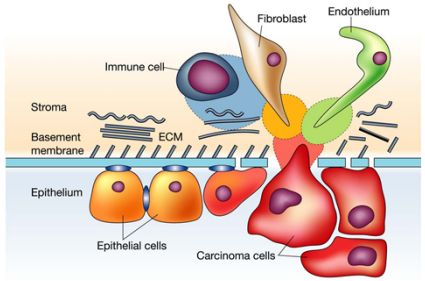
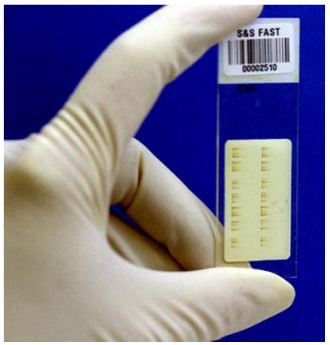


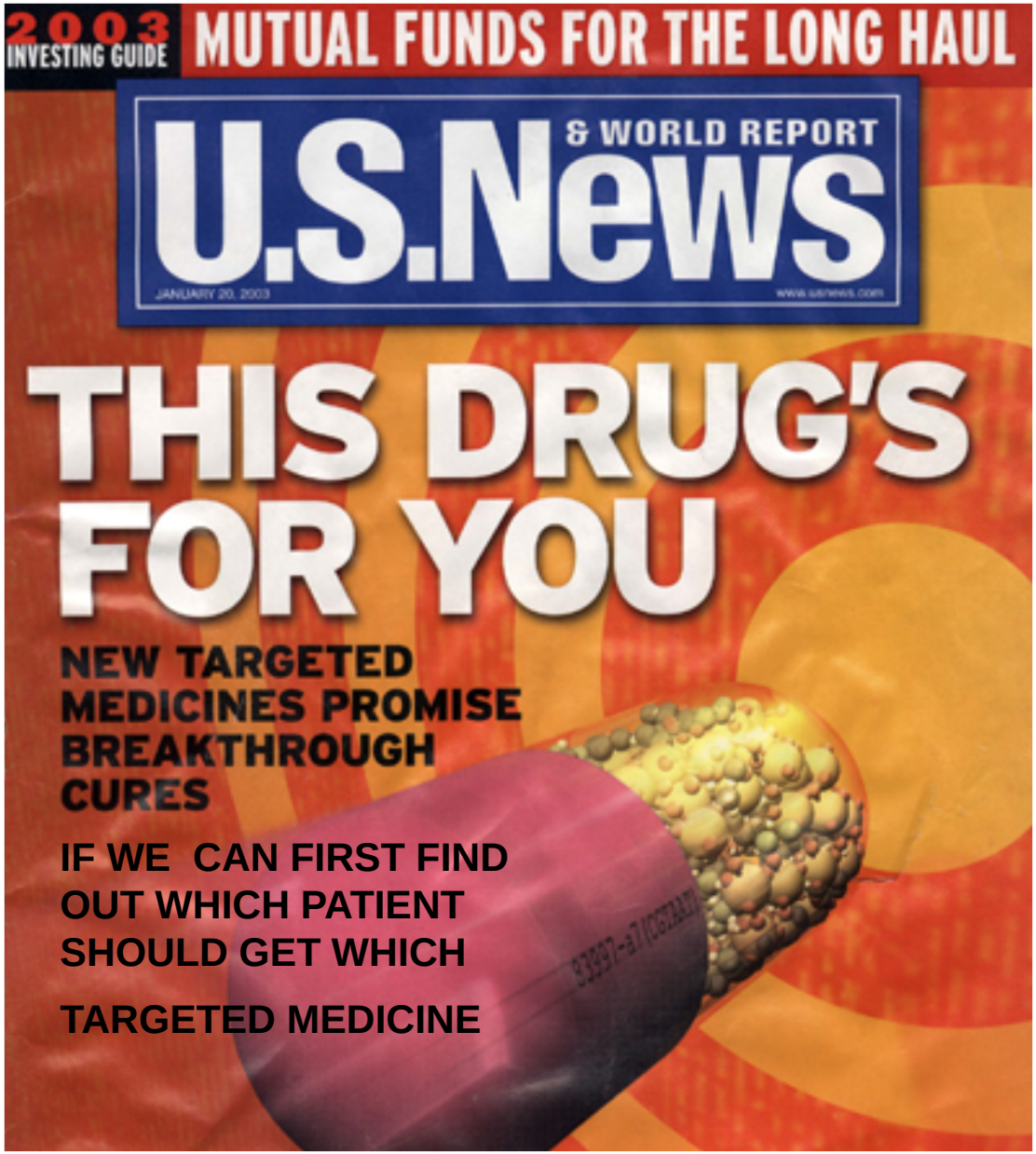


Mapping Molecular Network Interconnections:

Kinase Substrate Protein Microarray Analysis

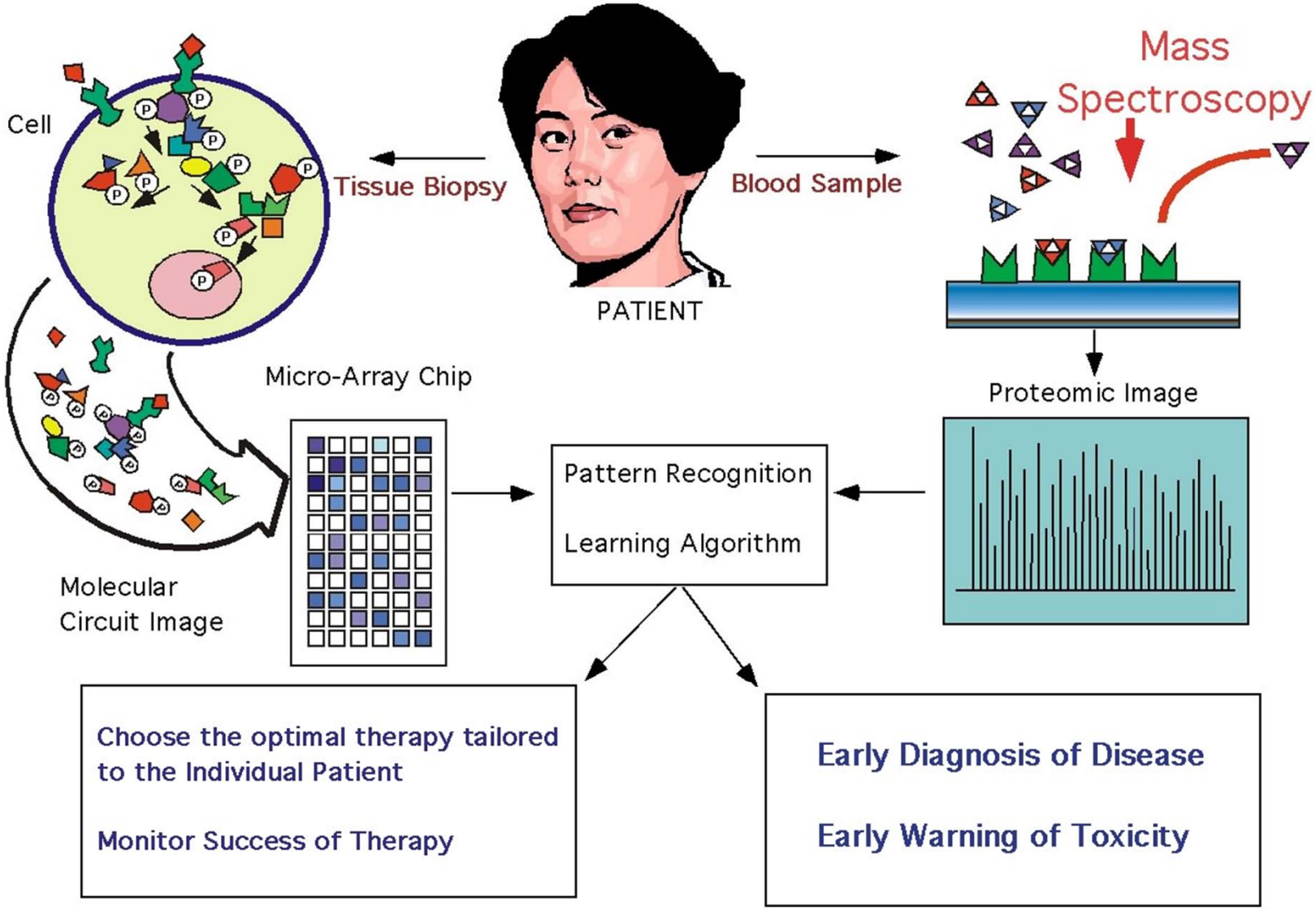
"If it were not for the great variability among individuals, Medicine might be a science not an art. (Sir William Osler, The Principles and Practice of Medicine 1892)

