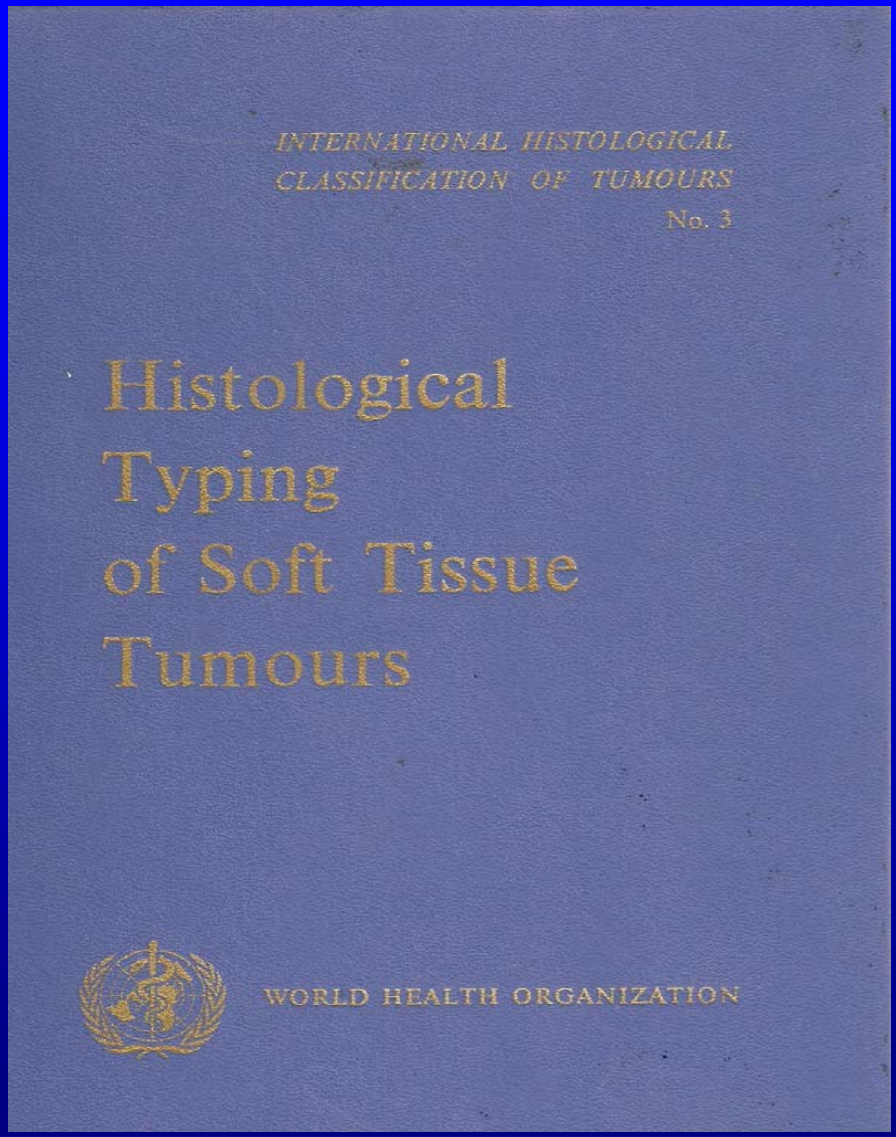
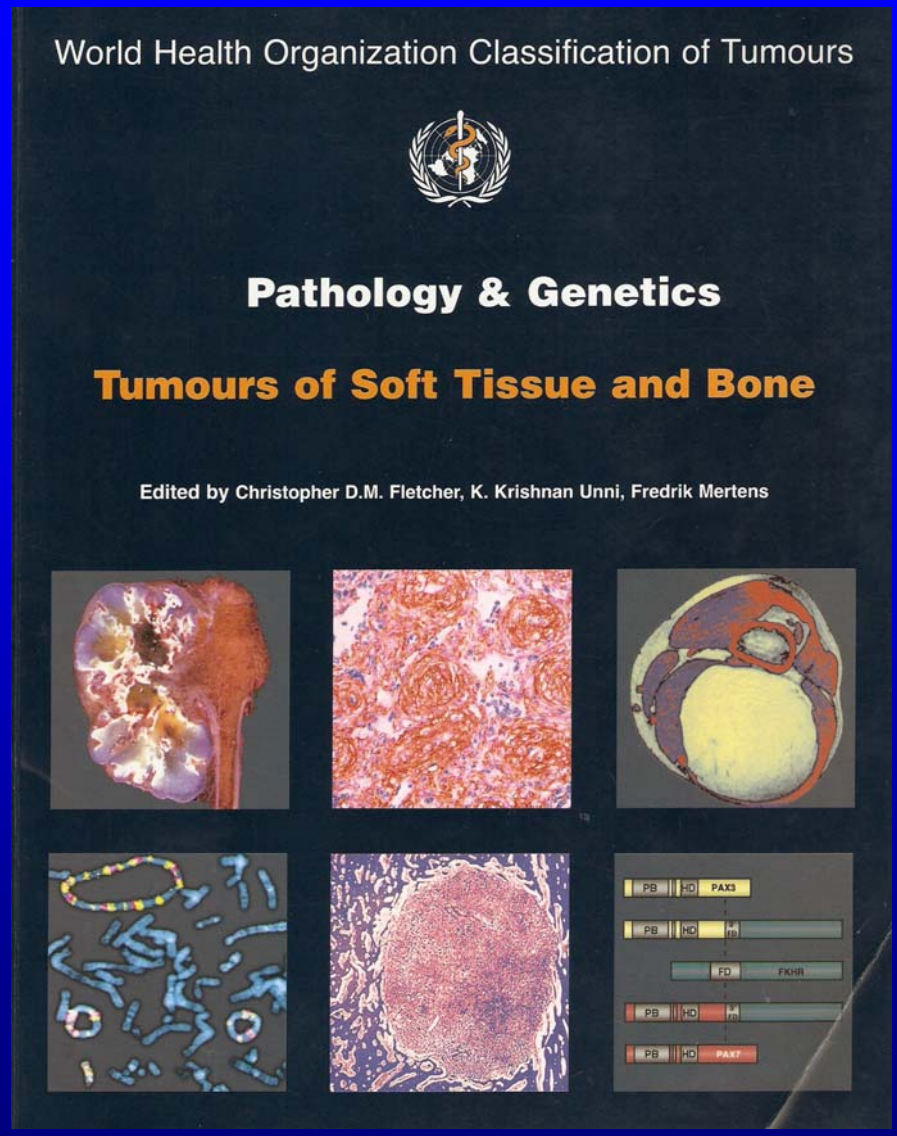
19th Scientific Meeting of
International Academy of Pathology – Arab Division

CELL SURFACE RECEPTOR MUTATIONS - EXPERIENCE AT KFSH&RC

Fouad Al Dayel, MD, FRCPA
Chairman
Department of Pathology and Laboratory Medicine
King Faisal Specialist Hospital and Research Centre
Riyadh, Saudi Arabia


INTERNATIONAL HISTOLOGICAL
CLASSIFICATION OF TUMOURS
No. 3
Histological
Typing
of Soft Tissue
Tumours
WORLD HEALTH ORGANIZATION


World Health Organization Classification of Tumours
Pathology & Genetics
Tumours of Soft Tissue and Bone
Edited by Christopher D.M. Fletcher, K. Krishnan Unni, Fredrik Mertens
PB
HD
PAX3
FD
FKHR
PAX7

