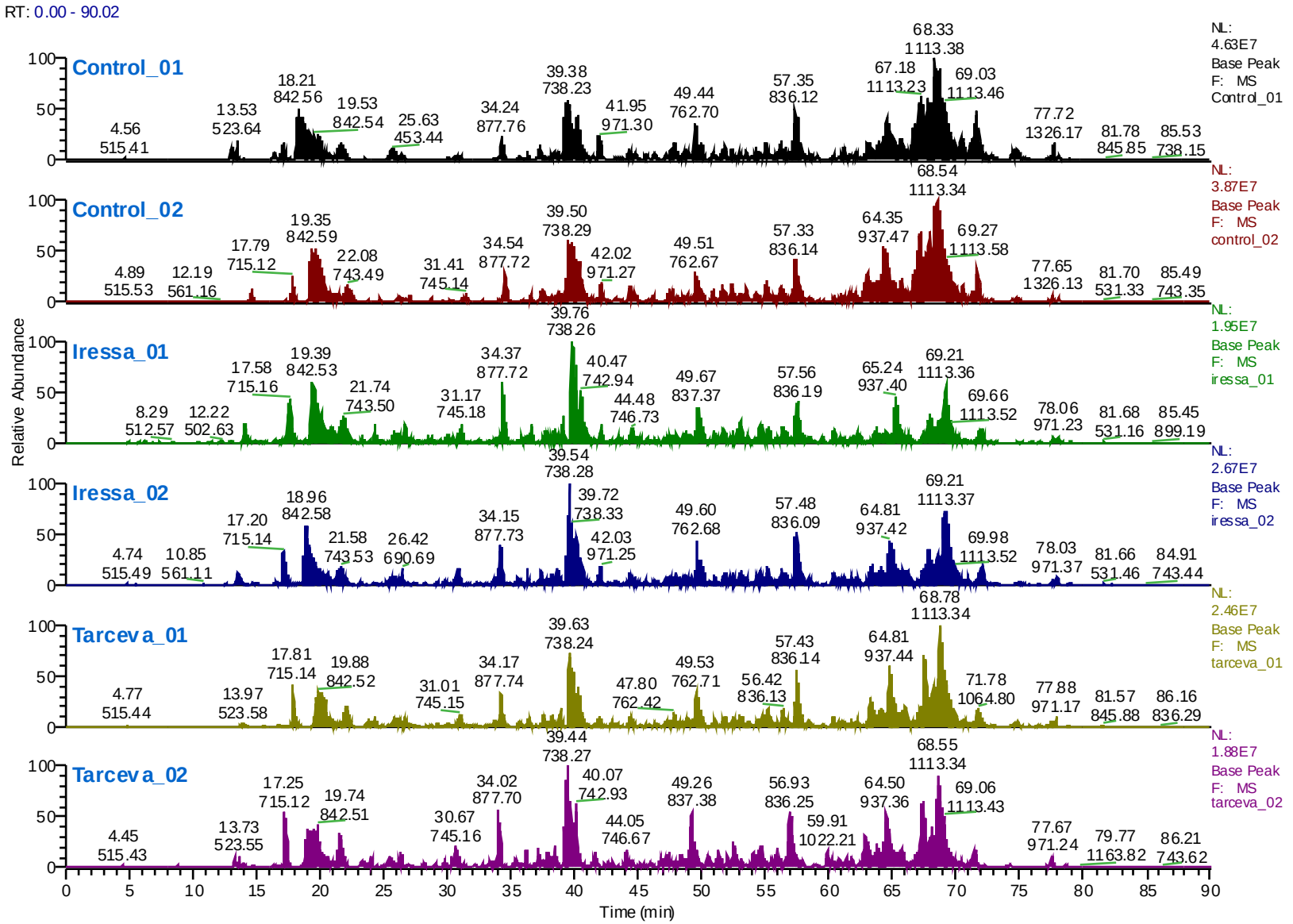
Identification of target proteins bind to EGFR-TKI

Base-peak Chromatograms of Chemical Pulldown Samples

Control

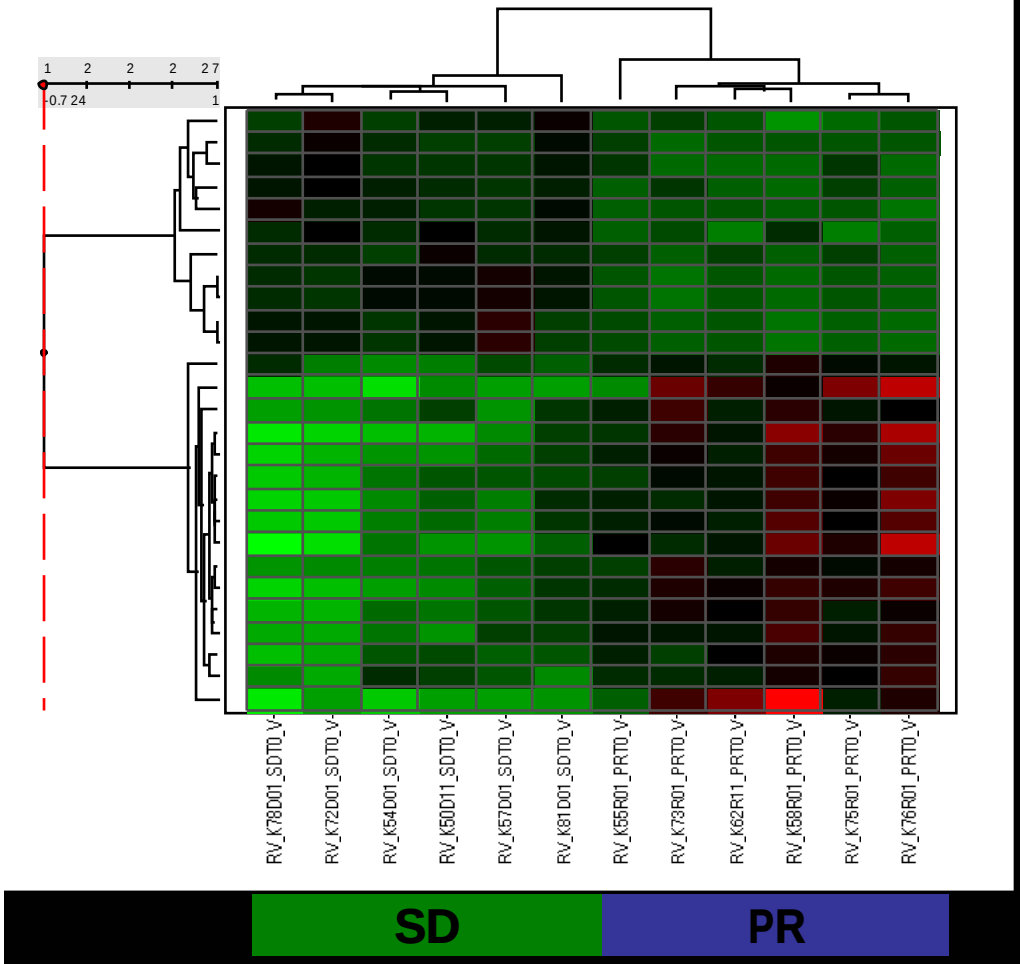
gerfitib (Iressa)

erlotinib (Tarceva)