Individualized Molecular Medicine

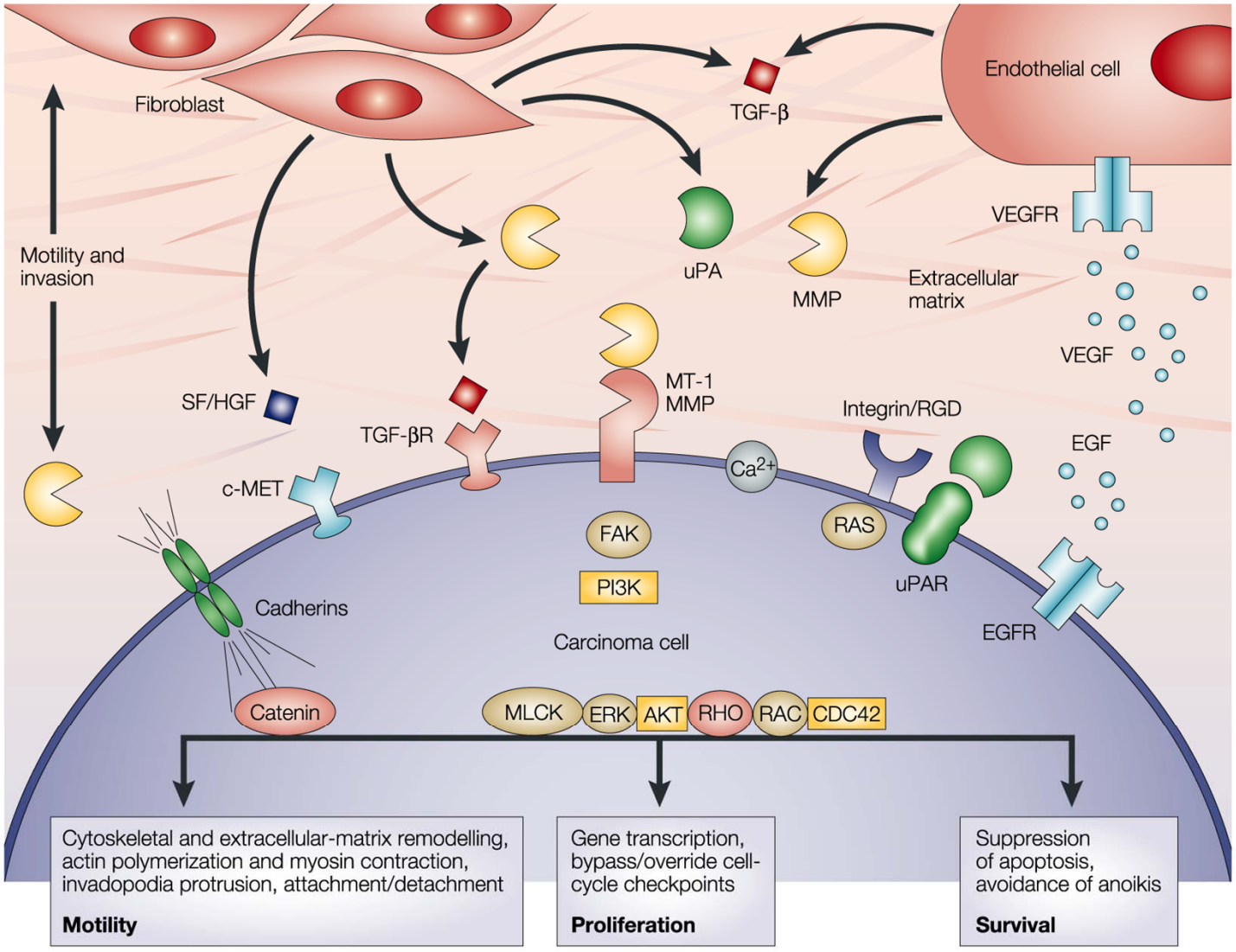


January 20, 2003

NCI / FDA Proteomic Technologies



Protein Microarrays “AI” Bioinformatic Tools Mass Spec



High-

Well-differ
carcin

Moderately
car

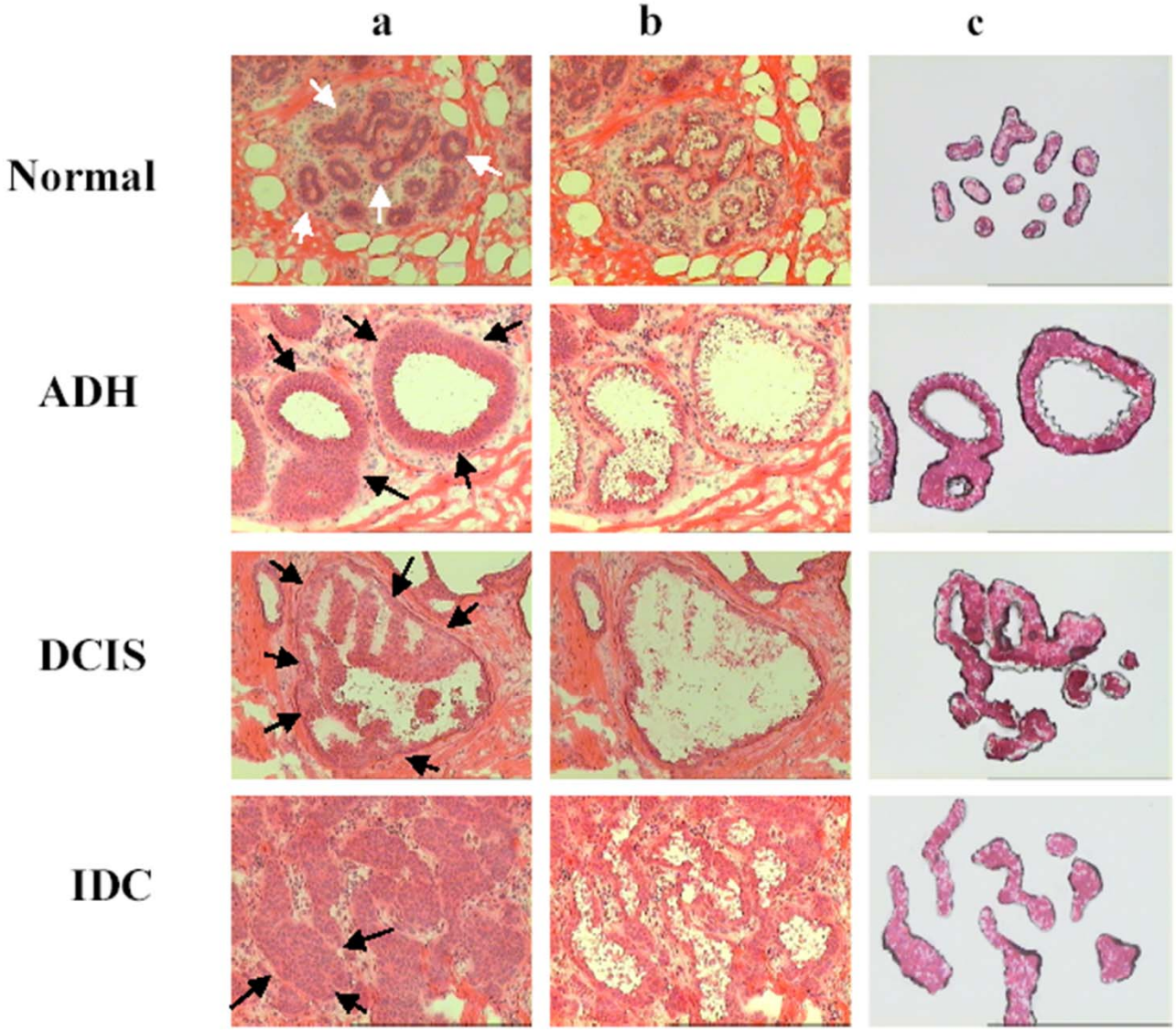
carcinoma

rve

Stroma

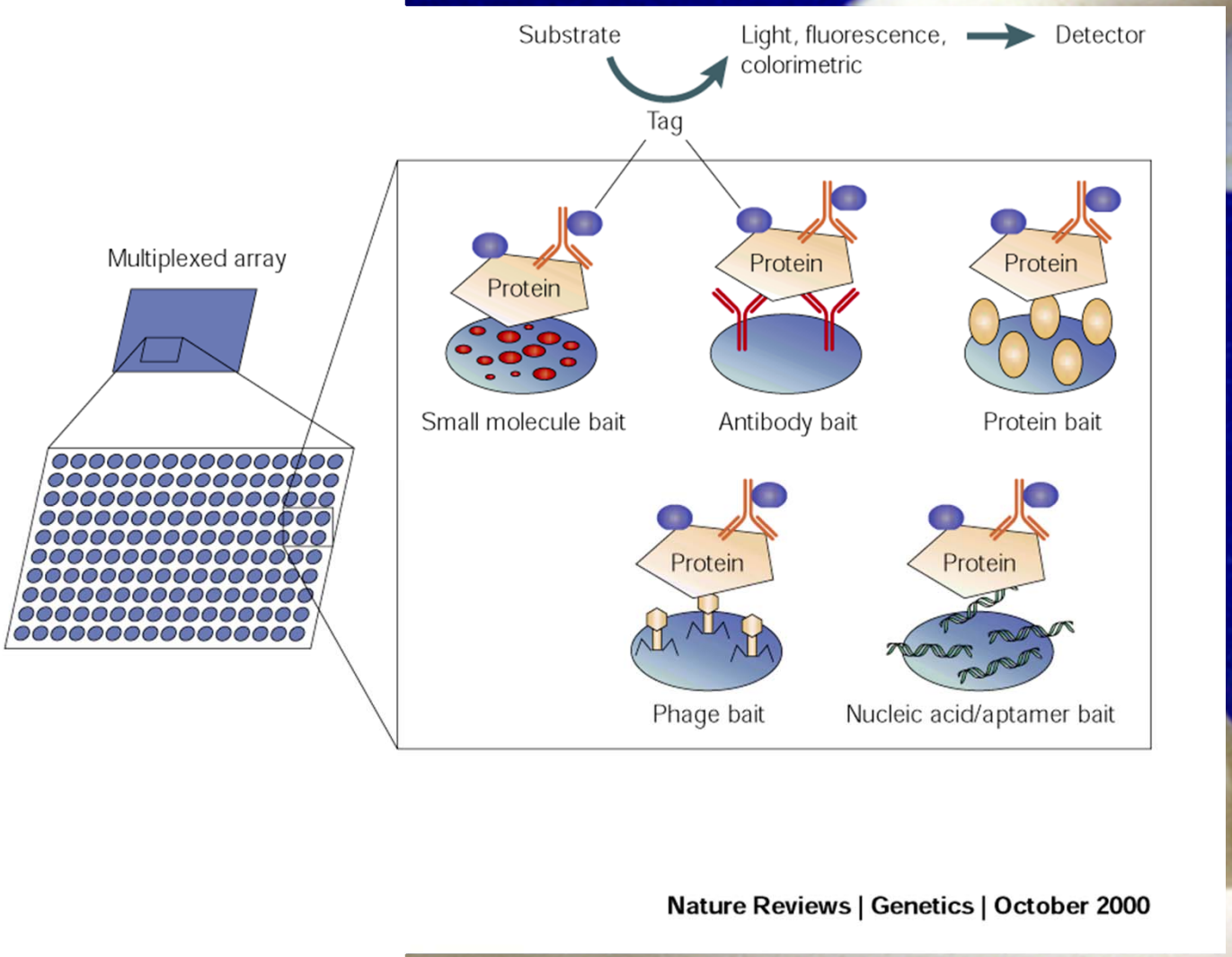
mation

Pathology of Premalignant Breast Cancer Progression



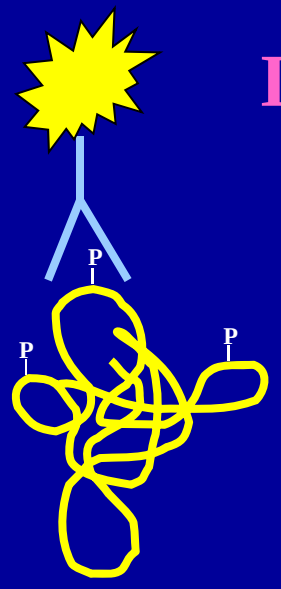
Ma &Sgroi et al

Microdissected
Pure cells

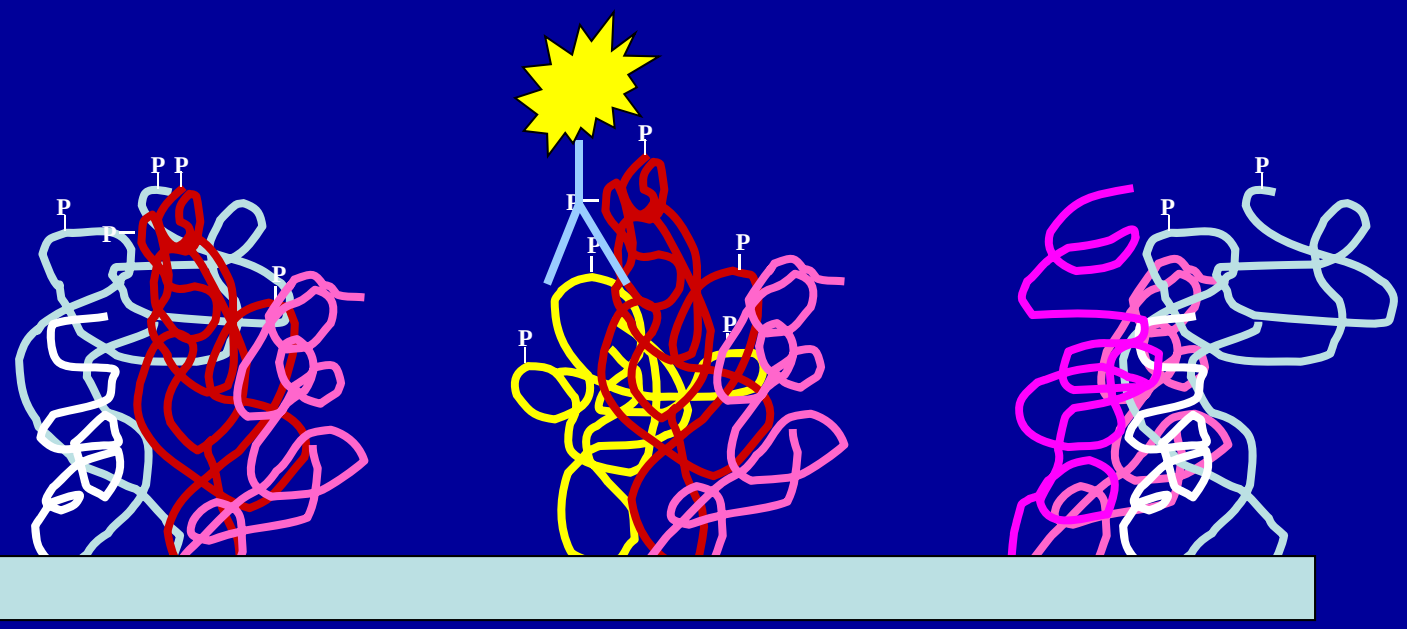


Protein Microarrays

IMMOBILIZED CELLULAR PROTEIN LYSATES: "REVERSE PHASE ARRAYS"



LCM
CELL
LYSATE
PROTEINS



NORMAL Pre-MALIG CANCER

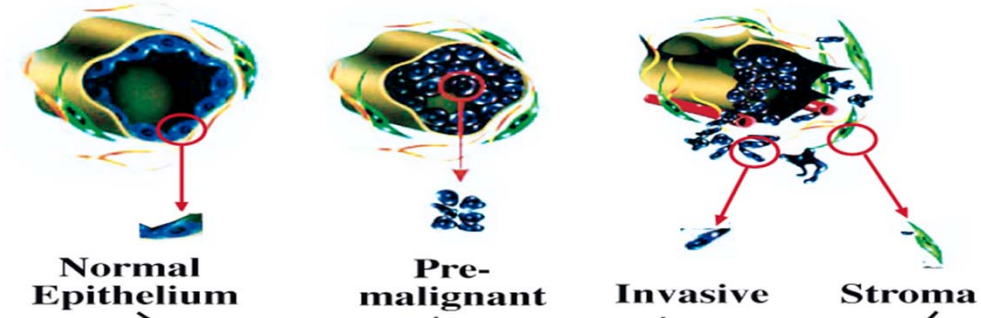
A New Protein Array Technology: Reverse Phase Protein Array

Oncogene 2001