INTERNATIONAL HISTOLOGICAL CLASSIFICATION OF TUMOURS
No. 15

Histological Typing of Intestinal Tumours

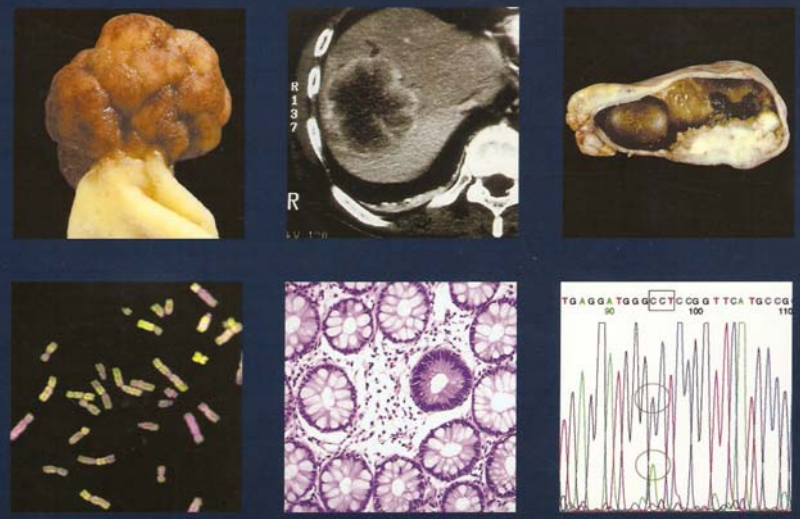
WORLD HEALTH ORGANIZATION

World Health Organization Classification of Tumours

Pathology & Genetics

Tumours of the Digestive System

Edited by Stanley R. Hamilton & Lauri A. Aaltonen

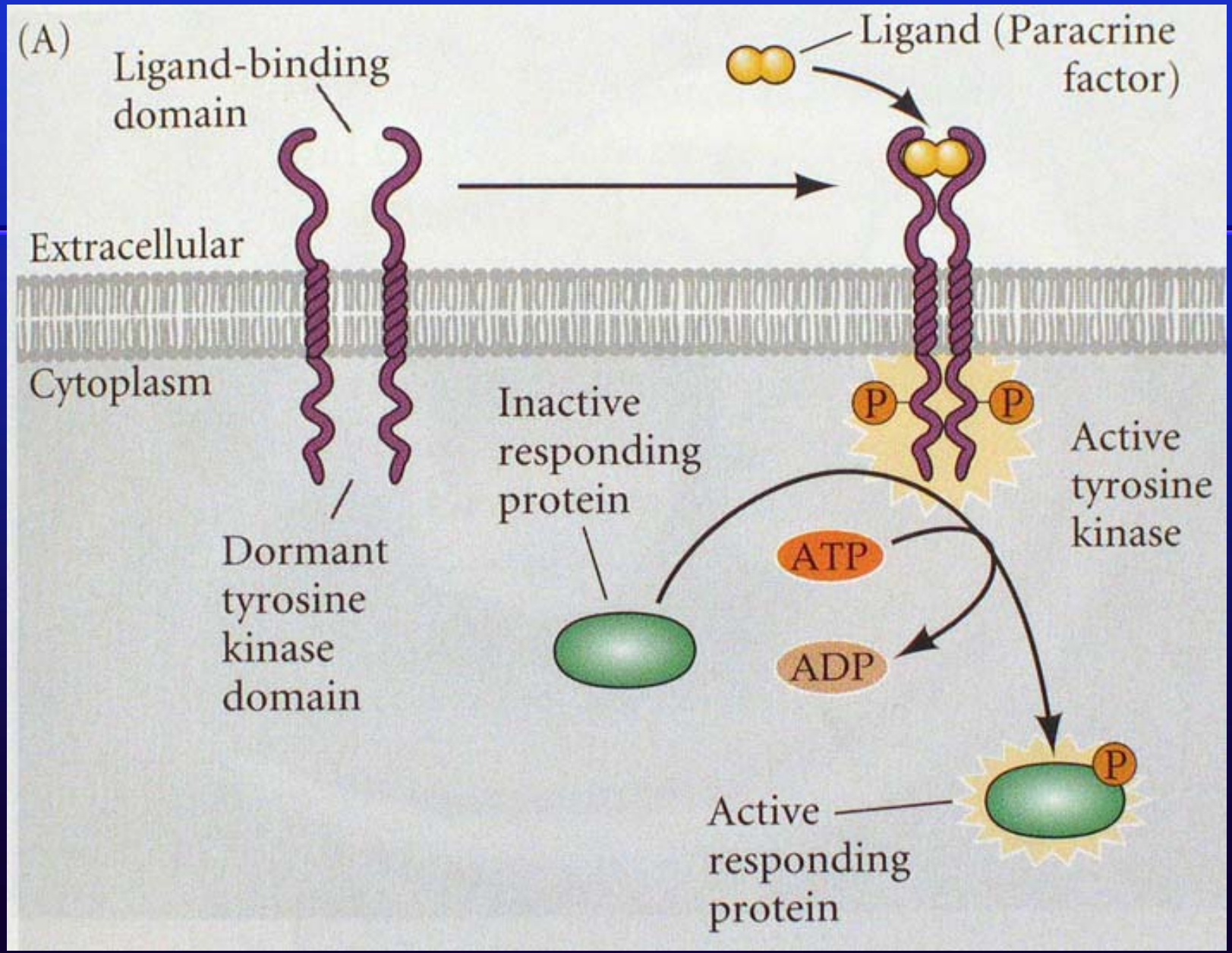


"Molecular Genetic" in Tumor Pathology

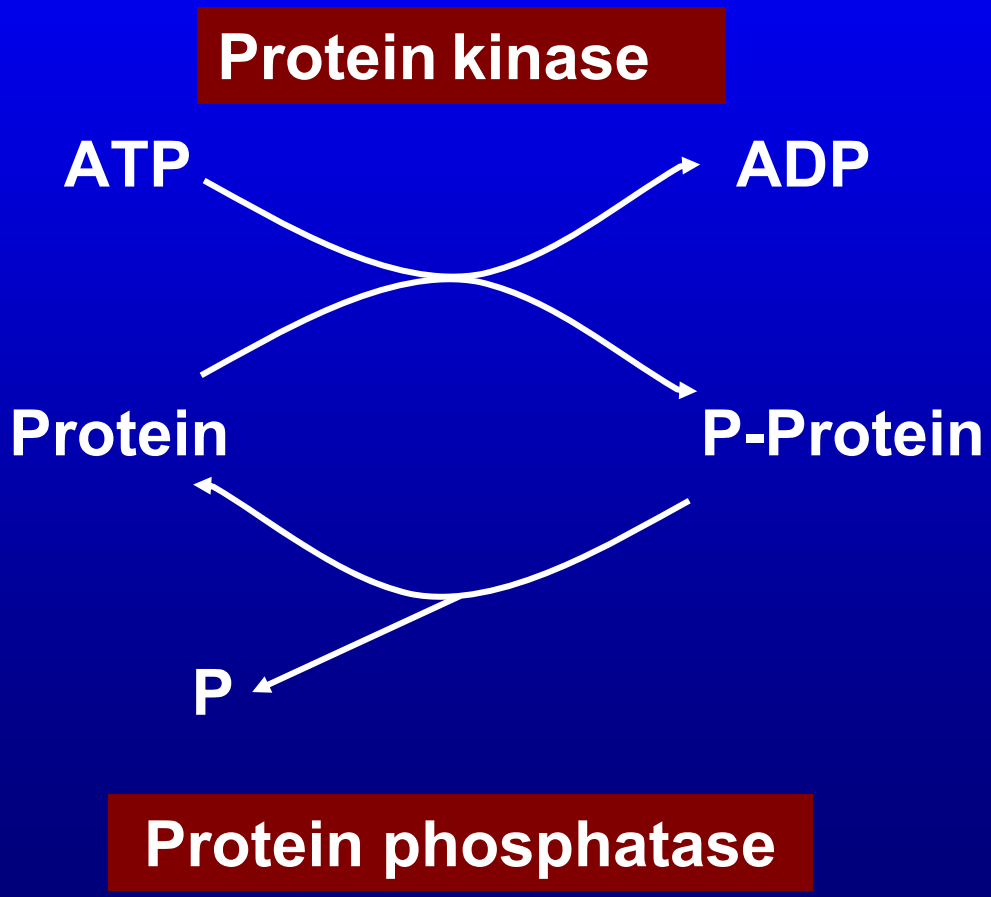
- Diagnosis and prognosis
- Molecular classification of tumors
  - Breast carcinoma
  - Lymphoma
  - Sarcomas
- Targeted therapy

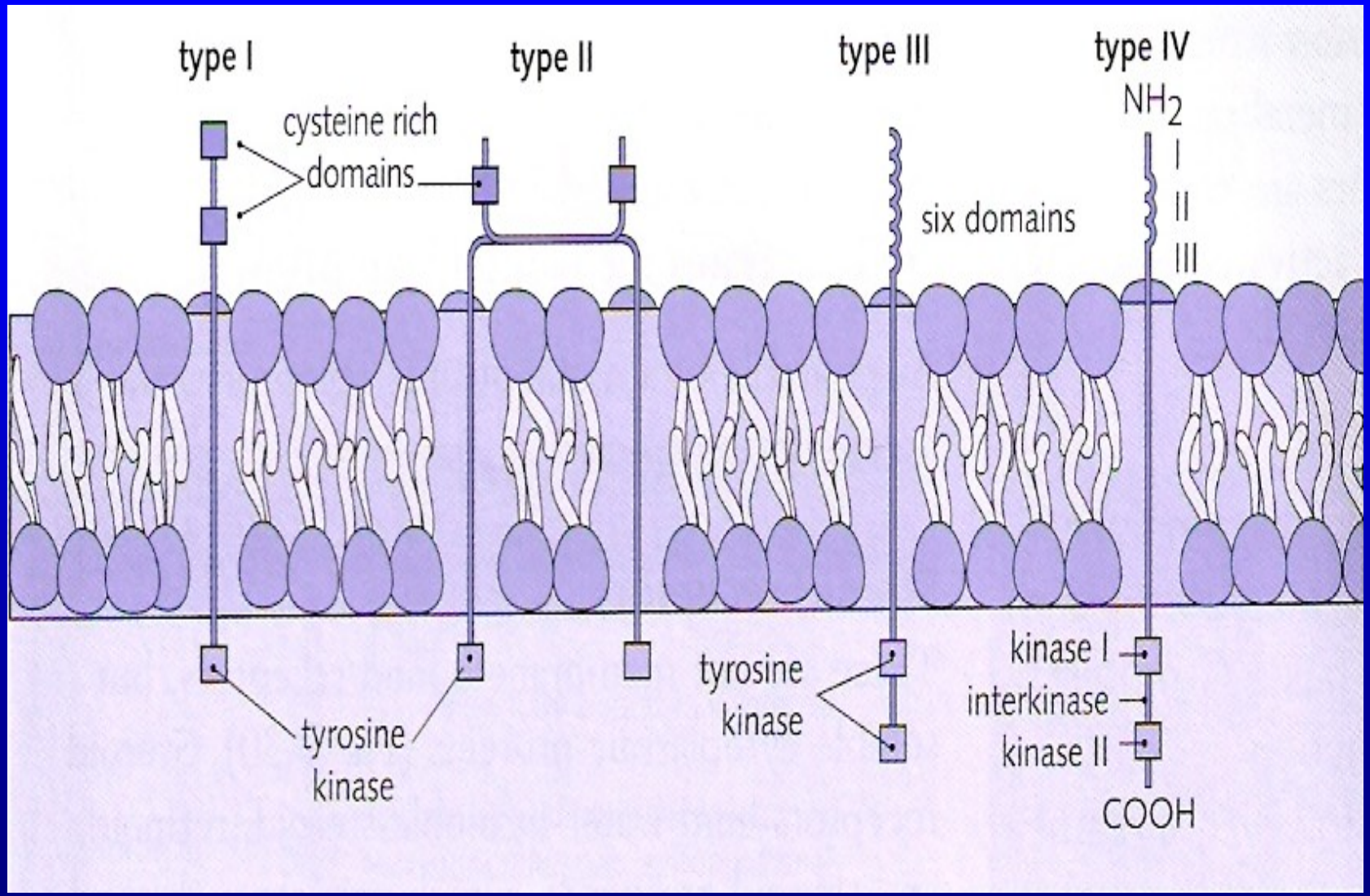
Protein phosphorylation is a fundamental cellular activity

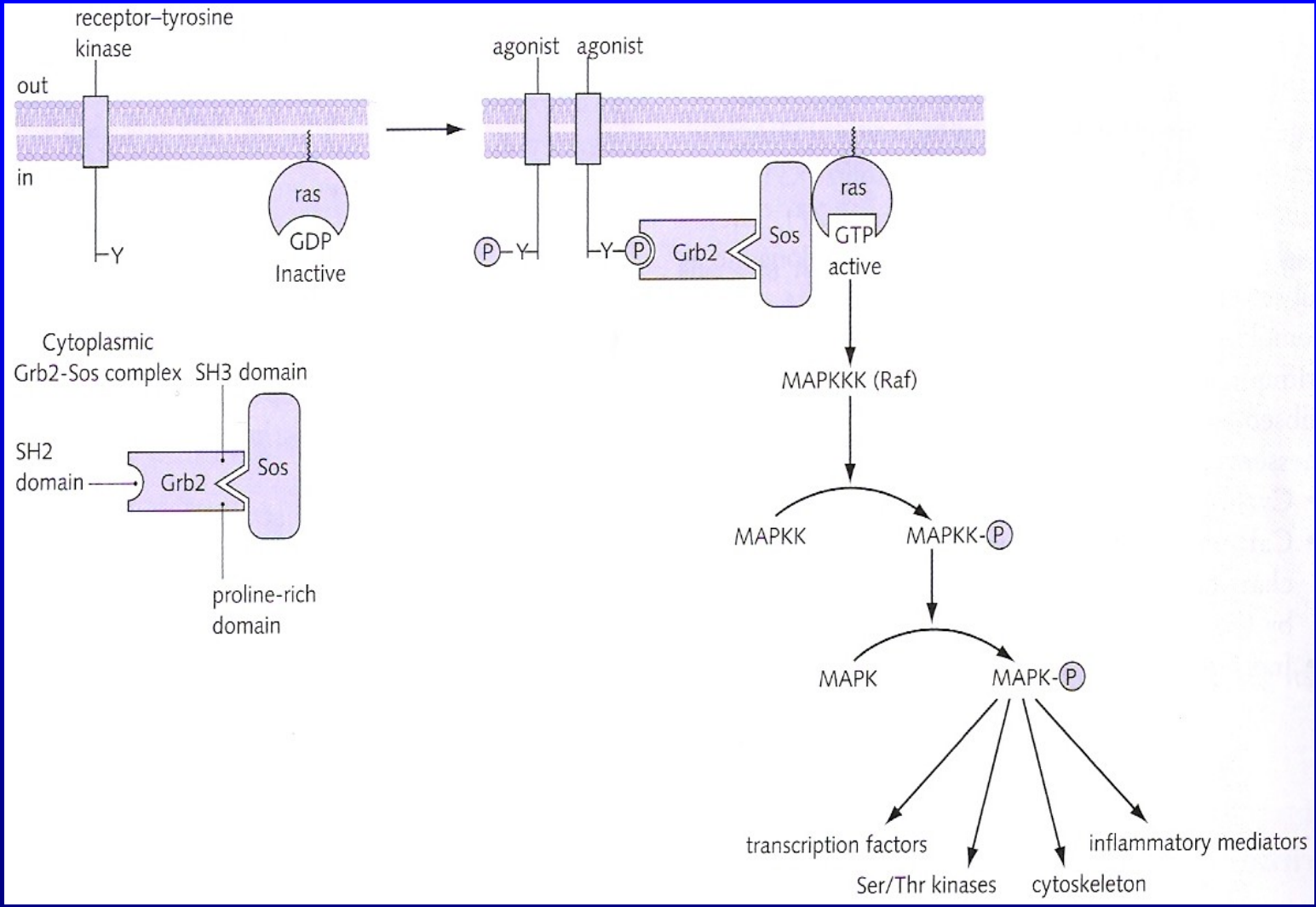
- There are 518 protein kinase genes, of which about 450 have kinase activity and 90 are tyrosine kinases. This represents about 2% of all human genes
- There are more than 140 protein phosphatases, and a large number of additional types of protein that recognize proteins once they are phosphorylated