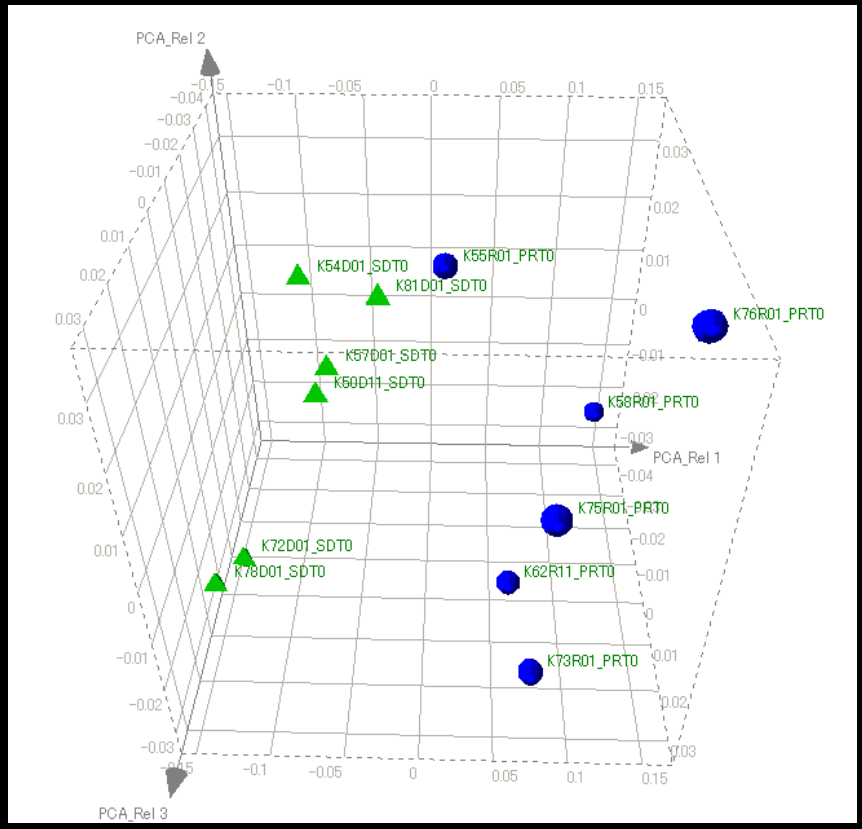


Proteomic approach to detect biomarkers to predict gefitinib-response

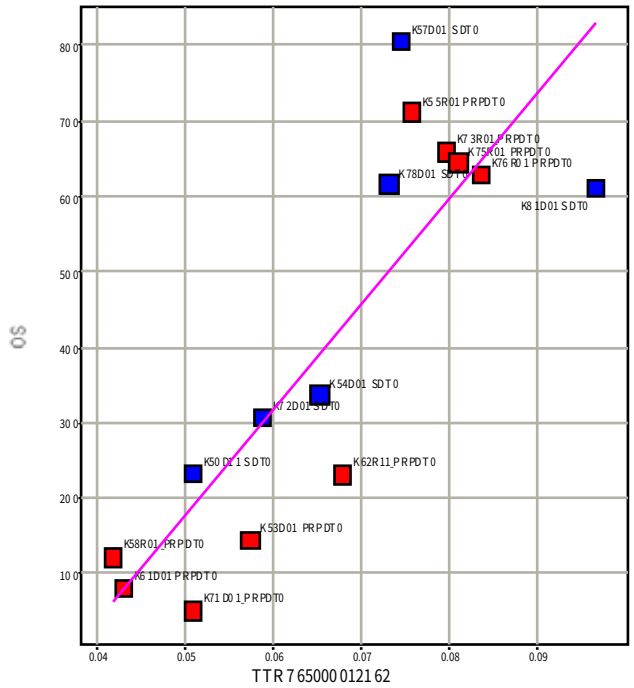
PR vs. SD (Pre)



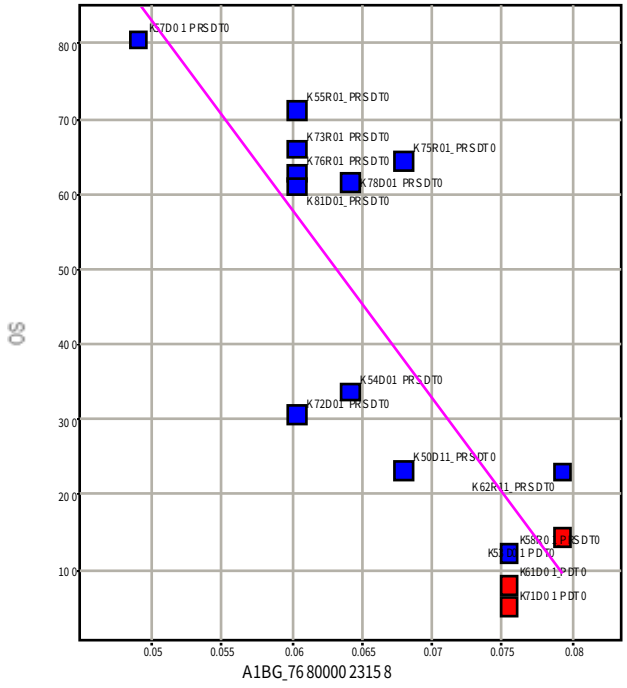
PCA



OS ~ T (818.1 / 38.1) pre



OS ~ G (1076.5 / 74.8) pre



R^2=0.73

Mass Spectrometry to Classify Non–Small-Cell Lung Cancer Patients for Clinical Outcome After Treatment With Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors: A Multicohort Cross-Institutional Study

Fumiko Taguchi, Benjamin Solomon, Vanesa Gregorc, Heinrich Roder, Robert Gray, Kazuo Kasahara, Makoto Nishio, Julie Brahmer, Anna Spreafico, Vienna Ludovini, Pierre P. Massion, Rafal Dziadziuszko, Joan Schiller, Julia Grigorieva, Maxim Tsypin, Stephen W. Hunsucker, Richard Caprioli, Mark W. Duncan, Fred R. Hirsch, Paul A. Bunn Jr, David P. Carbone

Conclusion This MALDI MS algorithm was not merely prognostic but could classify NSCLC patients for good or poor outcomes after treatment with EGFR TKIs. This algorithm may thus assist in the pretreatment selection of appropriate subgroups of NSCLC patients for treatment with EGFR TKIs.

J Natl Cancer Inst 2007;99:838–46

Table 3. Outcomes in the patient sets included in this analysis*

Outcome	Training set	Validation sets		Control sets		
	Italian A/Japan A and B (n = 139)	Italian B (n = 67)	ECOG (n = 96)	Italian C (n = 32)	VU (n = 61)	Polish early stage (n = 65)
Classification from MALDI MS algorithm, No. (%)						
Good	105 (75.5)	39 (58.3)	69 (71.9)	20 (62.5)	41 (67.2)	44 (67.7)
Poor	33 (23.7)	27 (40.3)	27 (28.1)	12 (37.5)	20 (32.8)	21 (32.3)
Undefined	1	1	0	0	0	0
Overall survival						
HR (95% CI)	0.45 (0.19 to 0.63)	0.5 (0.24 to 0.78)	0.4 (0.24 to 0.70)	0.74 (0.3 to 1.6)	0.81 (0.4 to 1.6)	0.9 (0.4 to 1.9)
Log-rank *P*	<.001	.0054	<.001	.42	.54	.79
Median time to death, days (good/poor)	441/148	207/92	306/107	163/141	729/312	1430/1233
Time to progression						
HR (95% CI)	0.5 (0.23 to 0.74)	0.56 (0.28 to 0.9)	0.53 (0.33 to 0.85)	N/A	N/A	N/A
Log-rank *P*	.0031	.02	.007	N/A	N/A	N/A
Median time to progression, days (good/poor)	161/63	84/61	98/58	N/A	N/A	N/A
Multivariable analysis of overall survival†						
HR (95% CI)	ND	0.74 (0.55 to 0.99)	0.53 (0.30 to 0.94)	ND	ND	ND
Wald *P*	ND	.048	.03	ND	ND	ND