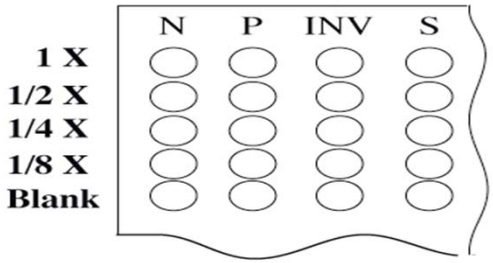
Coupling Laser Capture Microdissection
With High Throughput Protein Arrays

Patient biopsy tissue cells are microdissected:

2000 cells = 100 arrays

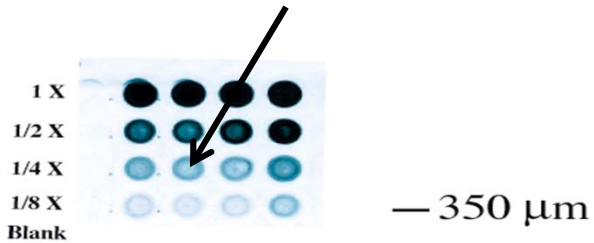


Each patient sample is arrayed in a miniature dilution curve:
Always in linear dynamic range of any antibody/ analyte pair



Arrays probed with labeled amplified antibody:

e.g. prostate cancer progression From one patient probed with Phospho-ERK antibody



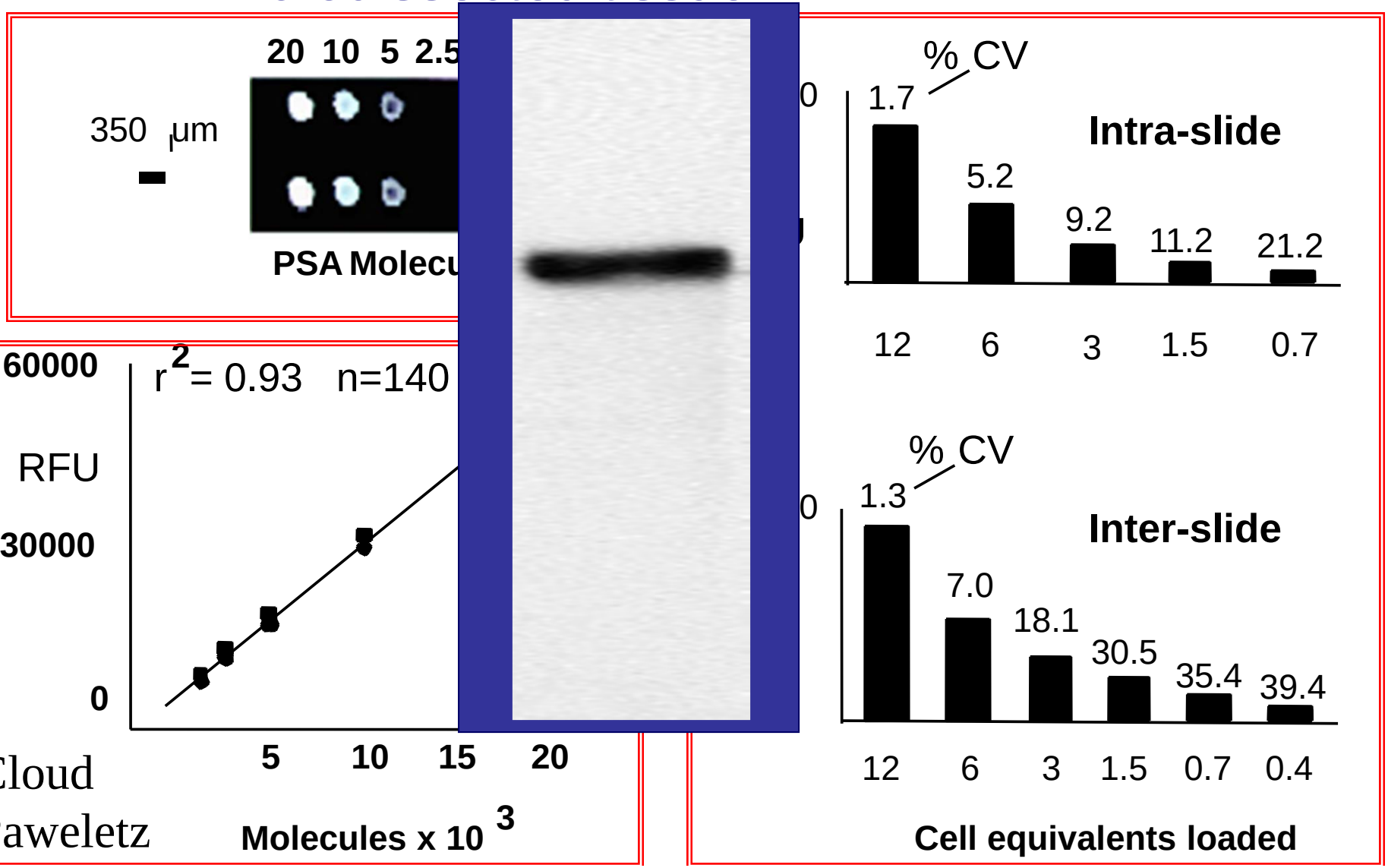
Microarray Sensitivity and Linearity

CV < 10% at 3 cell equivalents for microdissected tissue

Single-band on western blot for primary antibody on PSA

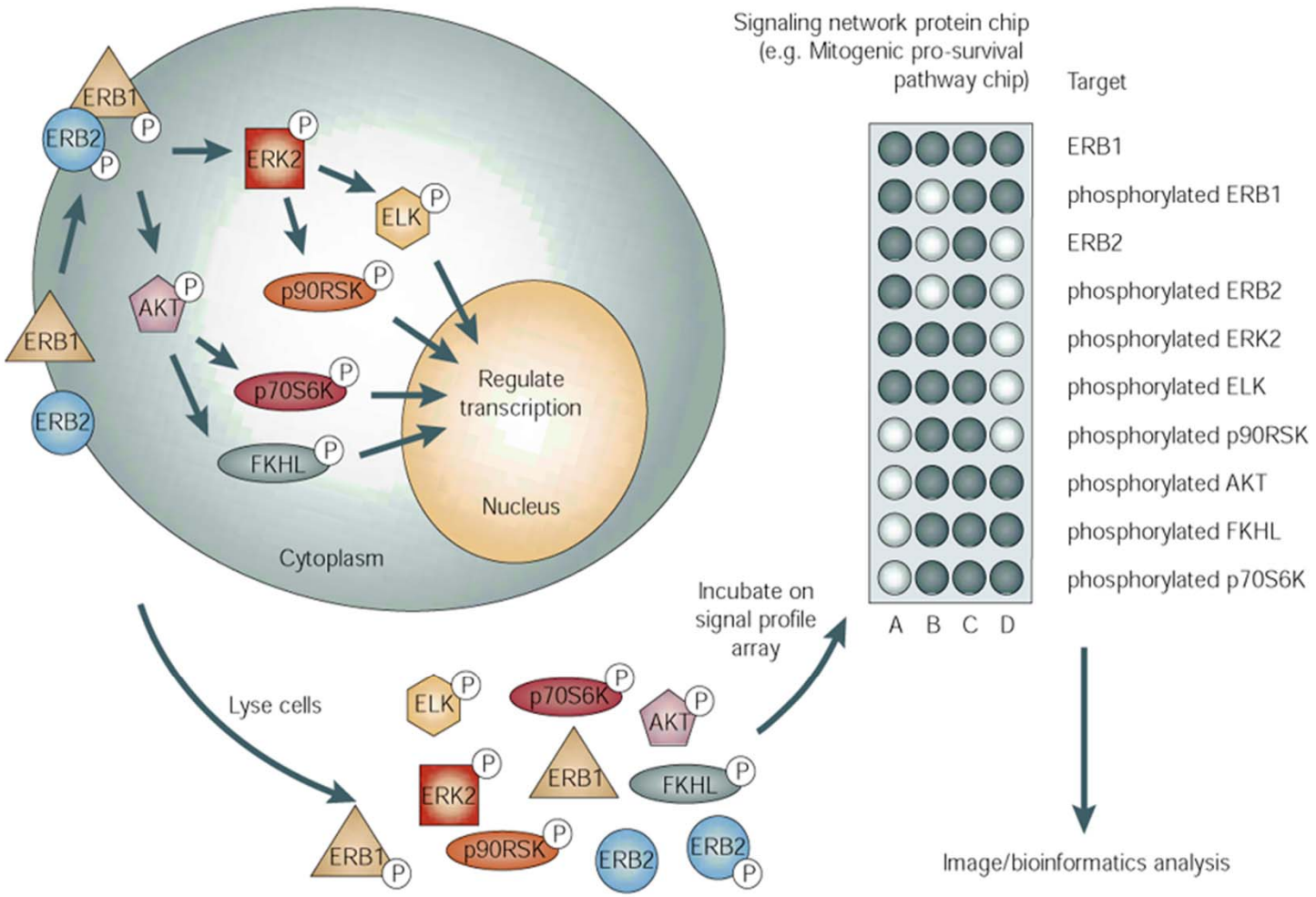
Sensitivity 50 to 100 cell equivalents with fluorescent