(A)
Ligand-binding domain
Ligand (Paracrine factor)
Extracellular
Cytoplasm
P
P
Inactive responding protein
Active tyrosine kinase
ATP
ADP
Dormant tyrosine kinase domain
P
Active responding protein

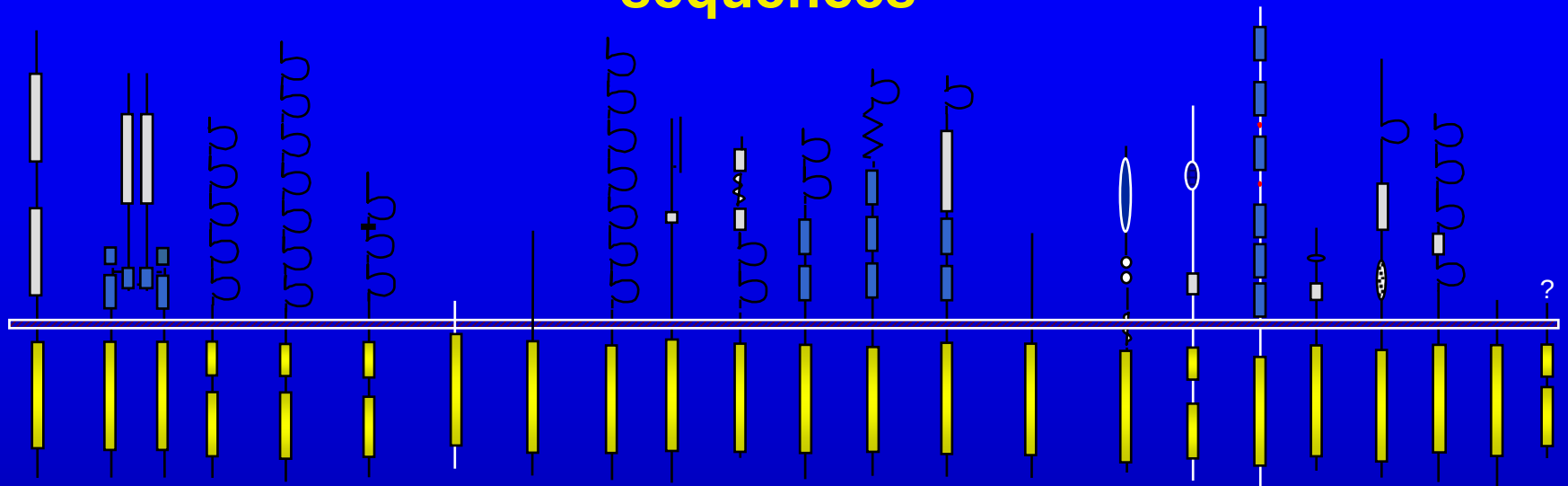
Protein phosphorylation/dephosphorylation provides a major mechanism for signal transduction




type I
type II
type III
type IV
NH2
cysteine rich
domains
six domains
I
II
III
tyrosine
kinase
tyrosine
kinase
kinase I
interkinase
kinase II
COOH


receptor–tyrosine kinase
out
in
Y
ras
GDP
Inactive
agonist
agonist
P
Y
Y
P
Grb2
Sos
ras
GTP
active
MAPKKK (Raf)
MAPKK
MAPKK-P
MAPK
MAPK-P
transcription factors
Ser/Thr kinases
cytoskeleton
inflammatory mediators
Cytoplasmic Grb2-Sos complex
SH3 domain
SH2 domain
Grb2
Sos
proline-rich domain

Receptor tyrosine kinases are grouped based on specific extracellular structural motifs as well as related kinase sequences



EGFR
HER2
HER3
HER4

INSR
IGF1R
IRR

PDGFRα
PDGFRβ
CSF1R
KIT
FLK2

FLT1
FLK1
FLT4

FGFR1
FGFR2
FGFR3
FGFR4

CCK4

MET
RON

TRKA
TRKB
TRKC

AXL
MER
SKY

TIE
TEK

EphA1
EphA2
EphA3
EphA4
EphA5
EphA6
EphA7
EphA8
EphB1
EphB2
EphB3
EphB4
EphB5
EphB6

RYK

DDR1
DDR2

RET

ROS

LTK
ALK

ROR1
ROR2

MuSK

RTK106

LMR1
LMR2
LMR3

LET-23 DAF-2

F59F3.1 EGL-15
F59F3.5
F40G9.13

KIN15
KIN16
TKR-1
C08H9.8
F59F5.3
M01B2.1
R09D1.12
R09D1.13

T01G5.1
T17A3.8
W04G5.6N
W04G5.6C
Y50D4B-4
ZK938.5
B0198.3
F54F7.5

VAB-1

C16B8.1

F11D5.3
C25F6.4

C16D9.2
T10H9.2

CAM-1

Human RTKs
58 (20 classes)

Worm RTKs
29 (11 classes)
11 Unclassified

(B0252.1, F11E6.8, F40A3.5, R151.4, T148.1, T22B11.3
Y38H6C.20, C24G6.2A, F08F1.1, F09A5.2, F09G2.1)

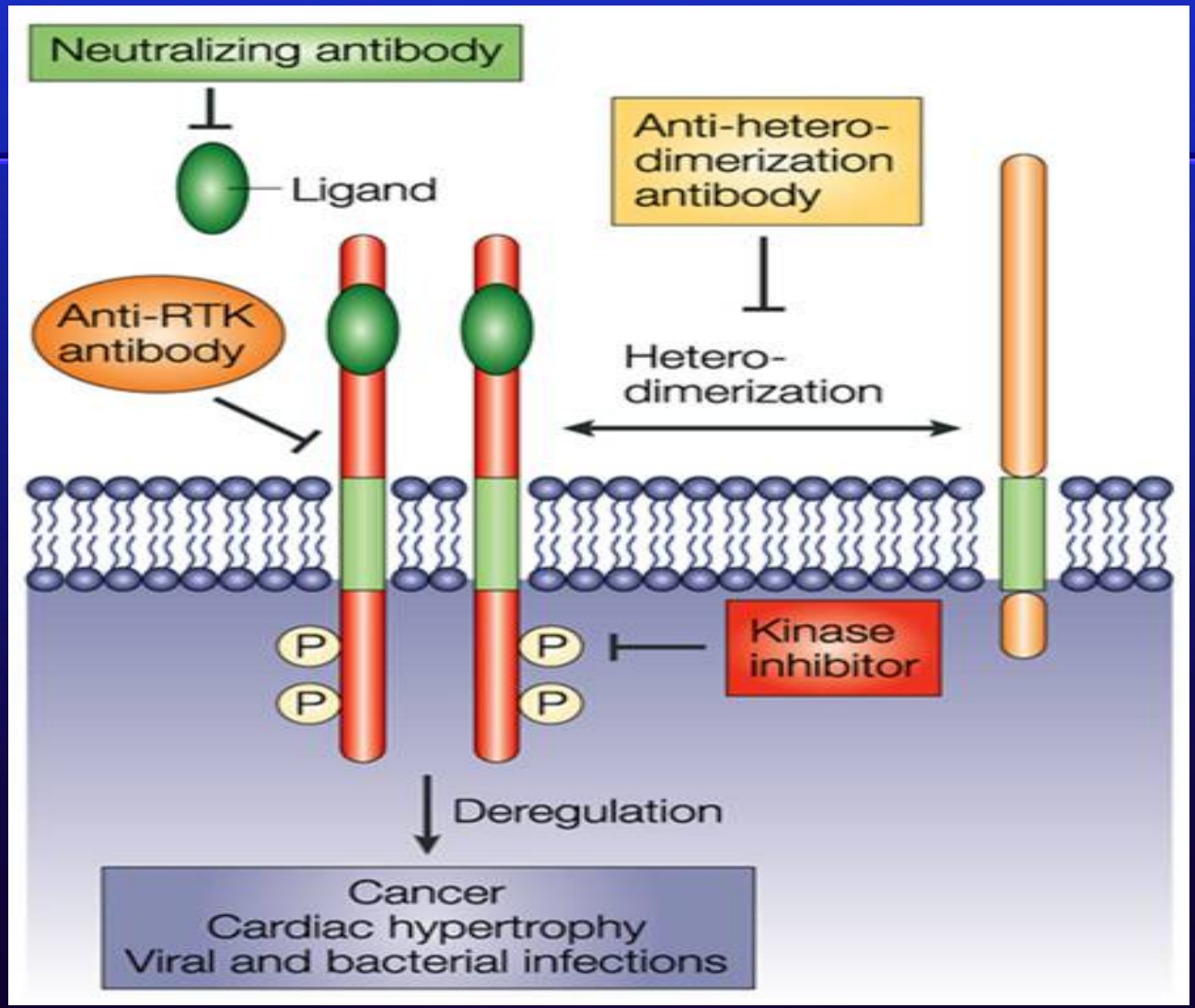
What is tyrosine phosphorylation used for?