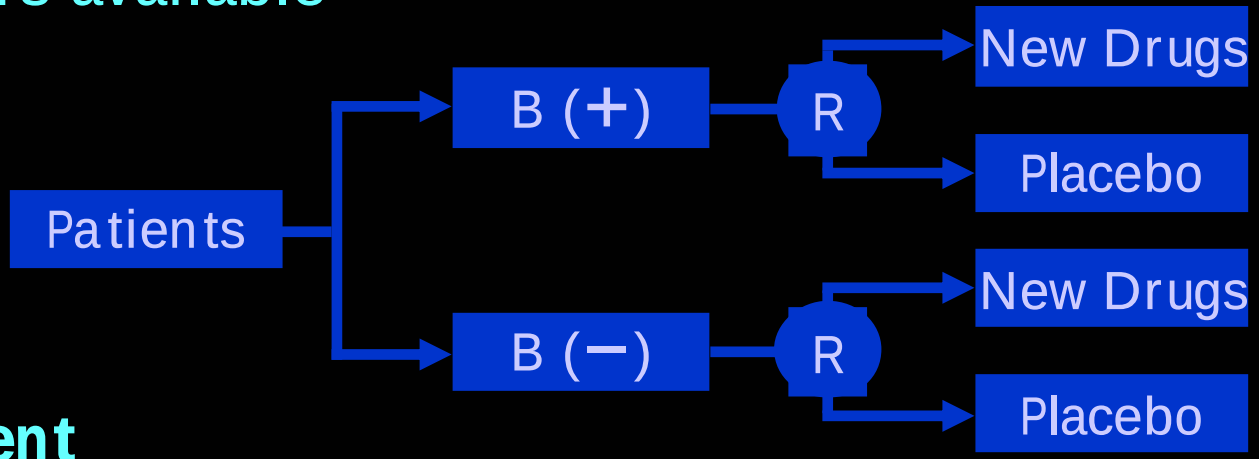
- Multicohort Cross-Institutional Study

- Study Design
  To distinguish prognostic and predictive biomarkers

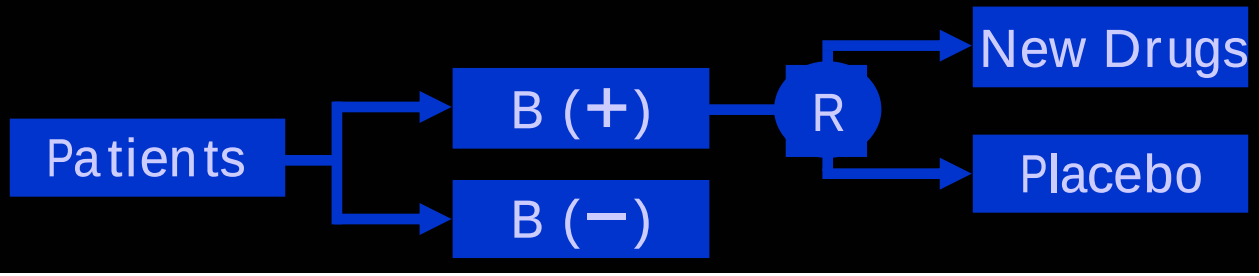
Study design

1. Biomarkers not available

2. Biomarkers available

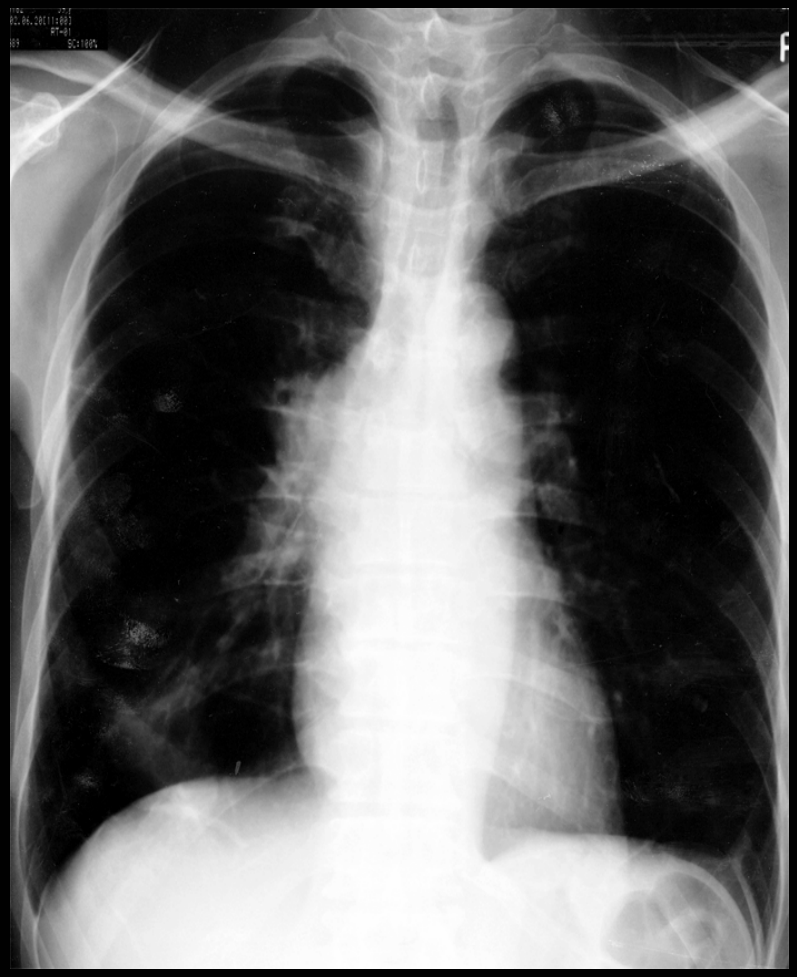


3. Enrichment

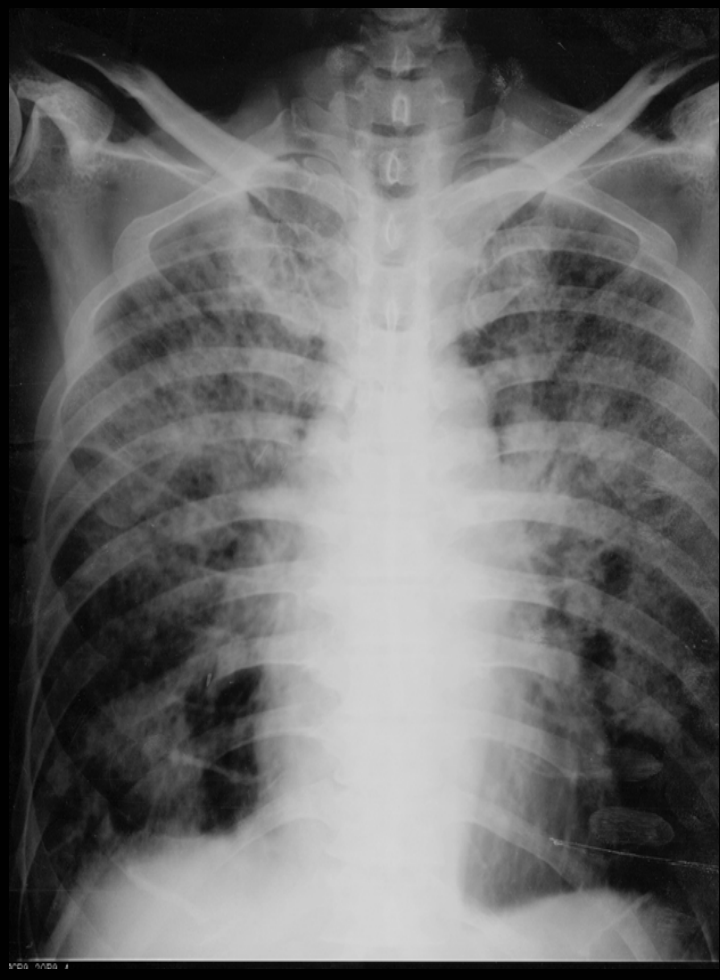


Pneumonitis induced by gefitinib

ILD: interstitial lung disease

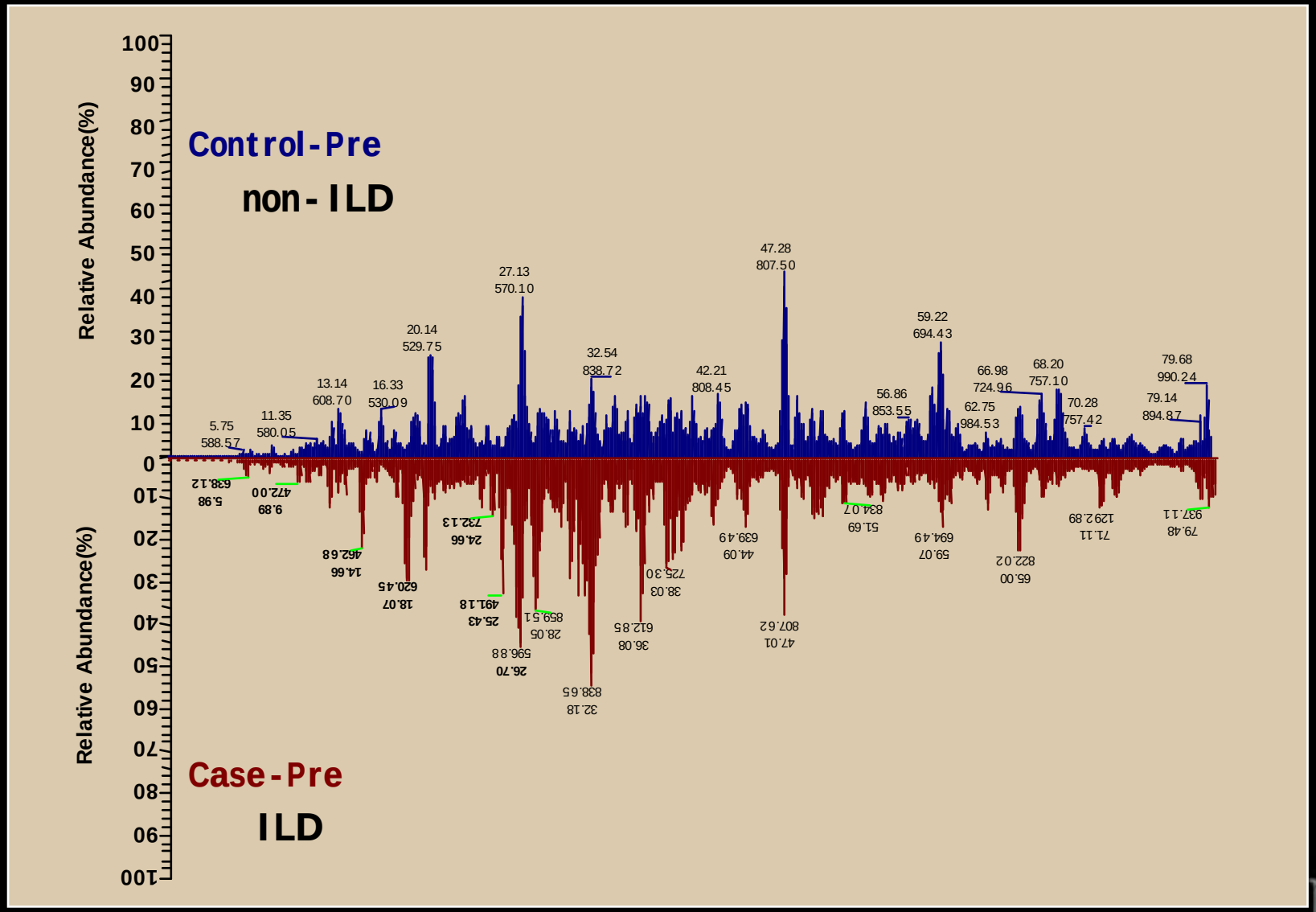


Pretreatment



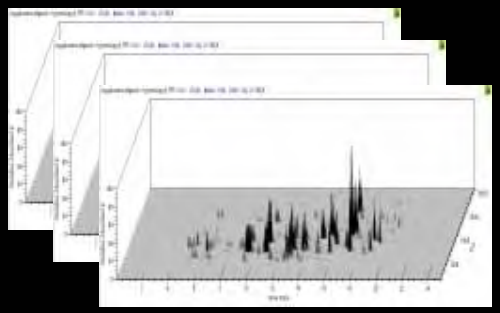
After 35 Days

Comparison of chromatogram of typical ILD / non-ILD baseline samples

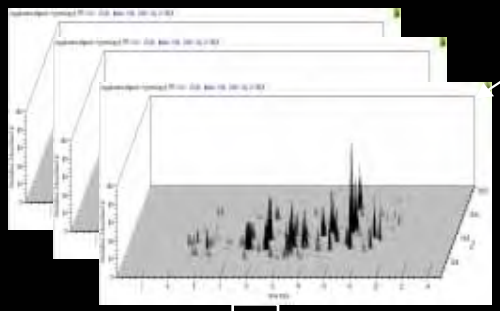


How to identify group-specific peptide signals
i-OPAL approach

Cases



Controls



A **Comprehensive Ion Signal Map** from each data point

The Map consists of quantitative signals and physicochemical properties (like m/z) derived from the LC-MS measurement