Linear with 0.03 correlation coefficient

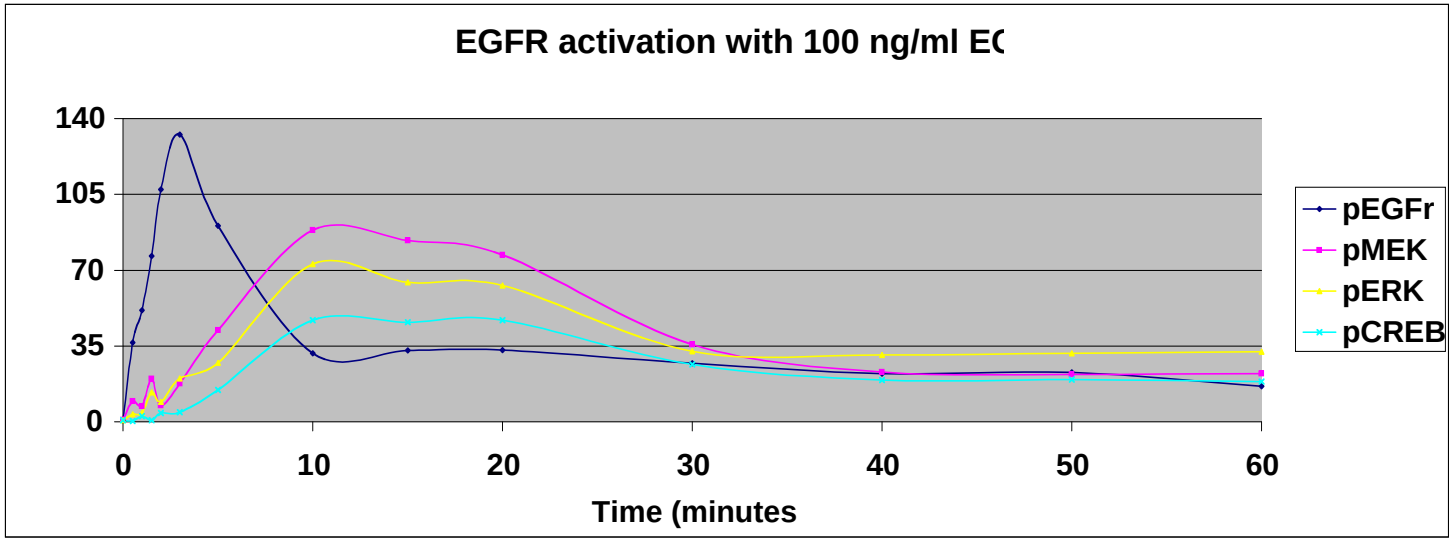


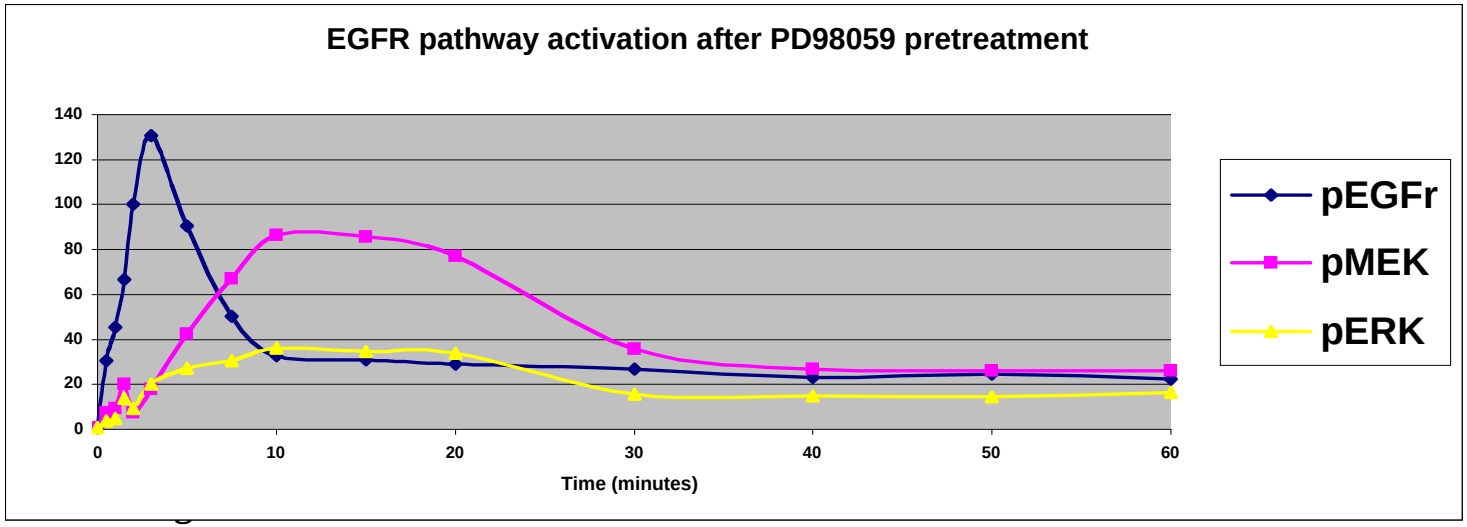
Cloud Paweletz

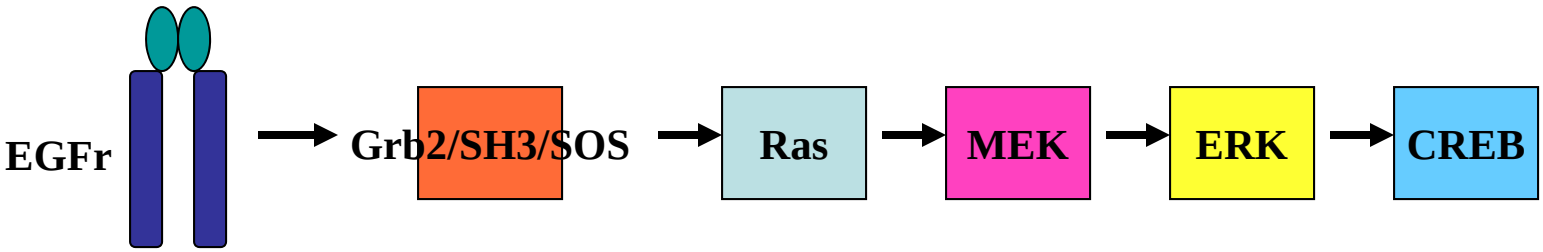
Signal Transduction Pathway Profiling



Nature Reviews | Genetics | October 2000

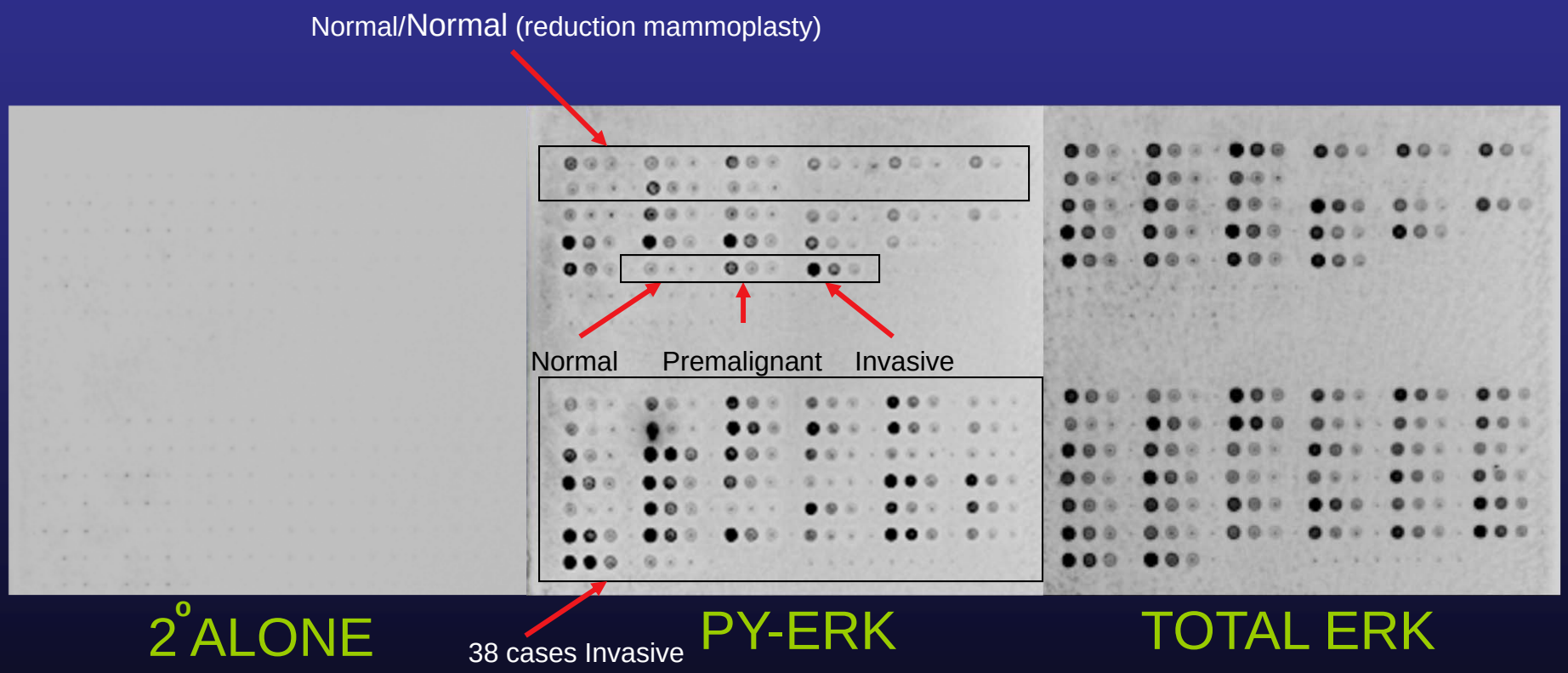

EGFR activation with 100 ng/ml EC
140
105
70
35
0
0
10
20
30
40
50
60
Time (minutes
pEGFr
pMEK
pERK
pCREB


EGFR pathway activation after PD98059 pretreatment
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Time (minutes)
pEGFr
pMEK
pERK