1. Growth factor signaling (and oncogenesis)
2. Cell adhesion, spreading, migration and shape
3. Cell differentiation in development
4. Cell cycle control
5. Gene regulation and transcription
6. Endocytosis and exocytosis
7. Insulin stimulation of glucose uptake
8. Angiogenesis (formation of new blood vessels)
9. Regulation of ion channels in nerve transmission

Protein Kinases/Phosphatases and Cancer

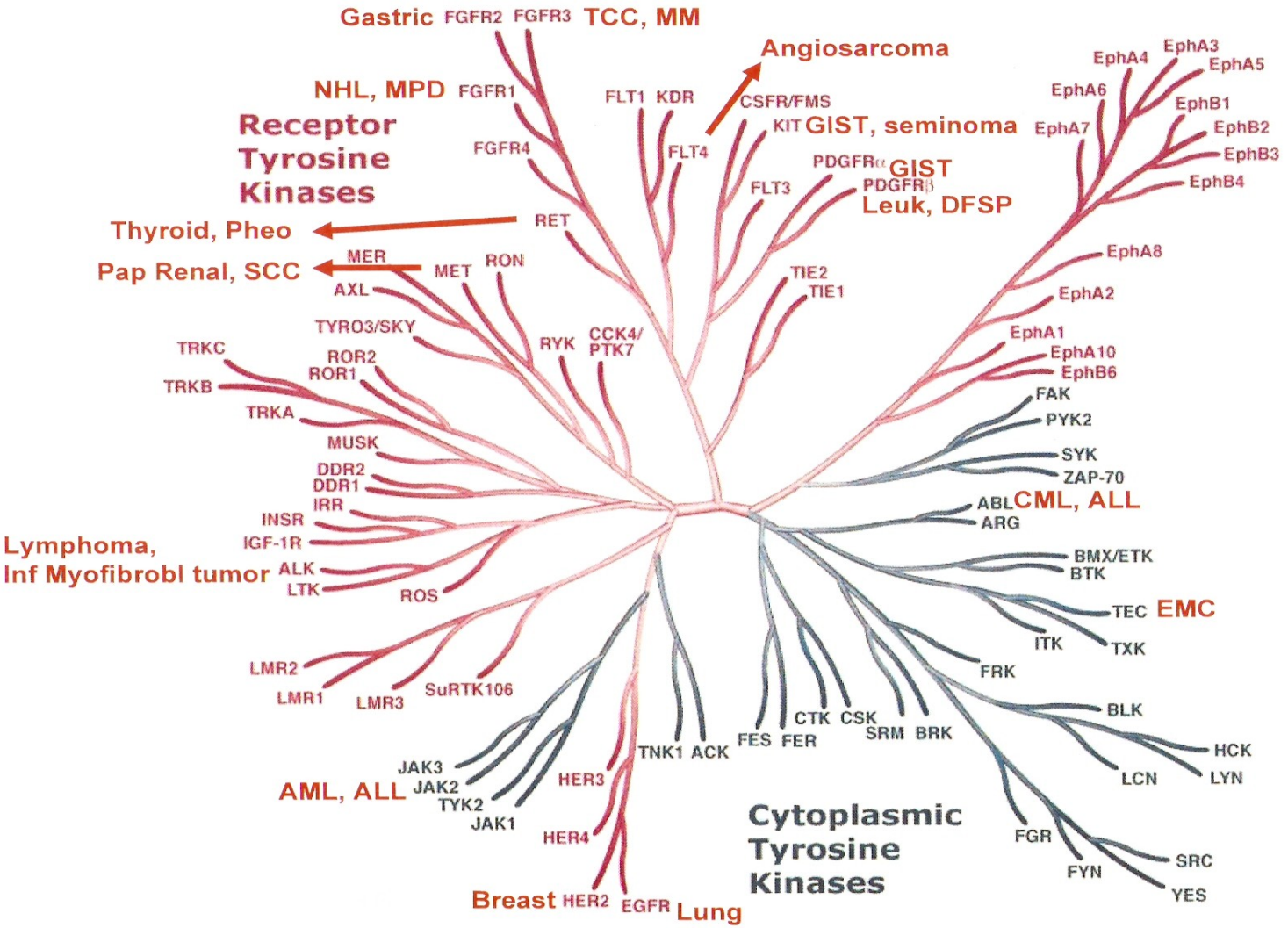
- Over half of the 90 tyrosine kinases are implicated in human cancer either through gain of function mutations (e.g. Bcr-Abl), gene amplification (e.g. EGF receptor) or overexpression (e.g. c-Src)
- Serine kinases are also implicated in cancer through activating mutations (e.g. B-Raf), overexpression (e.g. AuroraA), or loss of function mutations (e.g. Lkb1)
- 164 protein kinase genes map to amplicons found in tumors
- 80 protein kinase genes map to chromosomal disease loci and these are candidate genes for the causative mutation in hereditary disease (e.g. activating mutations in the Ret and Met RTKs in predisposition to cancer)
- Inactivating and activating mutations in protein/lipid phosphatases have also been implicated in cancer (e.g. PTEN, SHP-2, PRL-3, and Pr65/PP2A, PRL-3)(RPTPb, PTP-BAS, PEZ, RPTPg, LAR, PTPH1)

Therapeutic intervention for receptor tyrosine kinases

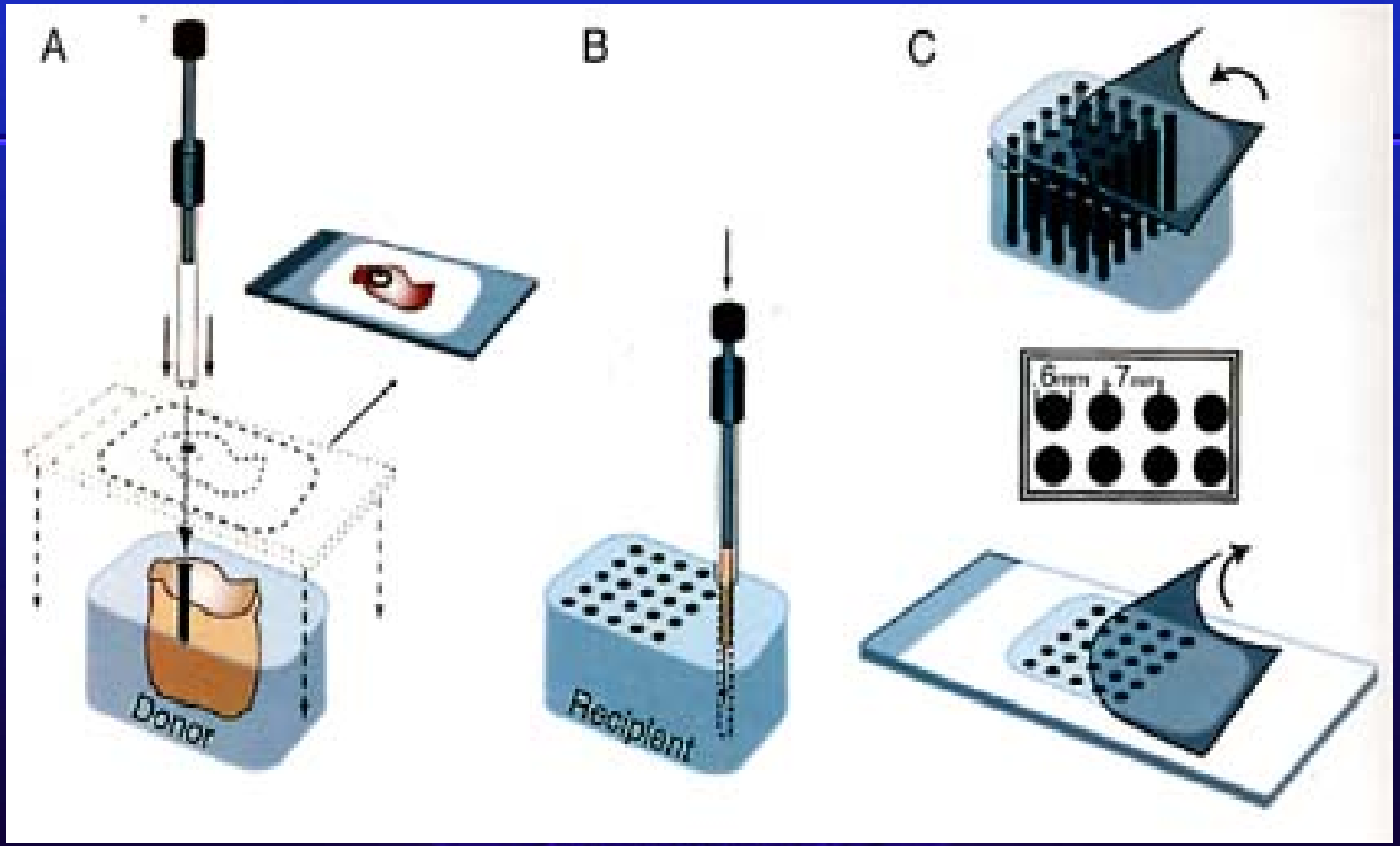


Gschwind et al. Nature Reviews Cancer 4:361

Tyrosine Kinases

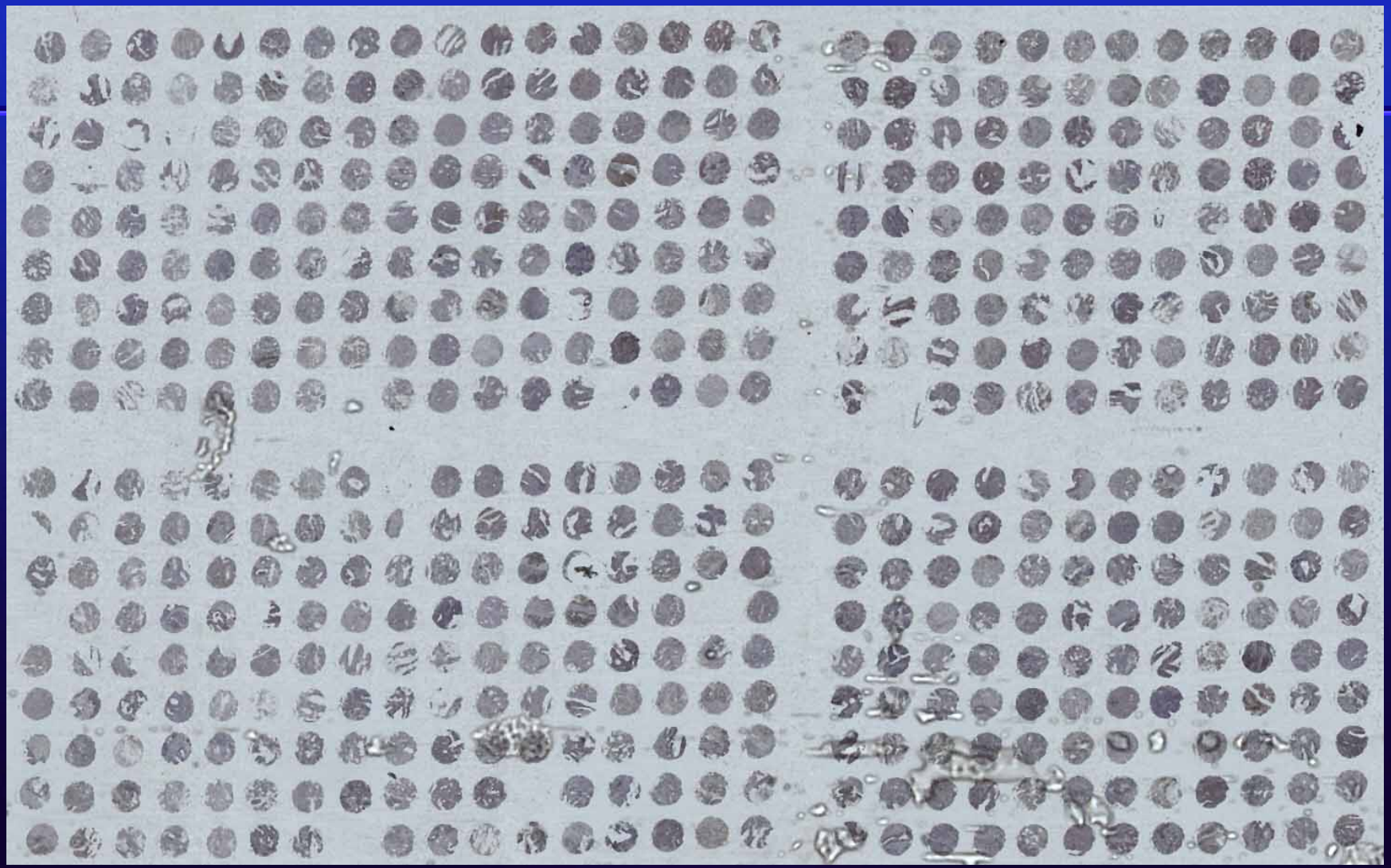


The Tissue Microarray Technology



Kononen et al., Nature Med. 4 (7), 844-847, 1998

Manufacturing TMAs



Epidermal Growth Factor Receptor (EGFR)