An 'averaged' map as a **template** of positive group

An 'averaged' map as a **template** of negative group

Mining significant signals by comparing the two templates

Significant LC-MS signals

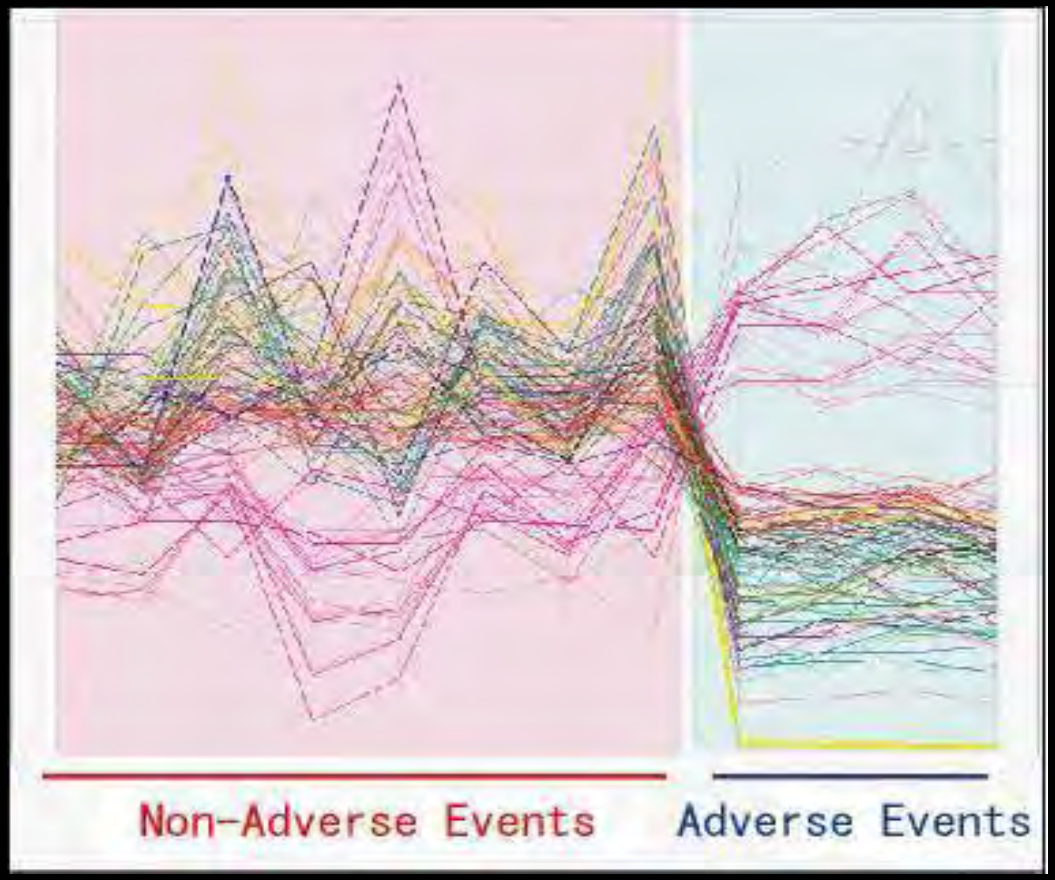
Target MS/MS to identify proteins from the signals

List of the proteins of which amounts are **significantly** different between the **two groups**

Kinki University School of Medicine
Dept Genome Biology

Application to the gefitinib ILD CCS

Profile chart of candidate marker signals



LC-MS measurement of case / control samples

↓

Align LC-MS signals of all samples

↓

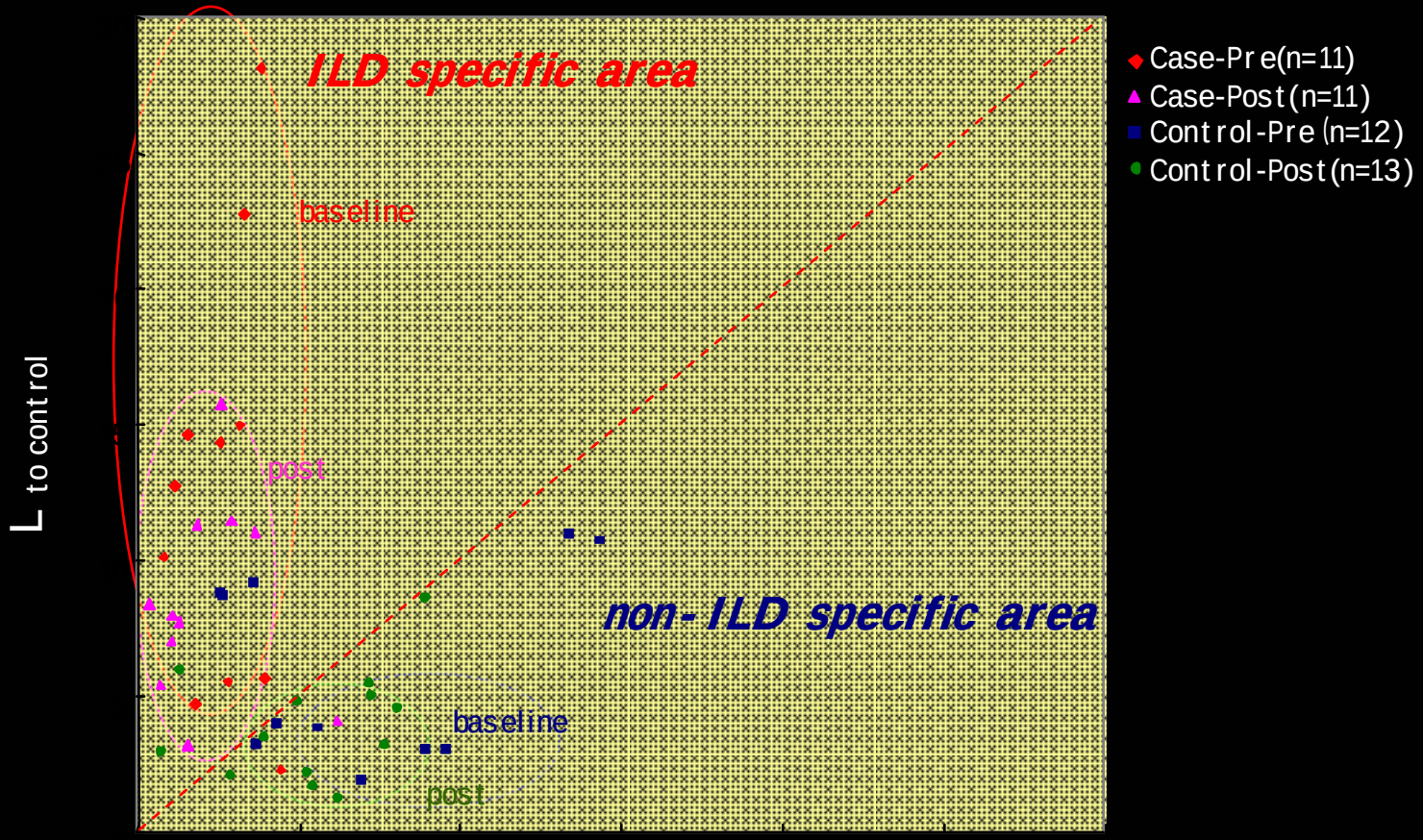
Apply statistical test to each aligned signal point

↓

Pickup signal points of which intensity is significantly different between cases & controls