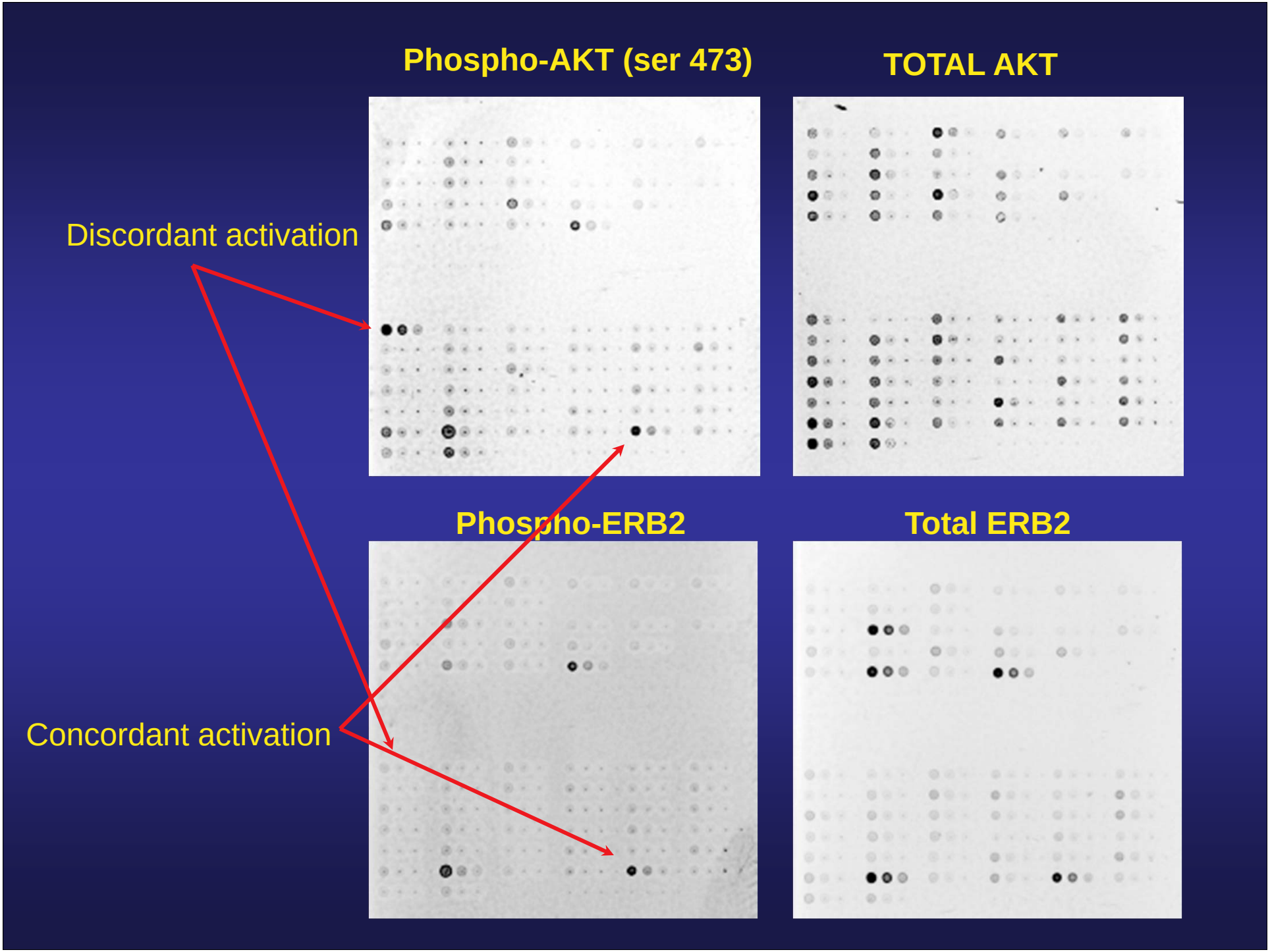

EGFr
Grb2/SH3/SOS
Ras
MEK
ERK
CREB

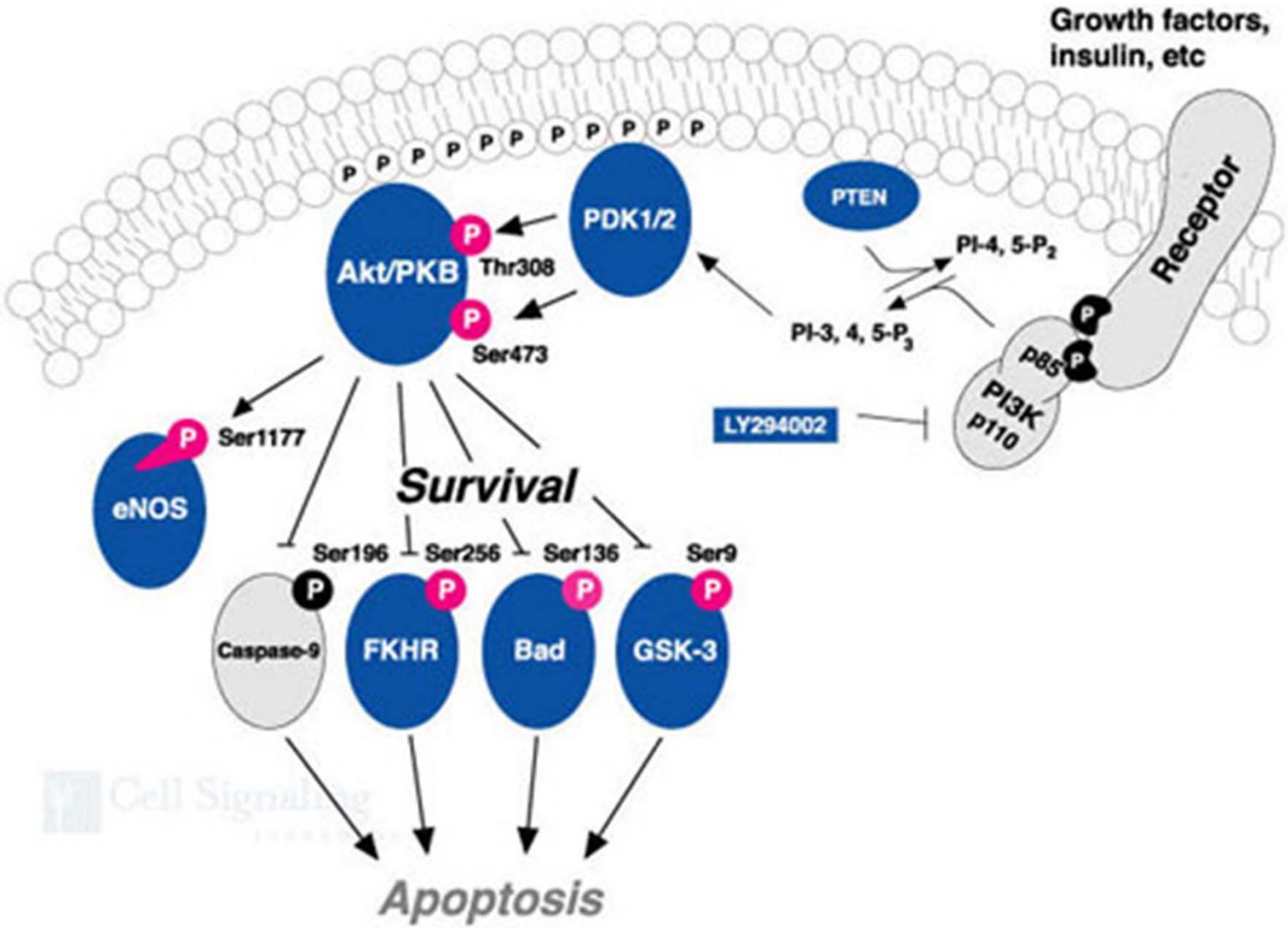
Use of Novel Protein Array Technology: Signal Pathway Profiling in Human Breast Cancer Biopsy Specimens

Coupling Laser Capture Microdissection With True Signal Pathway Profiling

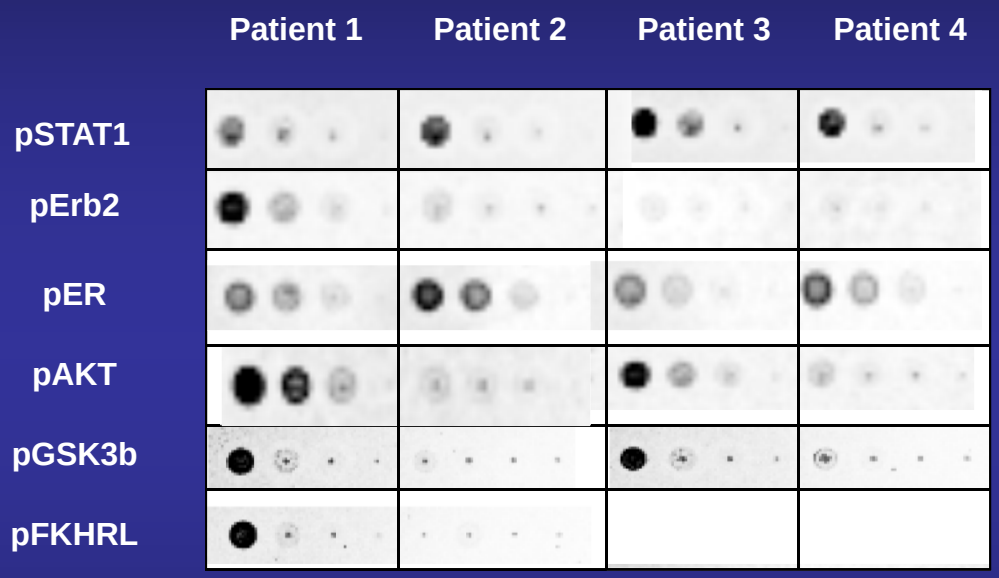


Ongoing work: cluster analysis with 135 phospho-specific endpoints, all normalized to the self protein for true signal pathway profiling


Phospho-AKT (ser 473)
TOTAL AKT
Discordant activation
Phospho-ERB2
Total ERB2
Concordant activation

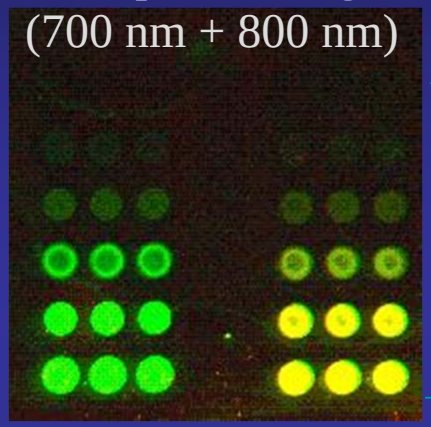

Growth factors, insulin, etc
Receptor
PTEN
PDK1/2
Akt/PKB
Thr308
Ser473
PI-4, 5-P2
PI-3, 4, 5-P3
p85
PI3K
p110
LY294002
eNOS
Ser1177
Survival
Ser196
Ser256
Ser136
Ser9
Caspase-9
FKHR
Bad
GSK-3
Apoptosis