- EGFR (ErbB1, ErbB, HER1)

3 known homulogues (forms heterodimers)

- ErbB2 (Neu, HER2)
- ErbB3 (HER3)
- ErbB4 (HER4)

Epidermal Growth Factor Receptor (EGFR)

EGFR (after binding to specific ligand)

↓

Activate tyrosine kinase

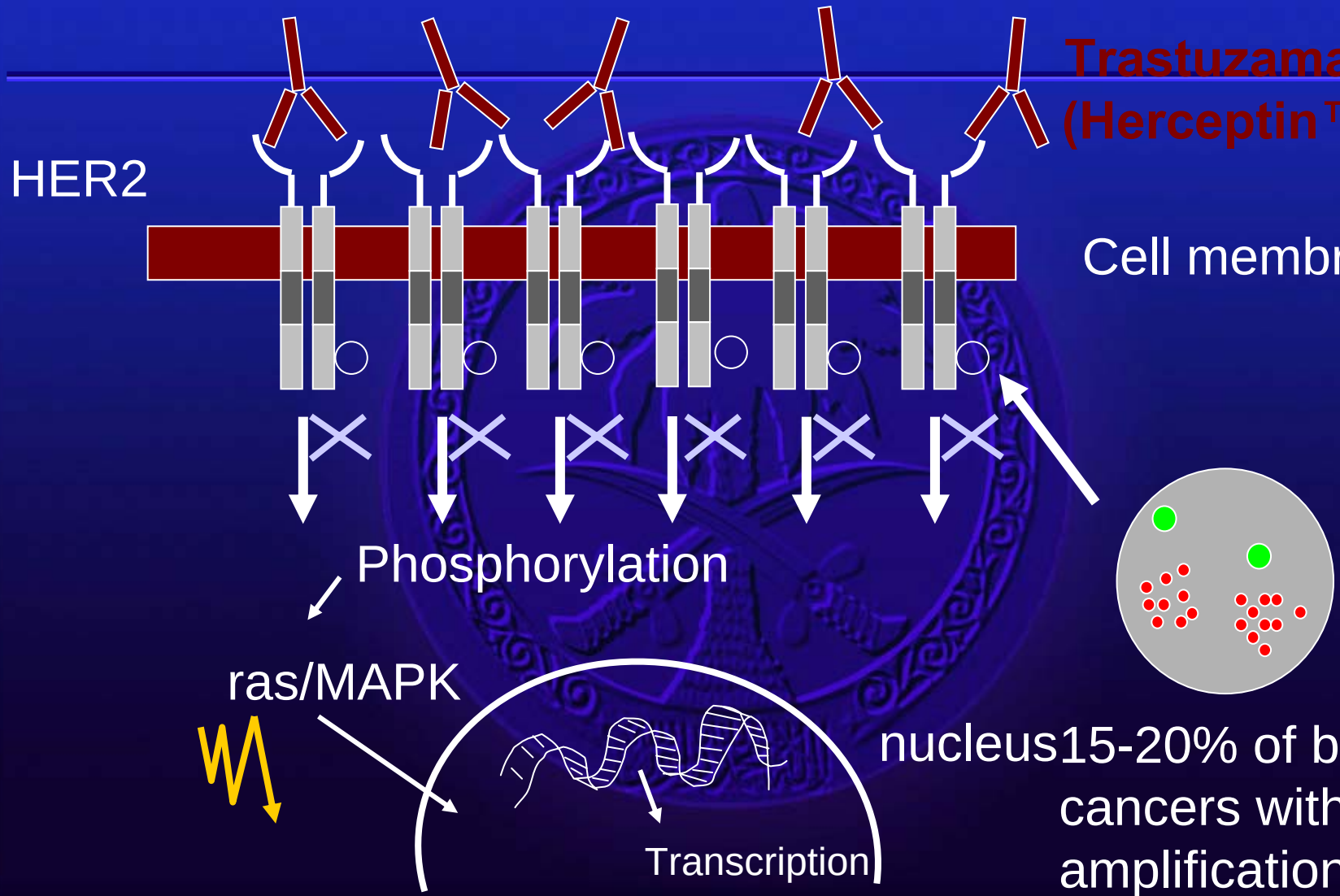
↓

Cell growth and multiplication

Tumor Progression

- ↓ apoptosis
- ↑ angiogenesis
- invasion

HER2


Trastuzamab
(Herceptin™)
HER2
Cell membrane
Phosphorylation
ras/MAPK
Transcription
nucleus15-20% of breast cancers with HER2 amplification

Breast Cancer TMAs

2 Sets of TMA

2197 cases
from Inst. of Clin. Pathology
(Switzerland)

(Median age 62 years)

204 cases from
Aramco Hospital
(Saudi Arabia)

(Median age 47 years)

- *FISH Testing for:*

HER2, CCNDI, MYC,
EGFR Amplification

Modern Pathology, 2005

Breast Cancer TMAs

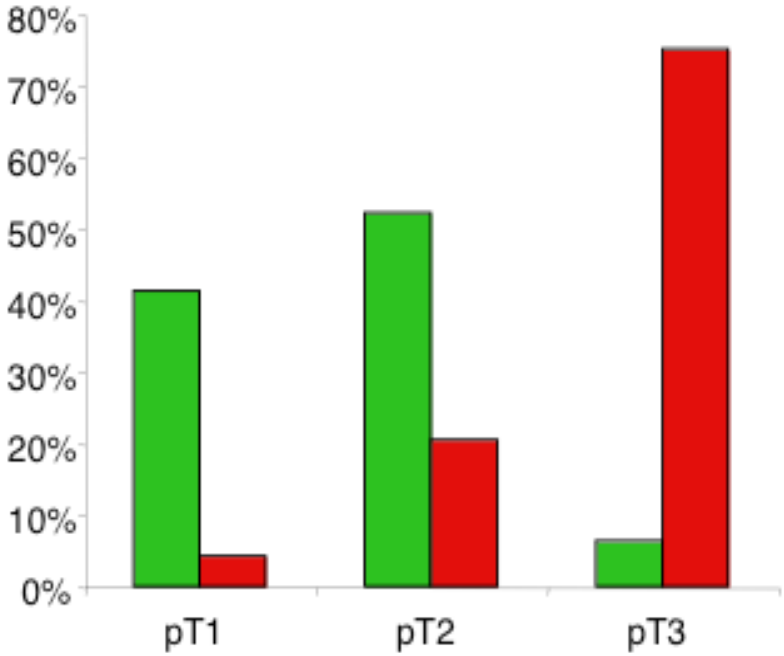
FISH Studies

Probe combinations

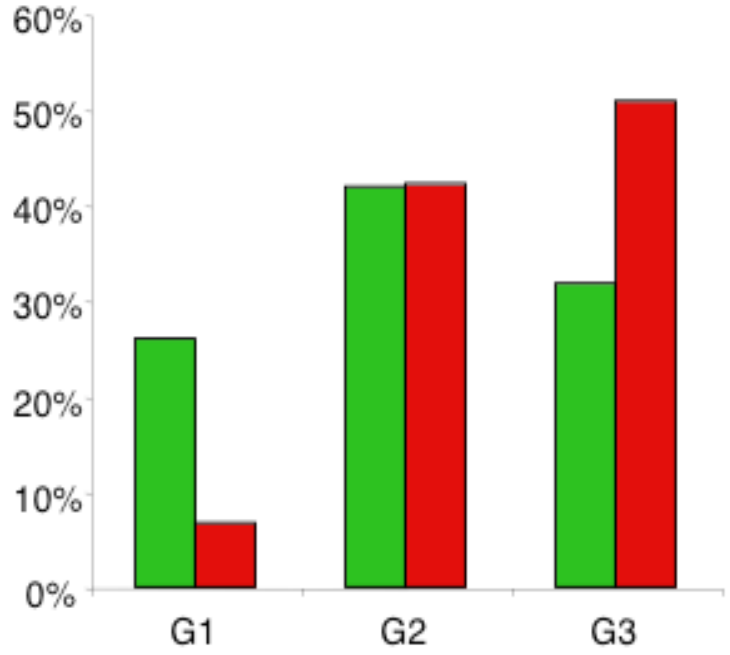
1. ***HER2*/centromere 17 (Path Vysion;Vysis)**
2. ***EGFR*/centromere 7 (Vysis)**
3. ***CCND1*/centromere 11 (Vysis)**
4. ***MYC*/centromere 8 (Vysis)**