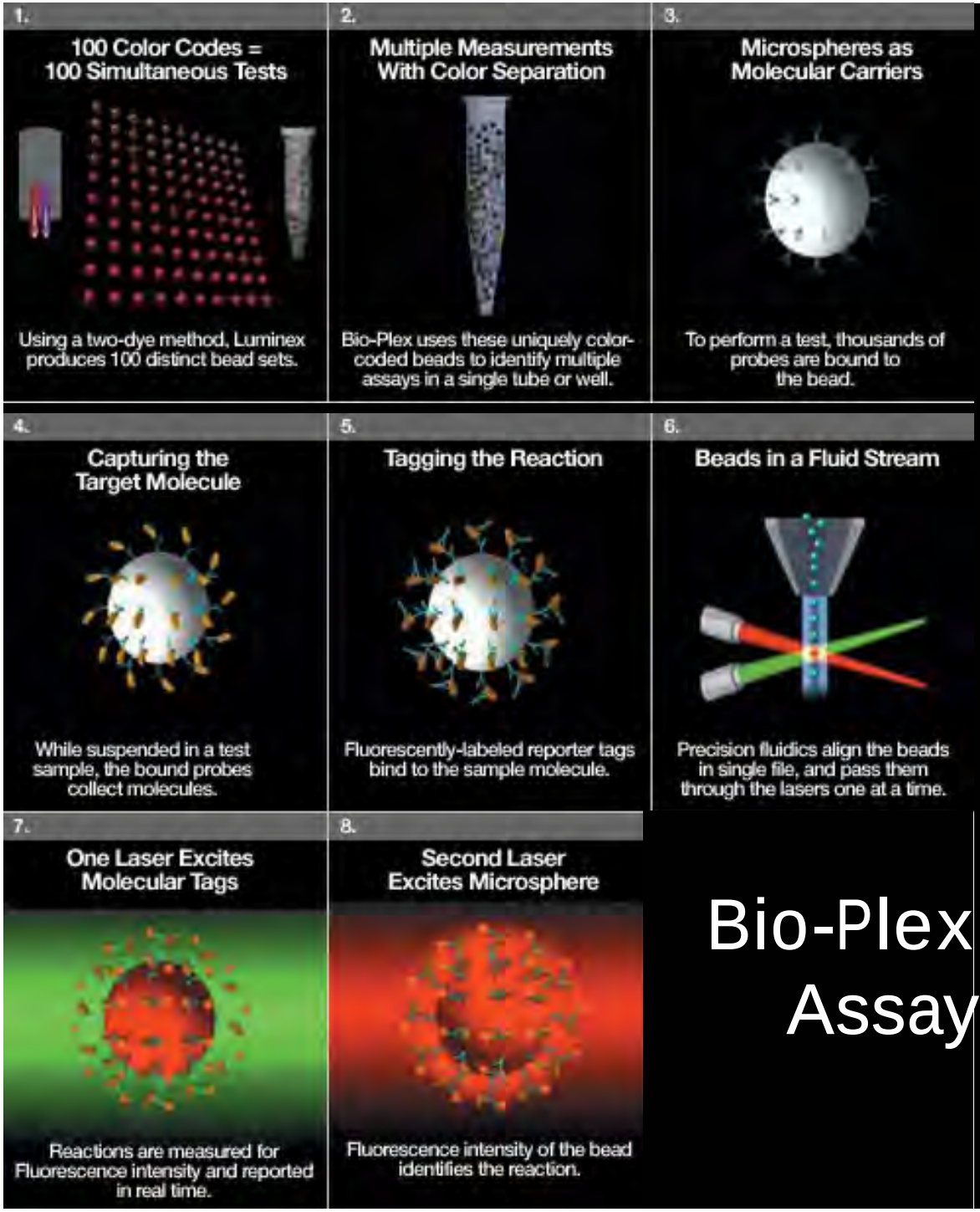
Distribution of 47 patients profiles using tentative signal set

(Case-Pre 11, Case-Post11, Control-Pre 12, Control-Post 13sample)



Search for biomarkers in surrogate tissue (circulating samples)

1. EGFR somatic mutation
   in circulating tumor cells in lung cancer
2. Gene expression profile in PBMC
3. Serum proteomics
4. Multiplex ELISA using bio beads
5. Glycoprofiling

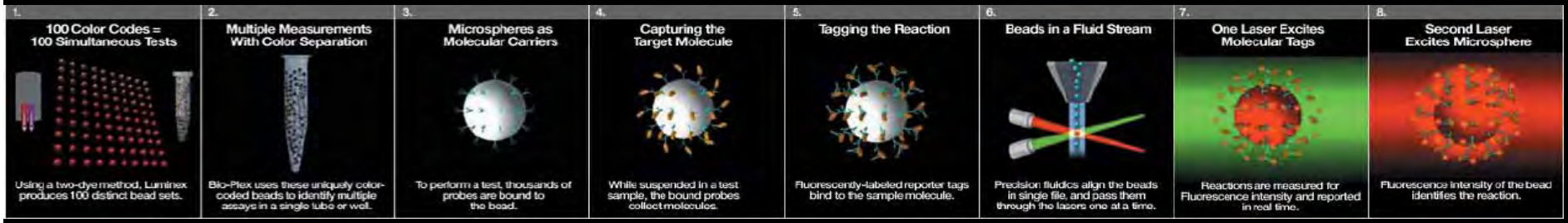


Multiplexing with Colored Bead Sets

Bio-Plex™ ELISA Assay System

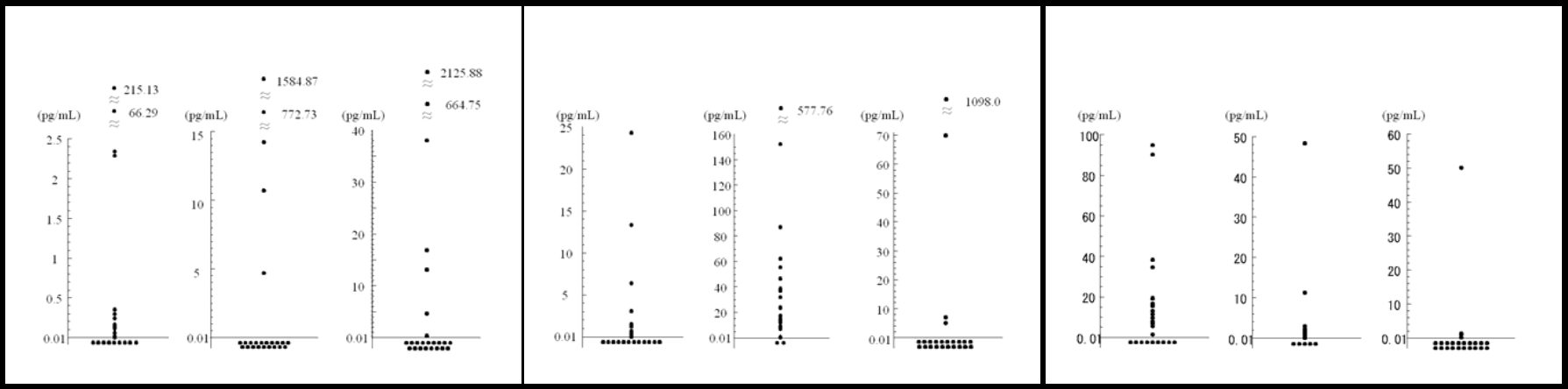
Kinki University School of Medicine
Dept Genome Biology

The levels of cytokines in plasma from NSCLC patients received gefitinib detected by BioPlex (Luminex)

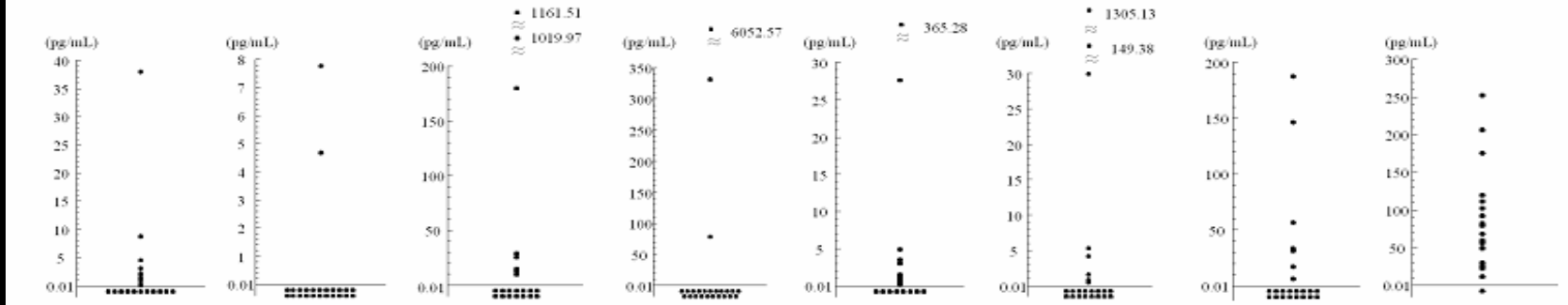


The cytokine levels at pre-treatment in NSCLC patients

IL-1b IL-2 IL-4 IL-5 IL-6 IL-7 IL-8 IL-10 IL-12



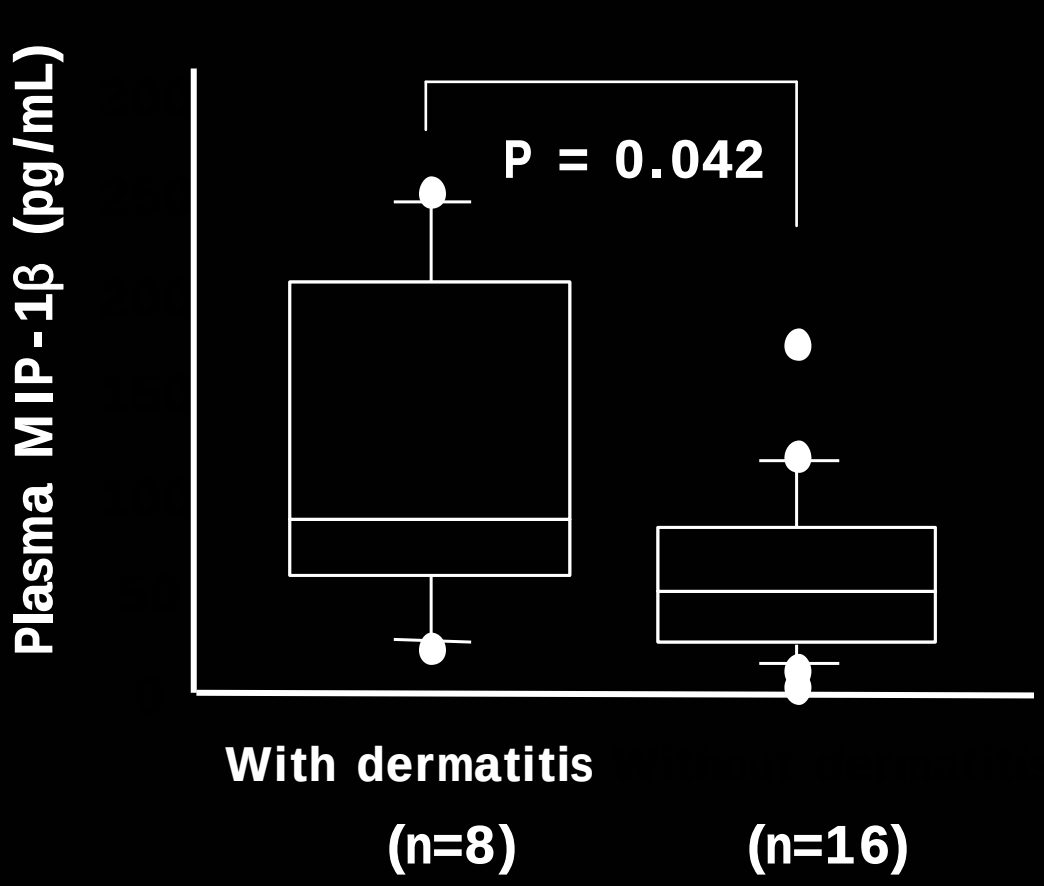
IL-13 IL-17 G-CSF GM-CSF TNF-a IFN-γ MCP-1 MIP-1b