Mapping Signal Transduction Pathways Using Reverse Phase Protein Microarrays



FasL Stimulation of Jurkat Cells & EGF stimulation of CCL250 Cells

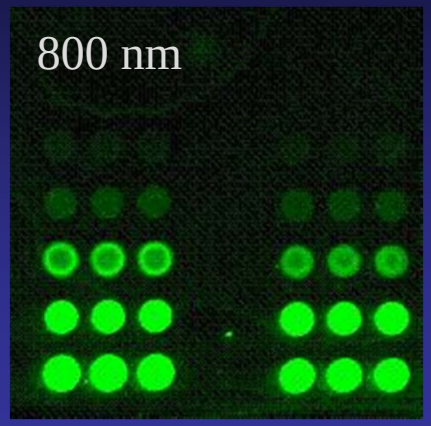
Composite Image
(700 nm + 800 nm)



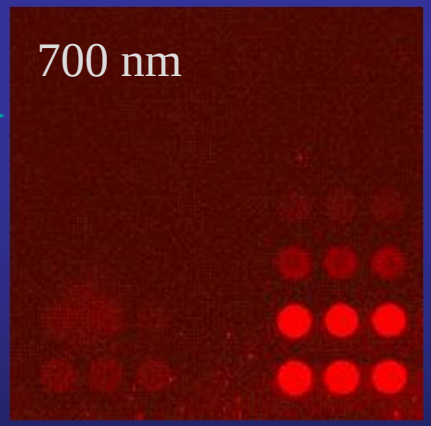
FasL-Stimulated Jurkat

EGF-Stimulated CCL250

Total ERK



P-ERK



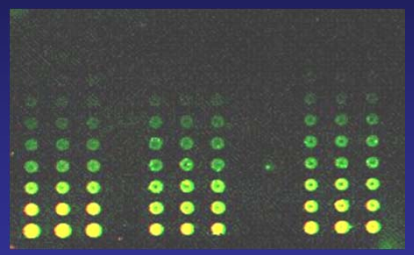
FasL-Stimulated Jurkat

EGF-Stimulated CCL250

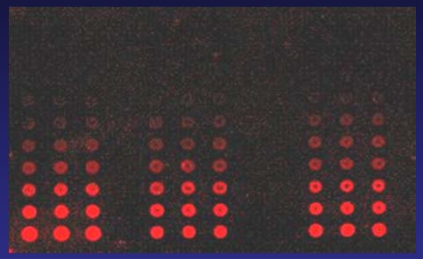
P-ERK/ERK in EGF-Treated CCL250 Cells

Dilution	CCL250
1/32	0.30
1/8	0.31
1/2	0.33
Neat	0.33

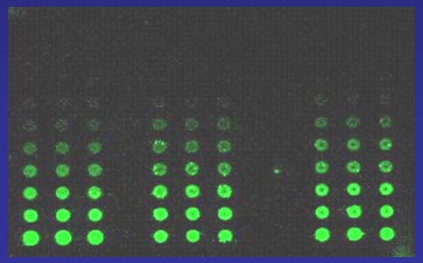
Two color ratiometric analysis of phospho- Stat-1 in breast tumors



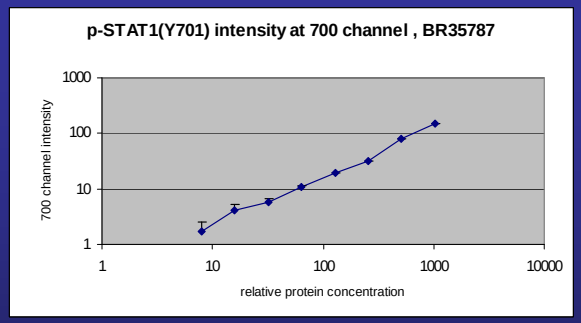
BR35787 BR35929 BR36015

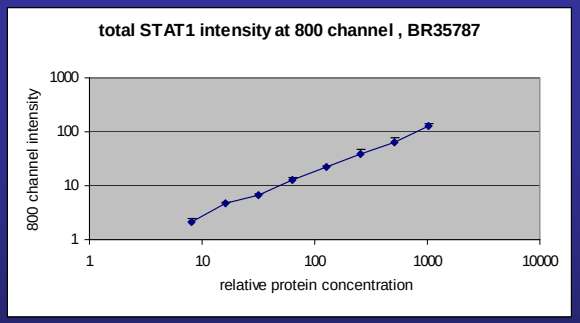


Phospho-Stat1

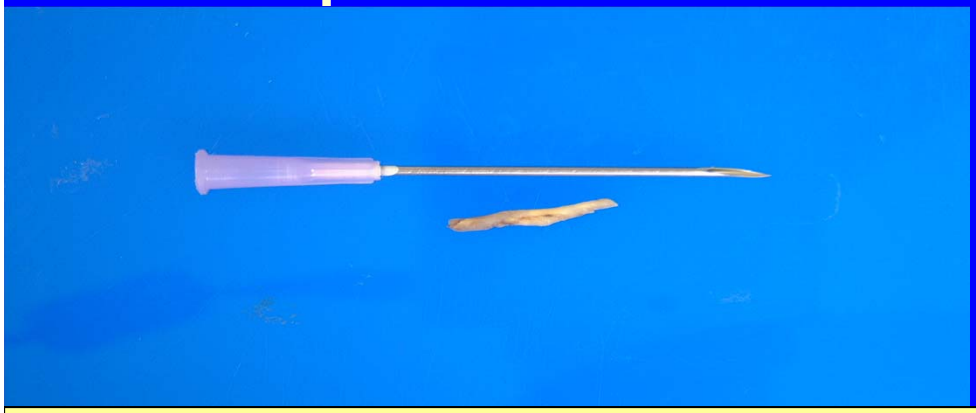


Total Stat1





Specimen/Pure Cell Procurement



2 cm x 0.5 cm tissue

10^9 cells in tissue section

10,000 tumor cells

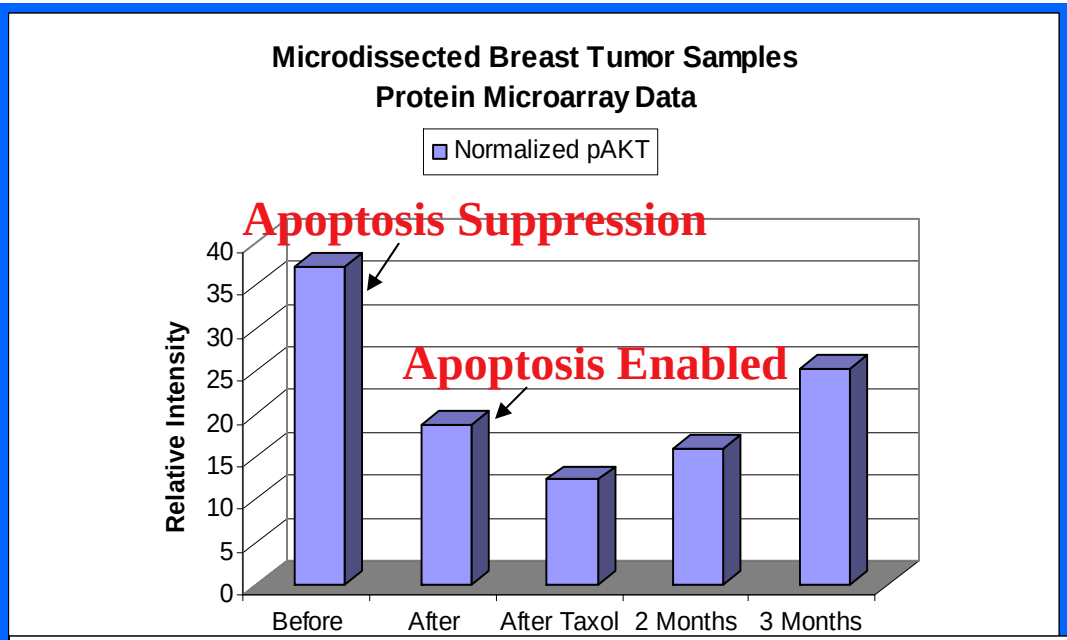
$$T = \frac{(A*s)}{x}$$

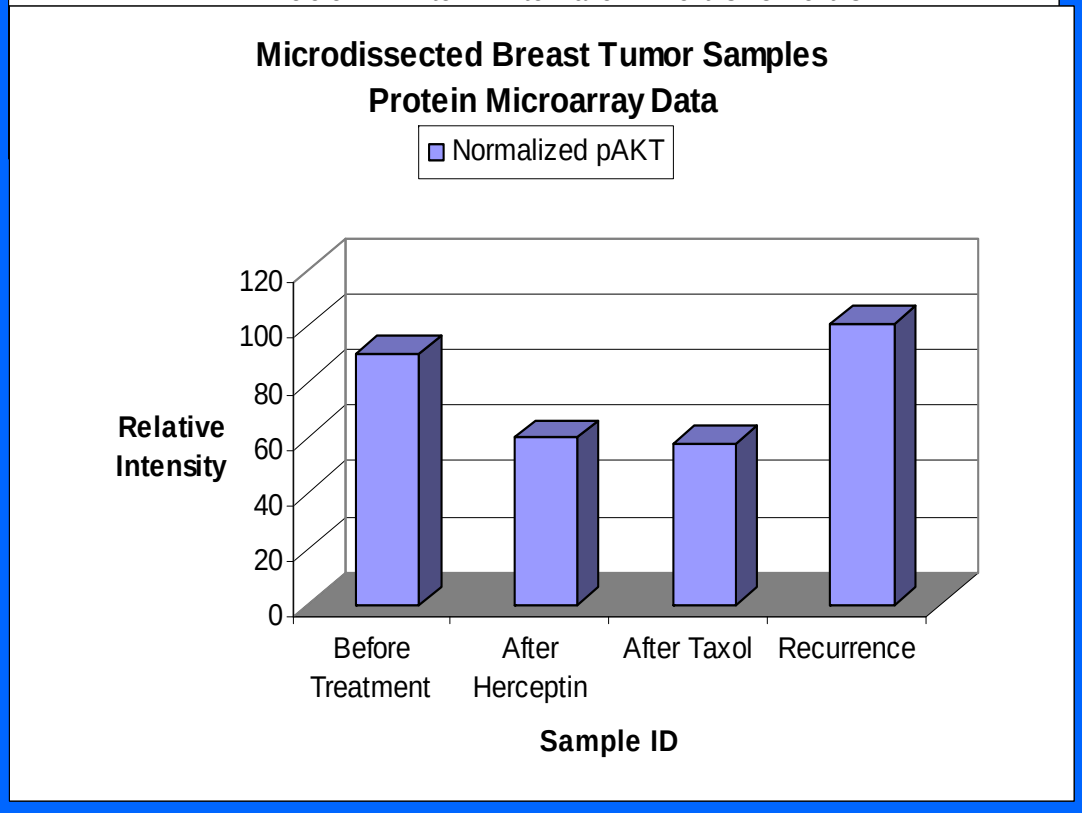
T = threshold for cell procurement per volume (cells/volume)

s = sensitivity of detection system (moles/volume)

A = Avogadro's number ($6.02*10^{23}$ molecules/mole)

x = # of analyte molecules per cell (molecules/cell)