Modern Pathology, 2005

Tumor stage

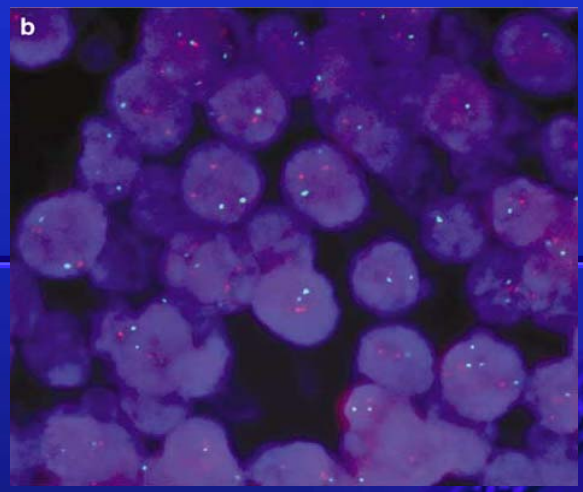


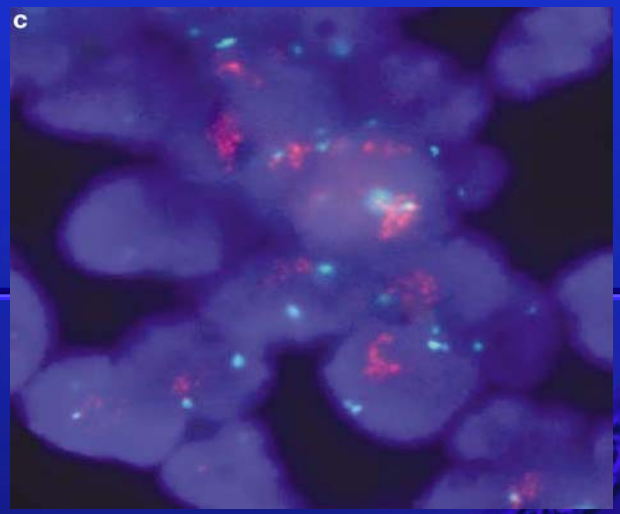
Tumor grade



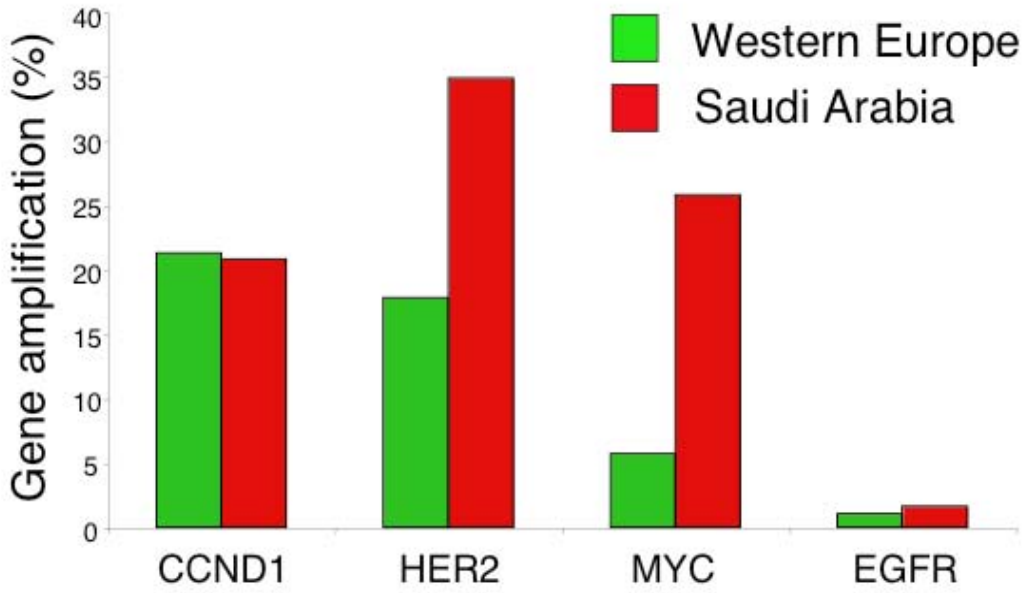
Western Europe

Saudi Arabia

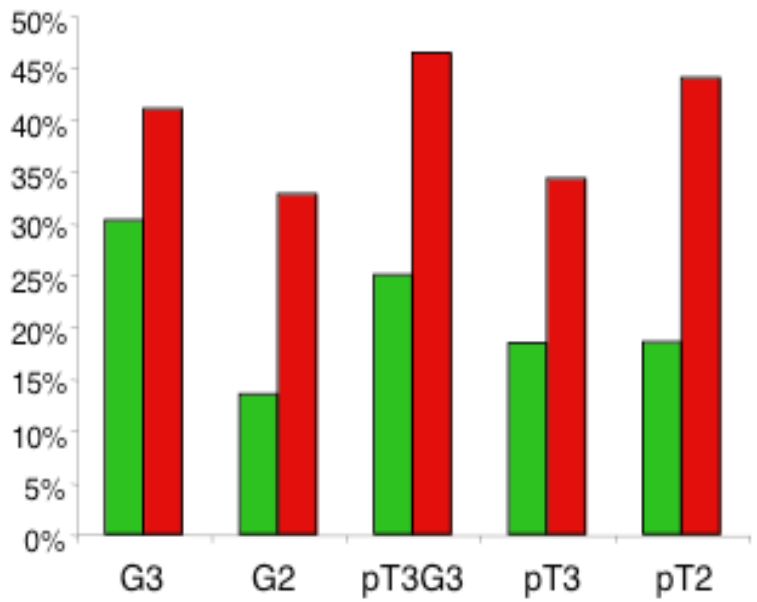

b


c

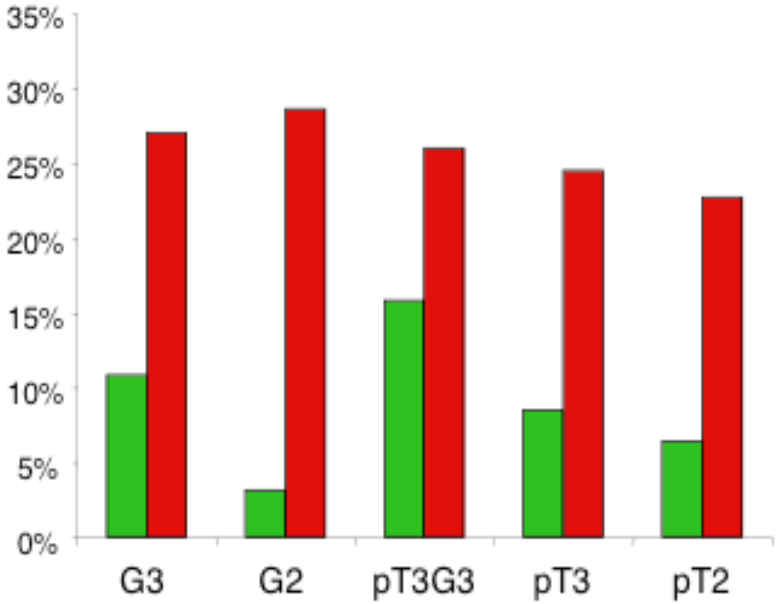
Gene amplification frequencies in breast cancer:
Western Europe versus Saudi Arabia



HER2 gene amplification and tumor phenotype



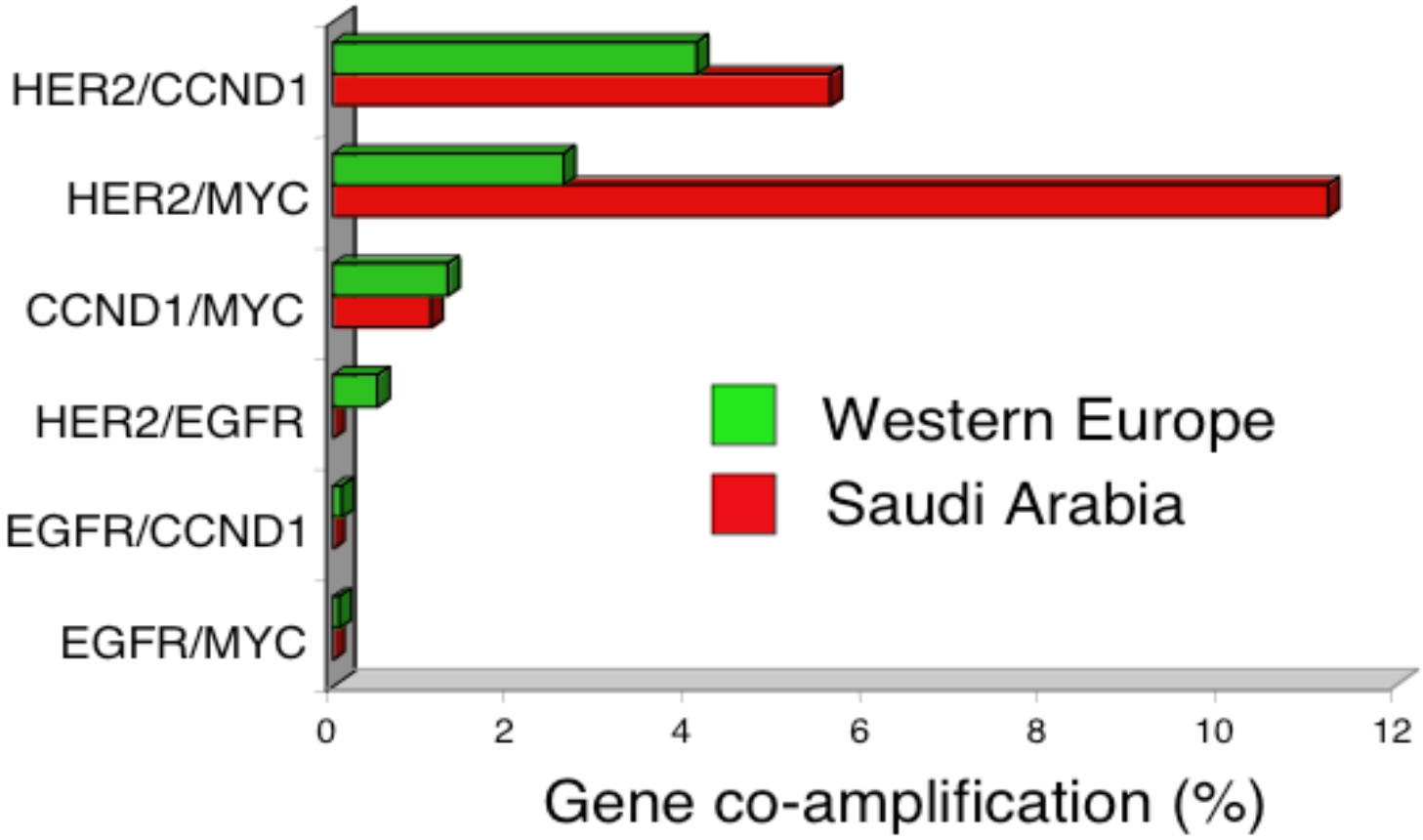
MYC gene amplification and tumor phenotype



Western Europe

Saudi Arabia

Gene co-amplification frequencies in breast cancer: Western Europe versus Saudi Arabia



Breast Cancer in Saudi Arabia

Conclusions

1) Saudi breast cancer cases show higher frequency of HER2 in comparison with Western cases (31% vs 17%) and MYC (16% vs 5%)

2) Higher percentage of Grade 3 carcinoma in Saudi women (65% vs 32%) with higher amplification of HER2 in Grade 3 (40% vs 30%)

3) Incidence of low grade breast cancer is 14 times lower in Saudi than Swiss women. The incidence of high grade breast cancer is comparable.

Modern Pathology, 2005

Molecular Classification of Breast Cancer

- Basal cell-like (expression of EGFR)
- HER2 over expression
- Luminal A, B, C subtypes

EGFR

Over expression associated with poor prognosis

- **lung cancer**
- **colon cancer**
- **high grade gliomas**
- **head and neck cancer**

EGFR in Lung Cancer

- Clinical trials revealed significant variability in response to target therapy (gefitinib) with higher response seen in Japanese patients than in European derived population (27.5% vs. 10.4%) in multi institutional Phase II trials.