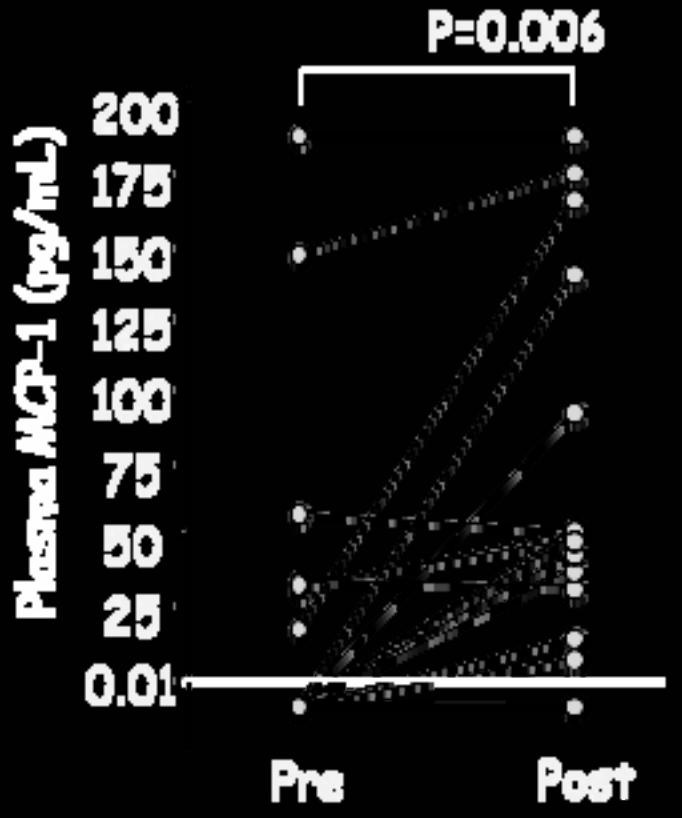


The minimum detectable dose for each cytokine was 0.01mg/ml.

Kinki University School of Medicine
Dept Genome Biology

MIP-1b levels between patients with and without gefitinib-induced dermatitis

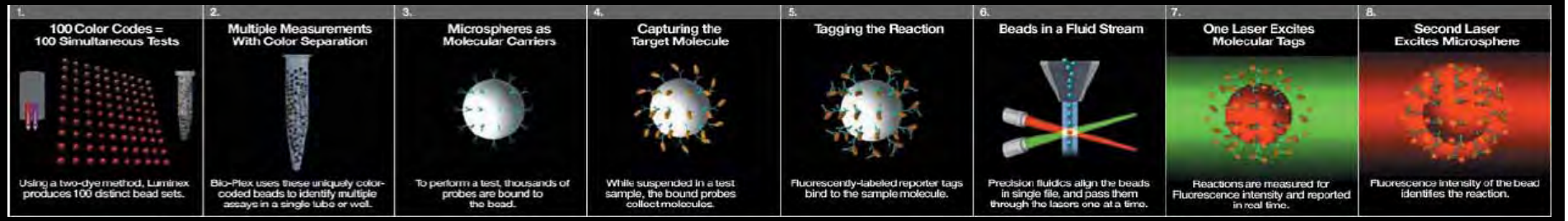




◆MIP-1b is a candidate for predictive marker to predict skin toxicity.

Kimura et al. Lung Ca, 2005

Bio-Plex™ ELISA Assay System
for angiogenesis inhibitors



1. Angio-1 panel
(angiopoietin-2, follistatin, G-CSF, HGF, IL-8, leptin, PDGF-BB, PECAM-1, VEGF)

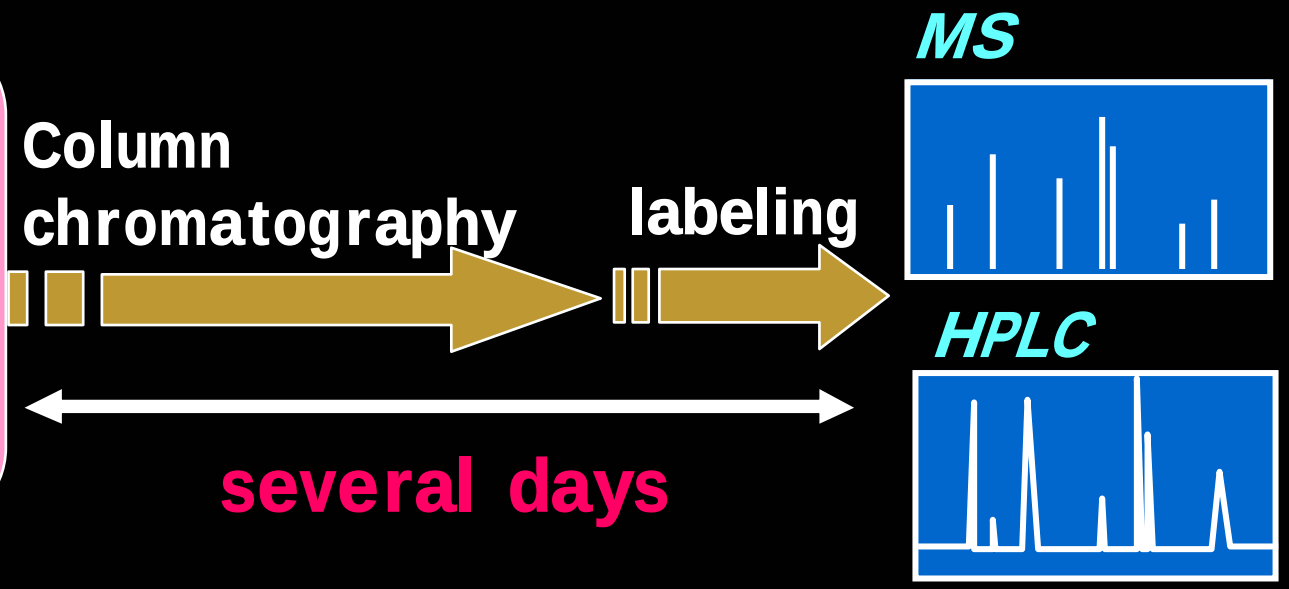
2. Angio-2 panel
(IL-6R、MMP-9、TIMP-1、TIMP-2、Endostatin、P-selectin、ICAM-1、VCAM-1、Tie-2、PAI-1、MIF、uPAR)

Search for biomarkers in surrogate tissue (circulating samples)

1. EGFR somatic mutation
   in circulating tumor cells in lung cancer
2. Gene expression profile in PBMC
3. Serum proteomics
4. Multiplex ELISA using bio beads
5. Glycoprofiling

Current situation of glycomics

Samples including released glycans.
(Ex.Glycoproteins
Serum, plasma
Cells,Tissue
treated by PNGase)



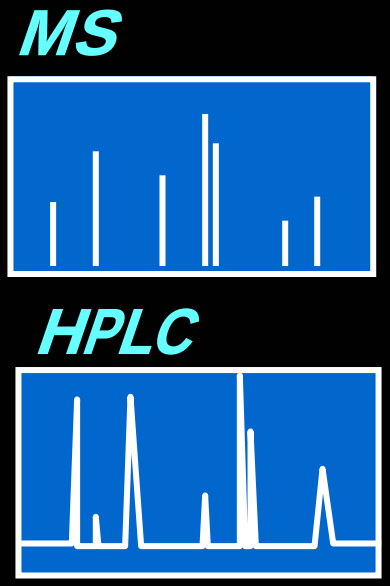
The bottleneck of current glycomics research is time-consuming and non-precise purification using column chromatography.

Kinki University School of Medicine
Dept Genome Biology

Concept of BlotGlyco

Samples Including
released glycans
(Ex.Glycoproteins
Serum, plasma
Cells,Tissue
treated by PNGase)

Precise purification
and various labeling,
5~7 hours in total



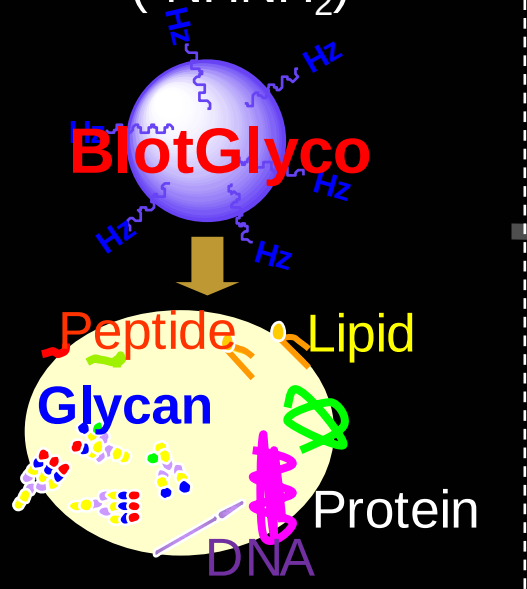
Speedy, one-pot solid phase process
to obtain perfectly purified and labeled glycan.