CLINICAL TRIAL

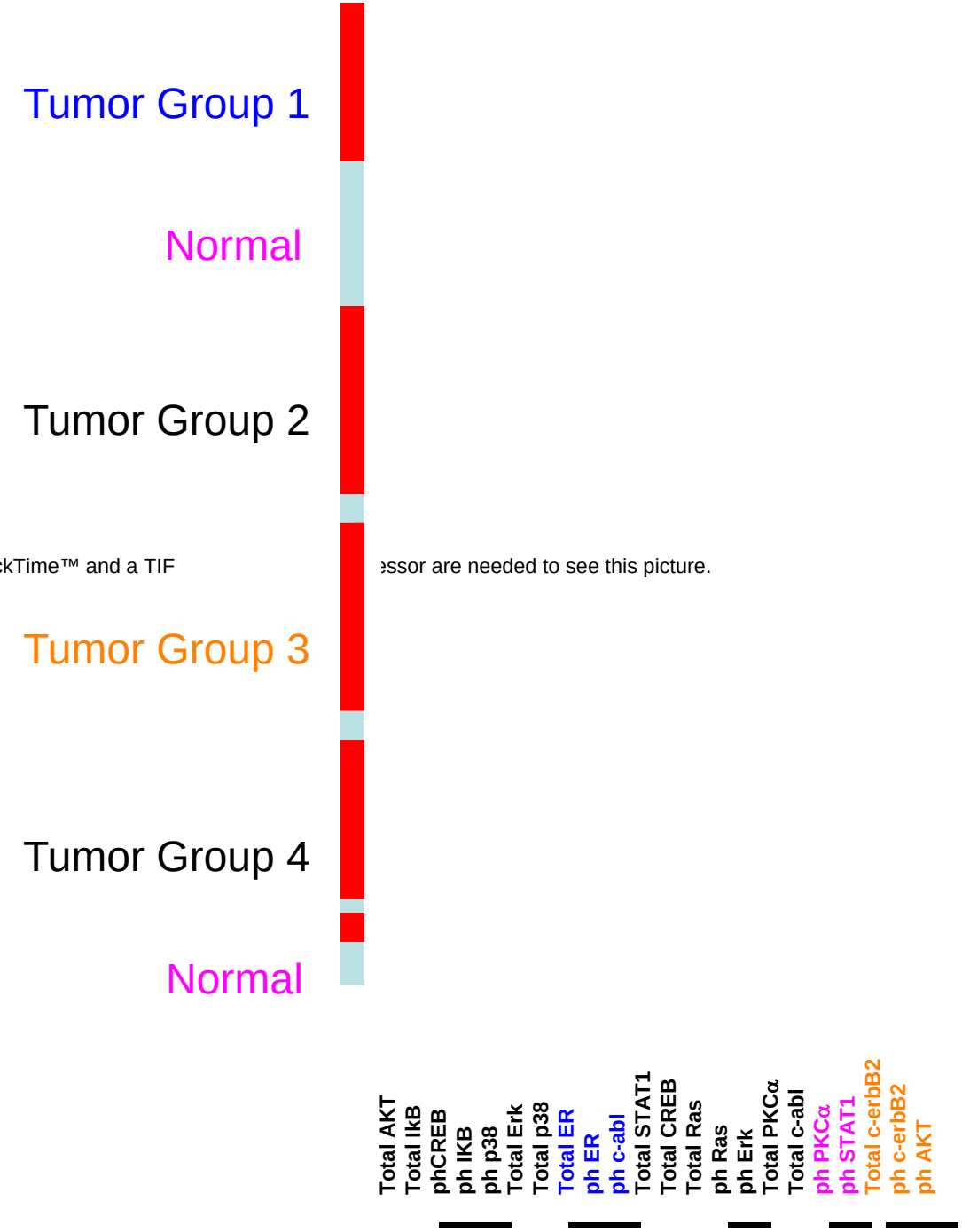
- Metastatic Breast and Ovarian Ca
- Therapy = Herceptin followed by Taxol
- Findings to date N=9

HERCEPTIN SUPPRESSES pAKT.
Virginia Espina

Hypothesis

Increased sensitivity to apoptosis inducing therapy (e.g. Taxol)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

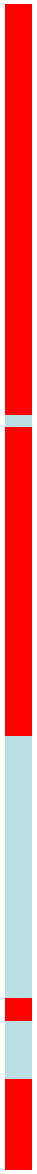


QuickTime™ and a TIF essor are needed to see this picture.

Principal Component Analysis
Of Human Breast Cancer
Phosphoproteome

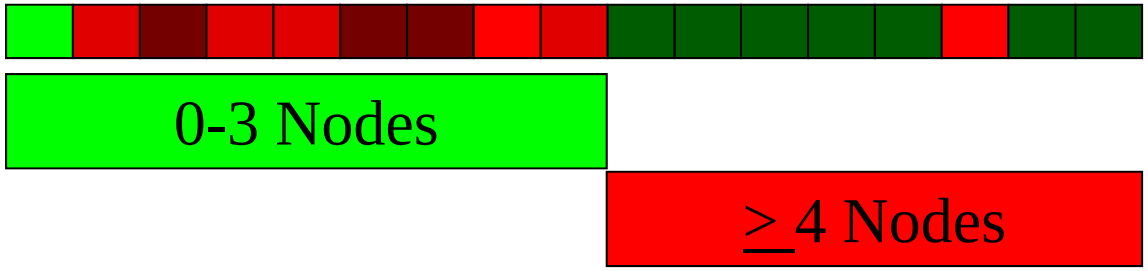
43/45 Cancer (96%)
15/16 Normal (94%)

Discriminated by a combination of phospho PKCα and phospho STAT1 levels



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Relative pPKCα



SIGNAL PATHWAY ANALYSIS OF HUMAN OVARIAN CANCER

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

ROADBLOCKS TO SUCCESS:

ANTIBODIES:
COLLABORATION WITH
BD TRANSDUCTION LABS

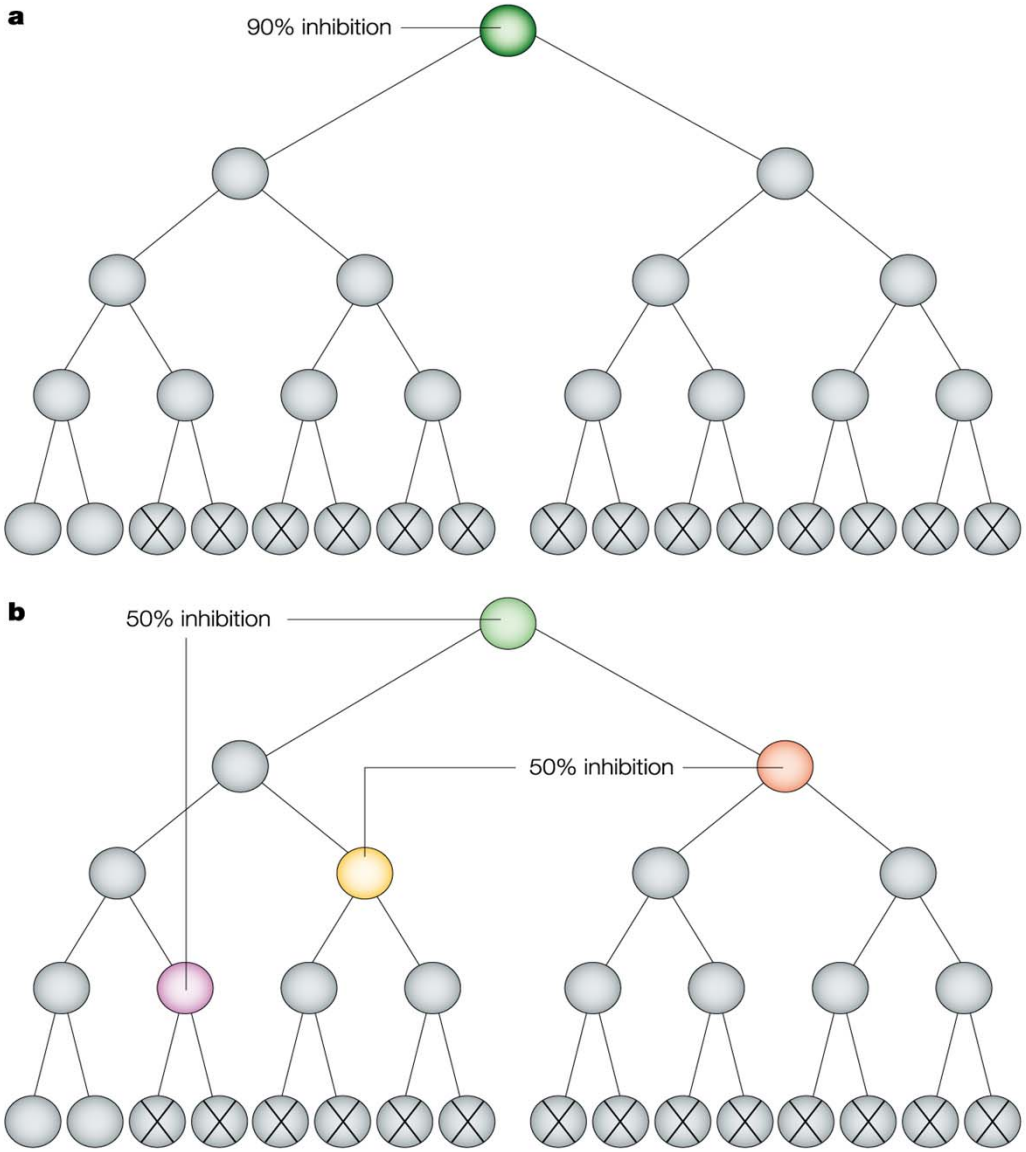
REFERENCE STANDARD:
- NIST
- BD TRANSDUCTION LAB

PLATFORM DEVELOPMENT:

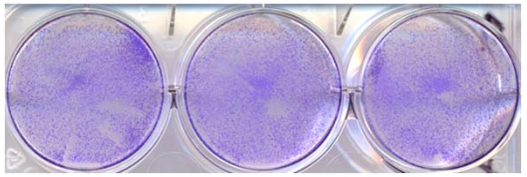
1. 3M
2. SCHLEICHER AND SCHUELL
3. DAKO
4. LICOR
5. Q-DOT
6. BIOFORCE
7. AFFYMETRIX

NOVEL INHIBITORS:

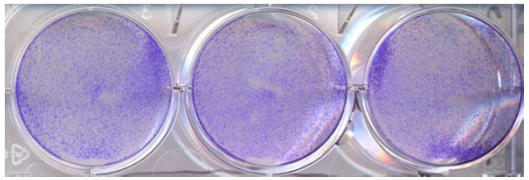
1. GSK
2. GENENTECH
3. AMGEN
4. BAYER
5. ELI LILLY
6. AVENTIS
7. NOVARTIS
8. MERCK



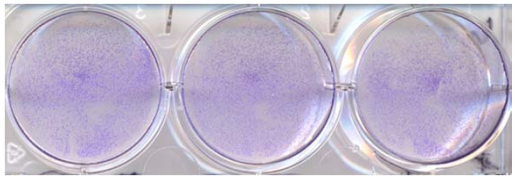
Nature Reviews | Drug Discovery
September, 2002