EGFR in Lung Cancer

In USA, partial response observed mostly in:

- Women
- Non smokers
- Adenocarcinoma patients

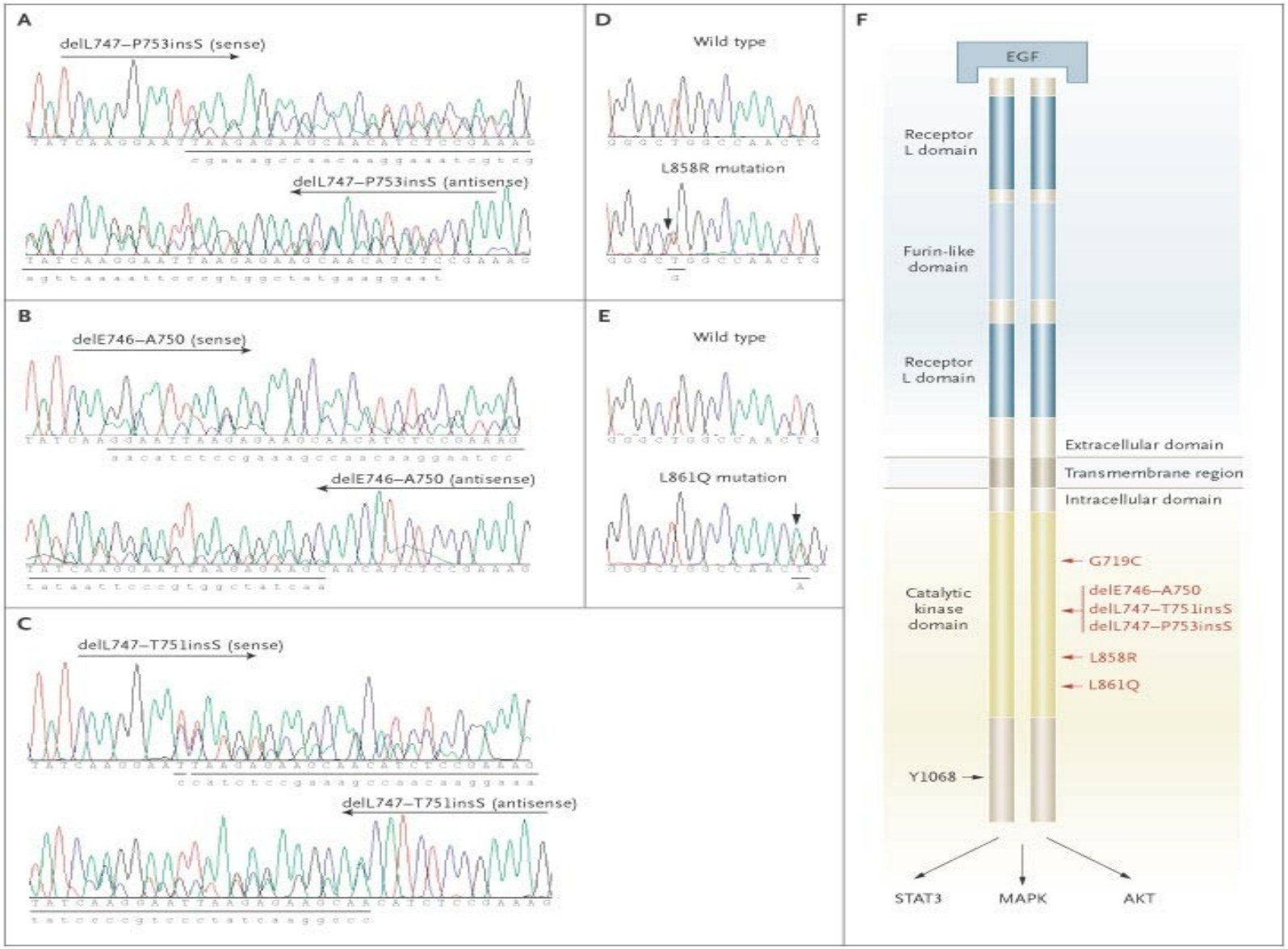
Lung Cancer 44; 221, 2004

EGFR in Lung Cancer

Mutations in EGFR (in NSCLC)

- Present in 8 out of 9 gefitinib-responsive
- Absent in 7 gefitinib-non responsive
- Mutation is present in 2 out of 25 NSCLC did not receive gefitinib (8%)

NEJM Vol. 350, No. 21, 2004


A
delL747–P753insS (sense)
delL747–P753insS (antisense)
B
delE746–A750 (sense)
delE746–A750 (antisense)
C
delL747–T751insS (sense)
delL747–T751insS (antisense)
D
Wild type
L858R mutation
E
Wild type
L861Q mutation
F
EGF
Receptor L domain
Furin-like domain
Receptor L domain
Extracellular domain
Transmembrane region
Intracellular domain
Catalytic kinase domain
G719C
delE746–A750
delL747–T751insS
delL747–P753insS
L858R
L861Q
Y1068
STAT3
MAPK
AKT

EGFR in Lung Cancer

- 10% of NSCLC have rapid response to tyrosine kinase inhibitor
- Specific mutations in EGFR leading to increase signaling
- Screening for mutation is important to identify responders to therapy

NEJM Vol. 350, No. 21, 2004

EGFR in Lung Cancer

- 90 consecutive NSCLC patient received gefitinib therapy
- 17 patients had EGFR mutation
- Response rate: 64.7% (with mutation)
  13.7% (without mutation)

Overall survival 30.5 months (with mutation)
6.6 monhhs (without mutation)

Cancer Research Institute, Seoul
J Clin Oncol 23, 2493-2501, 2005

EGFR in Lung Cancer

EGFR mutation in NSCLC

850 consecutive NSCLC examined

- **454 squamous cell carcinoma – no mutation**
- **31 large cell carcinoma – no mutation**
- **375 adenocarcinoma – 39 mutations (10%)**
  More in bronchioalveol (20%) then conventional adenocarcinoma (6%)

University of Chieti, Italy
J Clin Oncol 23; 857-865, 2005

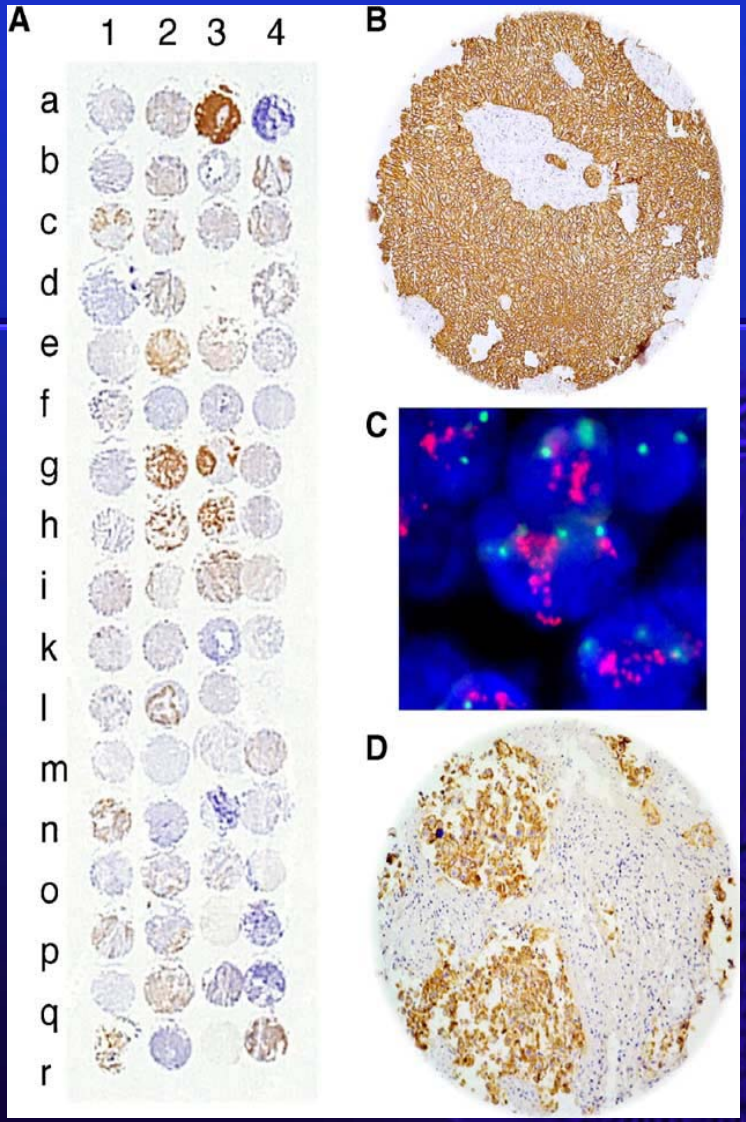
EGFR in Lung Cancer

Saudi Population

47 consecutive cases of NSCLC
(from pathology files, KFSH&RC)

- **27 adenocarcinoma**
- **17 squamous cell carcinoma**
- **3 large cell carcinoma**
- **1 giant cell carcinoma**

Human Pathology 37; 453-457, 2006


A
1 2 3 4
a
b
c
d
e
f
g
h
i
k
l
m
n
o
p
q
r
B
C
D

EGFR in Lung Cancer

Saudi Population

47 consecutive cases of NSCLC
(from pathology files, KFSH&RC)

- **Protein overexpression 69.8% of 43 cases**
- **Gene amplification 16% of 39 cases**
- **Gene mutation in one of 34 cases**

Human Pathology 37; 453-457, 2006

EGFR in Lung Cancer

Conclusion from Saudi NSCLC Study

- EGFR mutation is similar to Western countries
- EGFR amplification (16%) slightly higher than Western countries (6 – 9%)
  (EGFR amplification is linked to poor prognosis)

Human Pathology 37; 453-458, 2006

EGFR in Lung Cancer

Conclusions:

- No association between EGFR over expression and tumor histology, grade, stage or patient survival
- EGFR amplification is strongly linked to over expression.

GIST

- **Incidence**

  10–15 cases/million per year (European countries)
  6-8 cases/million per year (USA)

  ↓

  At least 100 cases/year in Saudi Arabia

STOMACH	**81**
Adenocarcinoma, NOS	29
Signet Ring Cell Carcinoma	19
Non-Hodgkin's Lymphoma	15
Intestinal Adenocarcinoma	8
Gatrointestinal Stromal Tumor, Malig	2
Mucinous Adenocarcinoma	2
Carcinoma, NOS	2
Tubular Adenocarcinoma	1
Carcinoid Tumor	1
Carcinoma, Diffuse Type	1
Squamous Cell Carcinoma	1