Kinki University School of Medicine
Dept Genome Biology

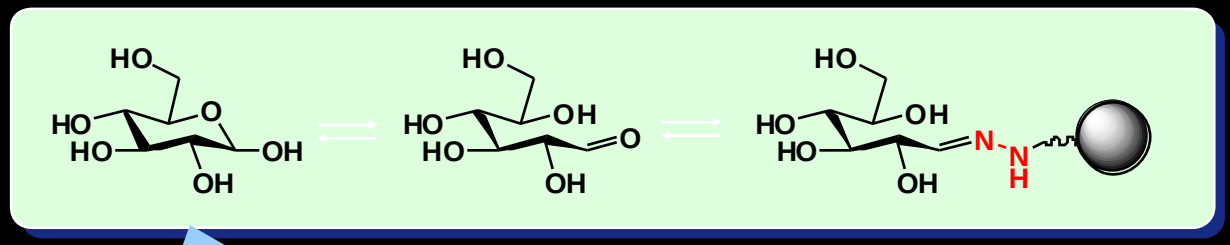
Reaction mechanism of BlotGlyco

Applying BlotGlyco into crude mixture

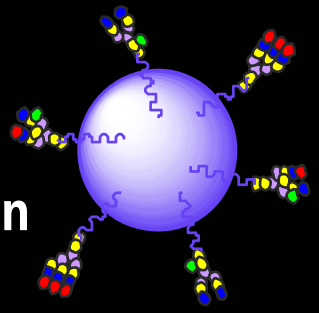
*Hz: hydrazide group ($-NHNH_2$)



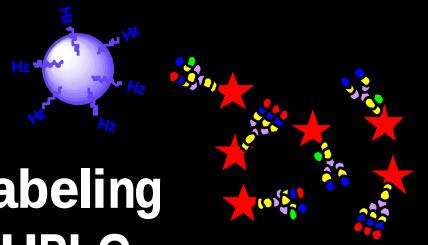
Aldehyde of reducing end of glycan is bound to hydrazide of BlotGlyco beads. (covalent bonding)



Purification



Various labeling for MS, HPLC



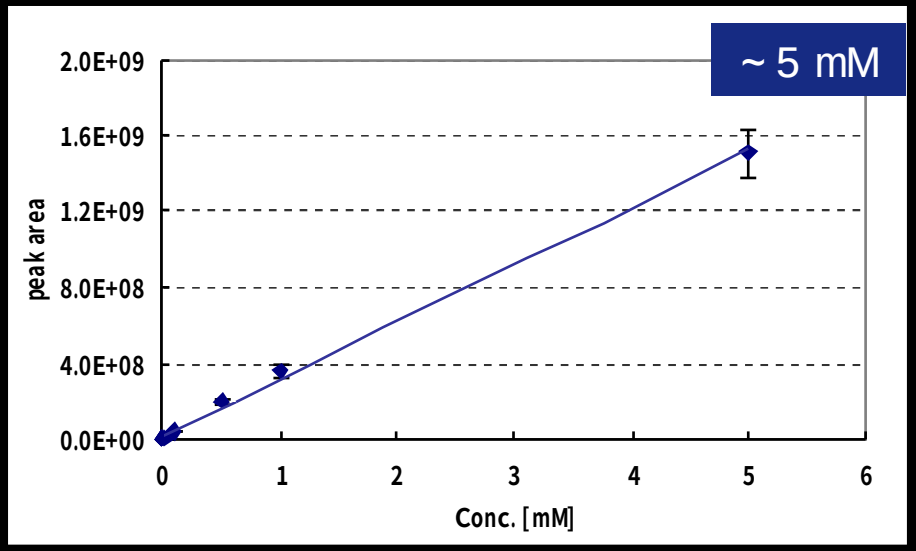
Labeled glycan

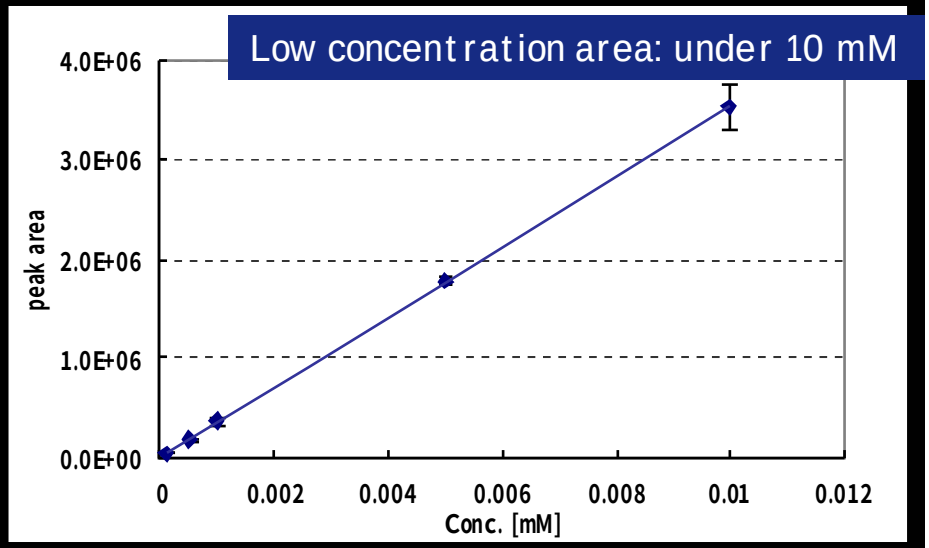
Glycan captured by BlotGlyo beads endure harsh wash. Every impurities even peptides can be completely removed.

Kinki University School of Medicine
Dept Genome Biology

Quantitative reliability of BlotGlyco

Correlation between concentration of glycan and peek area
*Maltoheptaose solution was used.

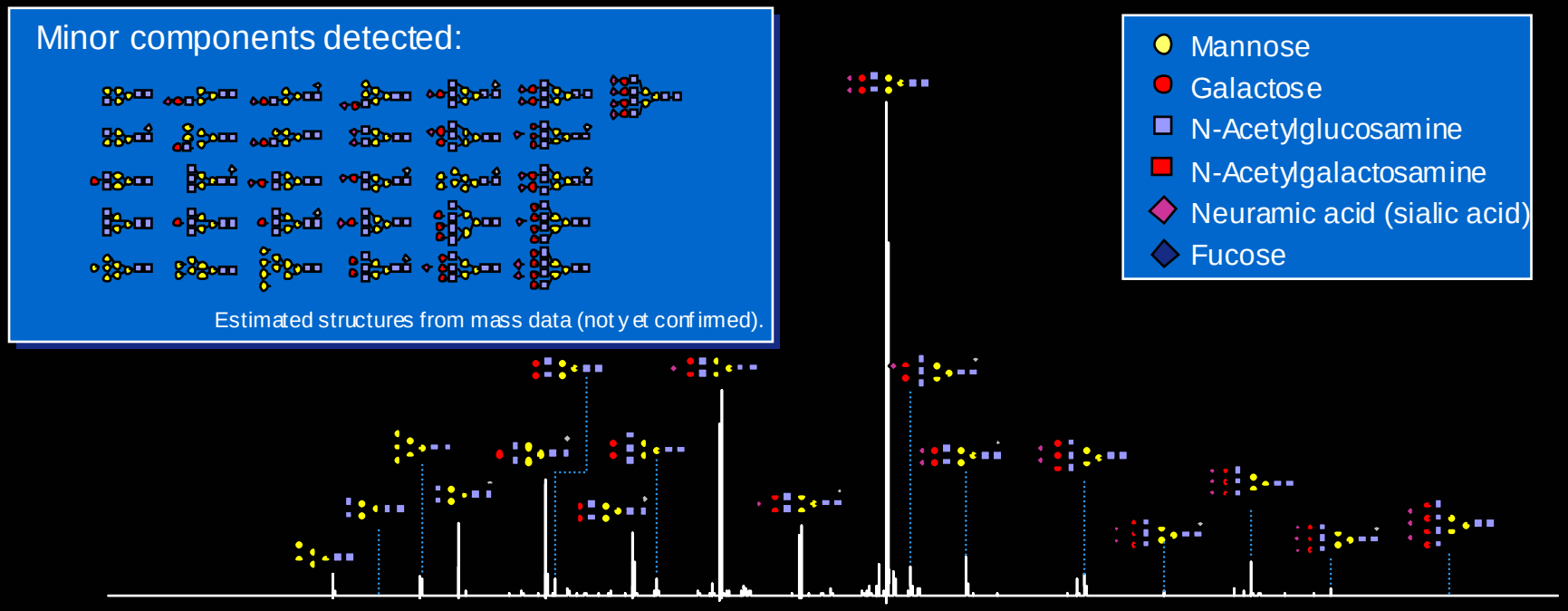




***Detectable from 0.1 mM solution (2 pmol Maltoheptaose)**
***Standard curve shows linearity in the range of 0.1 mM to 5 mM.**