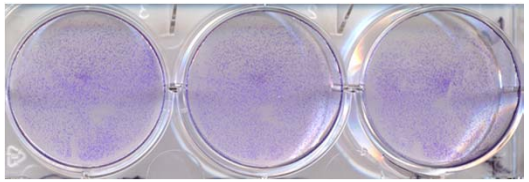
Media only



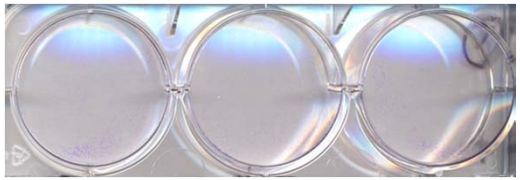
Media + EGf



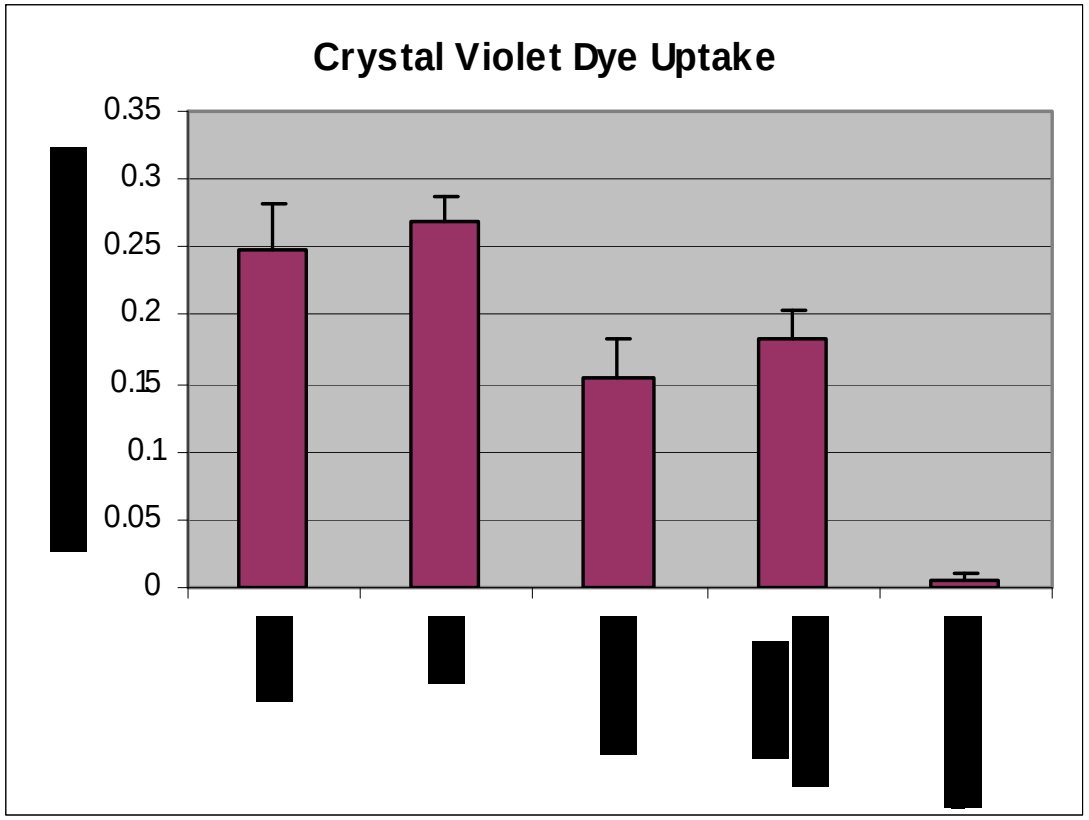
Media + EGF + CAI



Media + EGF + Cox II Inhibitor



Media + EGF + Combination



Cell growth is assessed by uptake of Crystal Violet dye in colon cells grown for 10 days with and without inhibitors. Profound inhibition of growth is seen in the CAI / COX-2 inhibitor treated cells..

Synergistic Pathway Therapy Colony Growth Assay

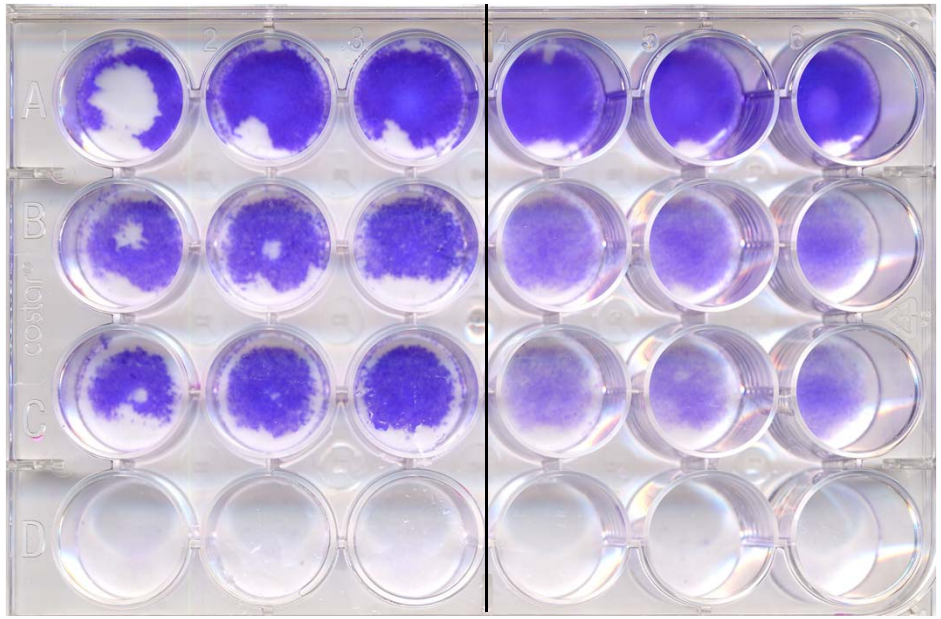
PANC-1 HT1080 ras +

Vehicle Control

CAI **Inhib NVG Ca Chnl IC50**

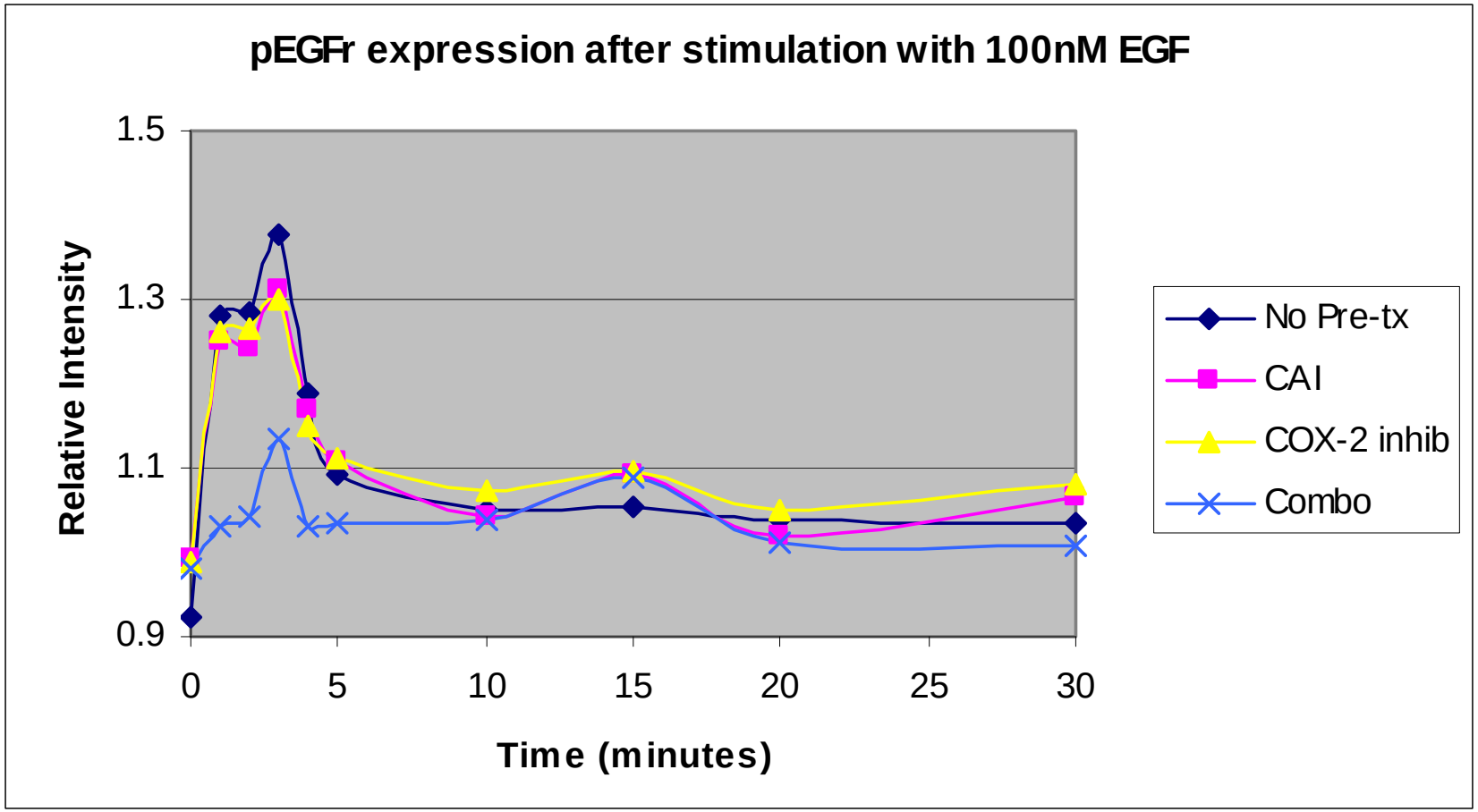
COX-2 **Inhib (LM1685) IC50**

Combination



Synergy of simultaneous inhibition of linked pathways
Pathway 1: COX-2 PGE2 pathway and
Pathway 2: Non voltage-gated calcium channel pathway

Combination therapy suppresses EGFr activation



CCL- 250 colon cancer cells were pretreated for 1 hour with the CAI / COX-2 inhibitor combination or either drug alone before stimulation with EGF. The combination shows dramatically less EGFr activation than either drug alone.

Clinical Proteomic Trials at the NCI Center for Cancer Research

Protein array based trials open to accrual:

- **Phase II Proteomic profiling of ovarian cancer patients during first clinical response (Elise Kohn):**

  Reverse Phase Multiplexed Phosphoprotein Array

  Serum Proteomic Pattern Analysis

- **Phase II Herceptin/Taxol combination therapy study for breast and ovarian cancer (Susan Bates):**

  Reverse Phase Multiplexed Phosphoprotein Array

- **Phase I and II EGFR inhibitors and Gleevec study for breast and ovarian cancer (Elise Kohn):**

  Reverse Phase Multiplexed Phosphoprotein Array

PLAN:
INCORPORATE KINASE SUBSTRATE PROFILING USING REVERSE PHASE PROTEIN MICROARRAYS INTO ALL CCR BASED TARGETED THERAPY TRIALS

FDA-NCI CLINICAL PROTEOMICS PROGRAM

SCIENTIFIC TEAM AND COLLABORATORS

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Correlogic Systems, Inc.

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Sally Ross

Julie Wufhuhle
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Clinical Director

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Director CCR, NCI