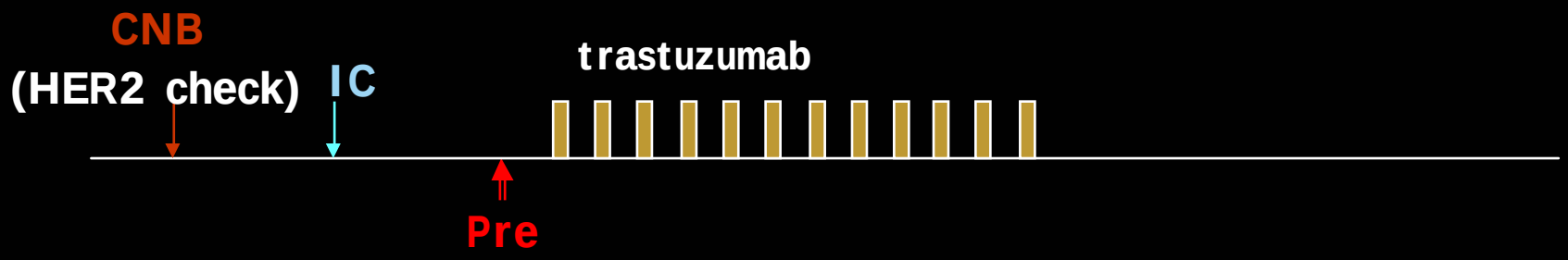
Kinki University School of Medicine
Dept Genome Biology

N-glycan profile obtained from 5μL human serum



49 kinds of N-glycans were detected from **5μL** human serum.

Identification of predictive biomarkers for response to trastuzumab using glycobiological analysis



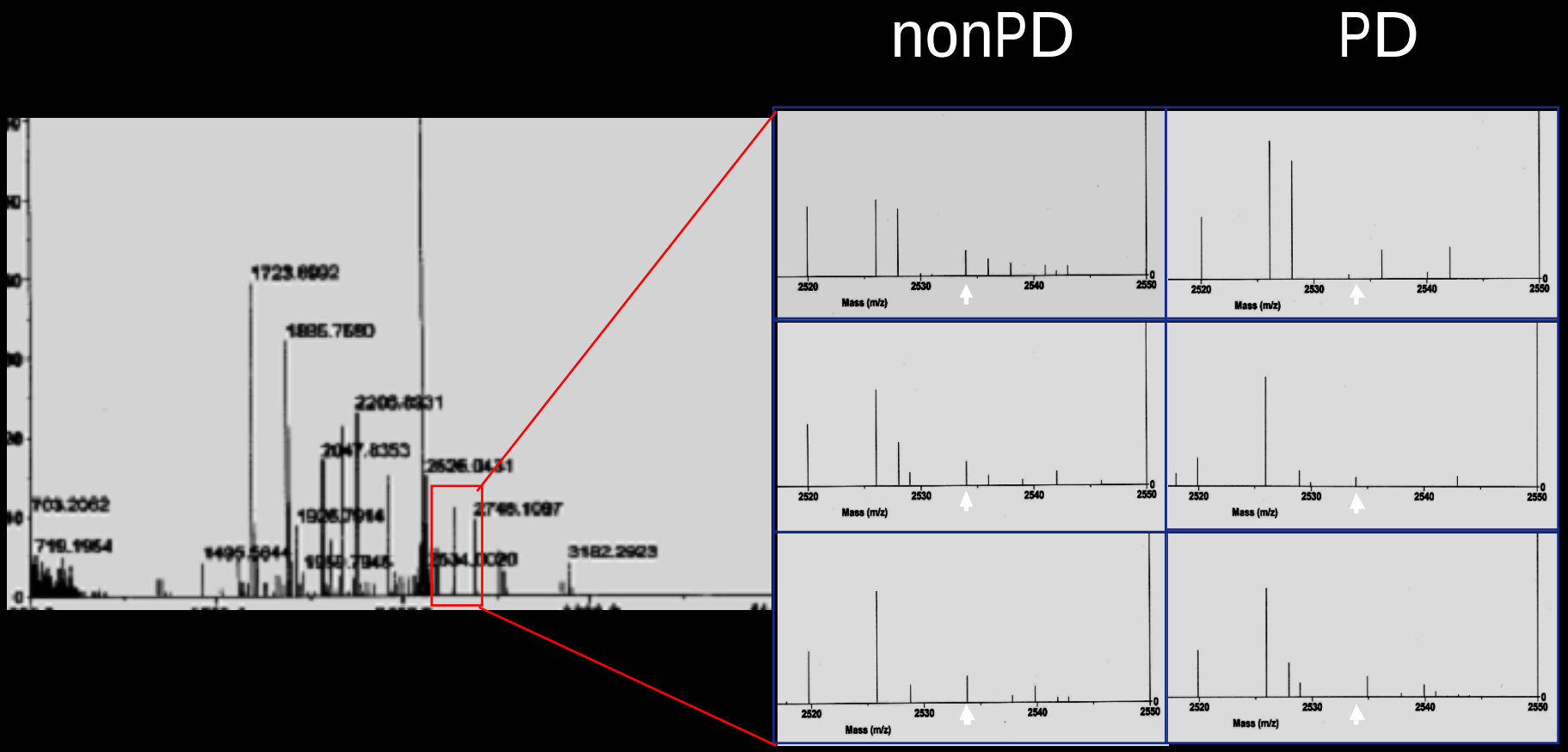
CNB:core needle biopsy

Serum samples of breast cancer patients received with trastuzumab monotherapy

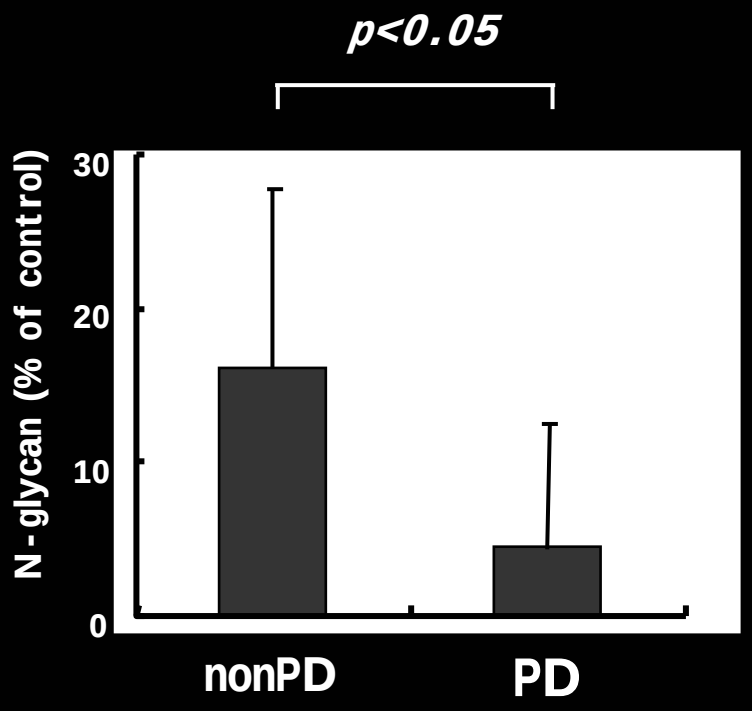
Trastuzumab (Herceptin): anti-HER2 Ab

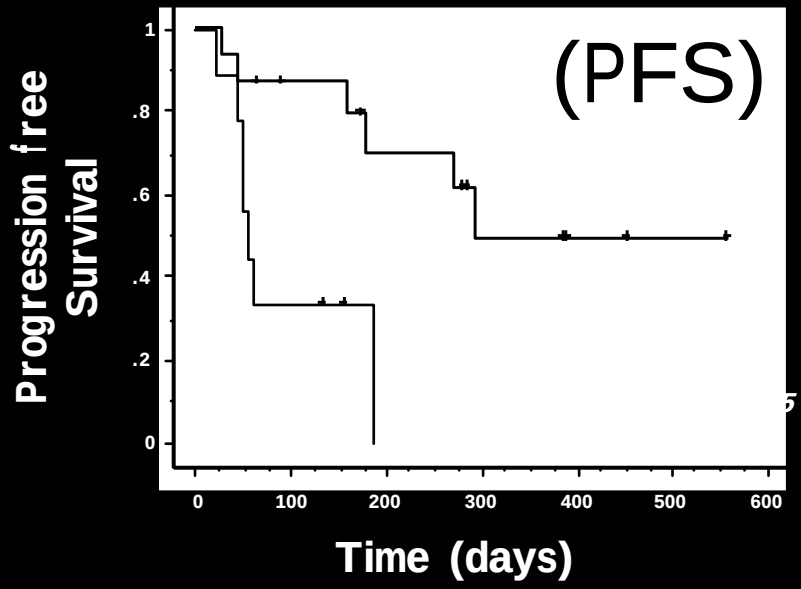
Kinki University School of Medicine
Dept Genome Biology

Representative data of plasma *n*-glycan profile measured using MALDI-TOF-MS



Plasma N-glycan and clinical outcome





(Left)
Expression of plasma N-glycan and clinical response.
The expression of plasma 2534 m/z N-glycan was significantly lower in patients with progressive disease (PD).
(Right)
Kaplan-Meier curve of high (detectable) or low (not detectable) plasma N-glycan groups for progression-free survival (PFS) after trastuzumab treatment.