Tumor Registry, KFSH&RC, 2004

Soft Tissue Sarcomas

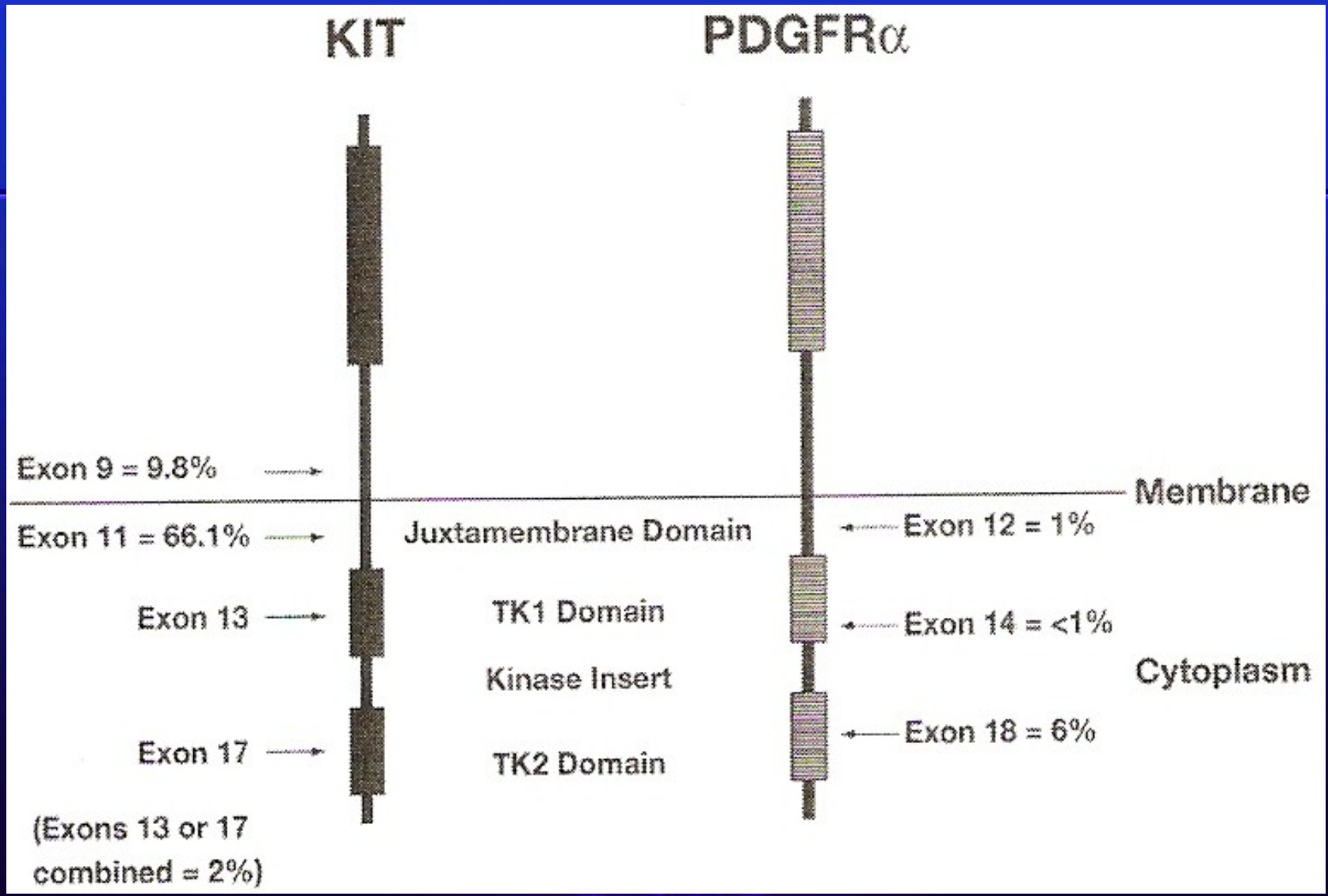
Soft tissue sarcoma incidence is 30 per million per year

At least 400 cases should be reported per year in Saudi Arabia

88 Cases Reported at KFSH&RC Tumor Registry, 2004

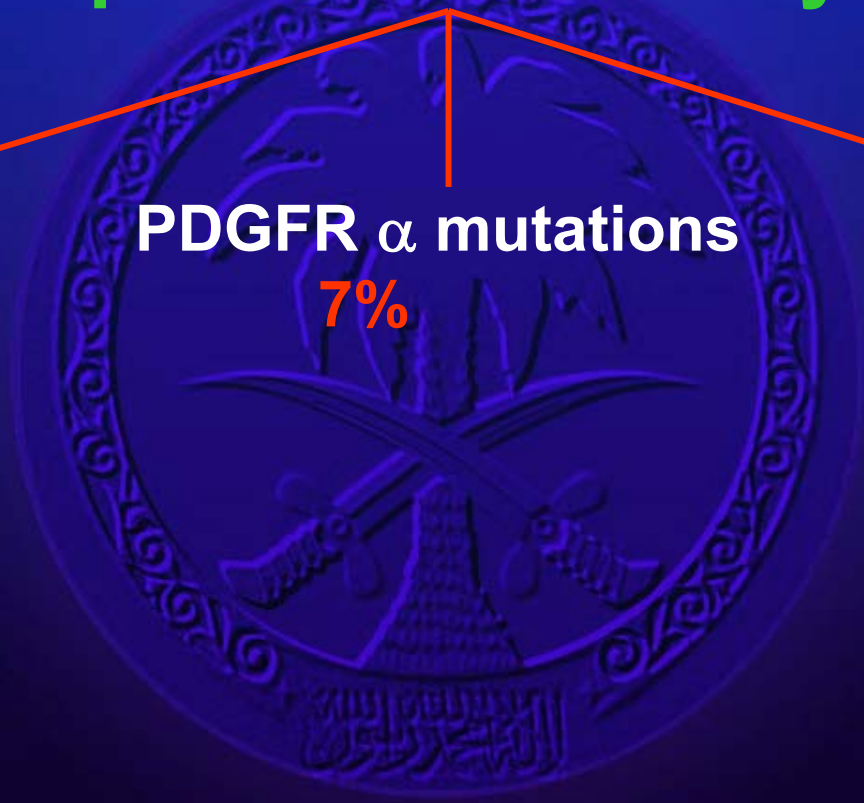
Type III Receptor Tyrosine Kinase

- KIT
- PDGFRA/B
- c-FMS
- BCR-ABL
- ABG
- ARG


KIT
PDGFRα
Exon 9 = 9.8%
Exon 11 = 66.1%
Exon 13
Exon 17
(Exons 13 or 17 combined = 2%)
Juxtamembrane Domain
TK1 Domain
Kinase Insert
TK2 Domain
Membrane
Exon 12 = 1%
Exon 14 = <1%
Exon 18 = 6%
Cytoplasm

GIST

KIT positive tumors by IHC



KIT mutations
80-85%

PDGFR α mutations
7%

Wild type genes encoding KIT and PDGFR α
10-15%

KIT – Negative GIST

- About 5% of GIST are KIT negative by IHC

25 tumors CD117 –ve by IHC mutational analysis revealed

- PDGFRA mutations in 18 cases
- KIT mutations in 4 cases
- 3 – no mutation

- More likely to have epitheliod morphology
- Mostly in stomach

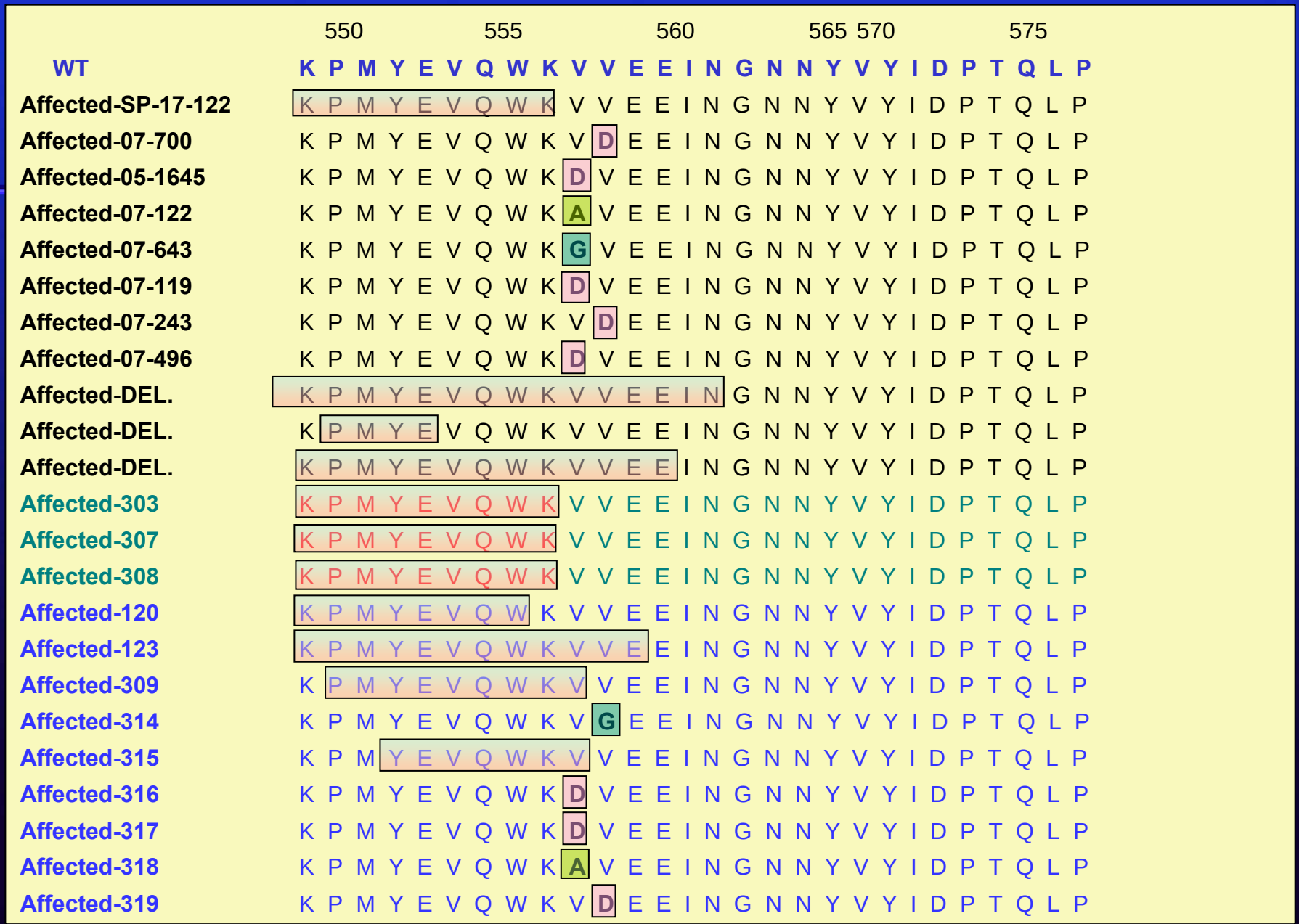
Am J Surg Path, Vol. 28, No. 7, July 2004

Results

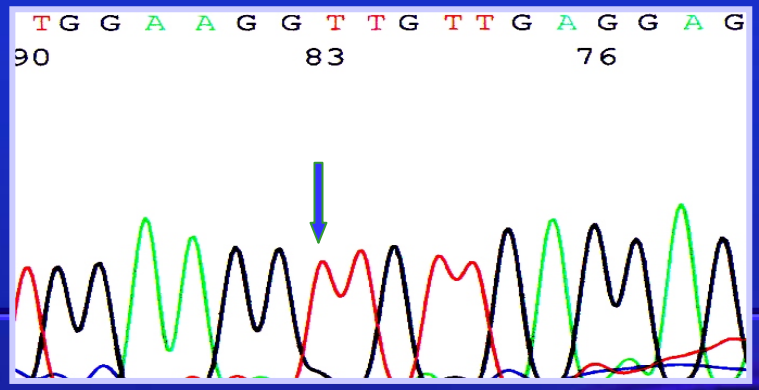
Sequence analysis revealed:

- *c-KIT* mutations in 23 out of 44 available tumor samples.
- 8 were previously reported mutations in exon 11, Valine to Aspasrtic Acid at codon 559 and 560.
- 4 samples exhibited novel mutations, in 2 cases Valine changes into Alanine while in other 2 cases it changes into Glycine on the same codon 559.
- 11 tumor samples had novel inframe deletions in the exon 11.
- No mutation was identified in 12 tumor samples in the exon 11, 9, 13 and 17 of the *c-KIT* gene and 12 & 18 of the *PDGFR* gene.
- DNA quality was not optimal for analysis in the remaining 9 samples.

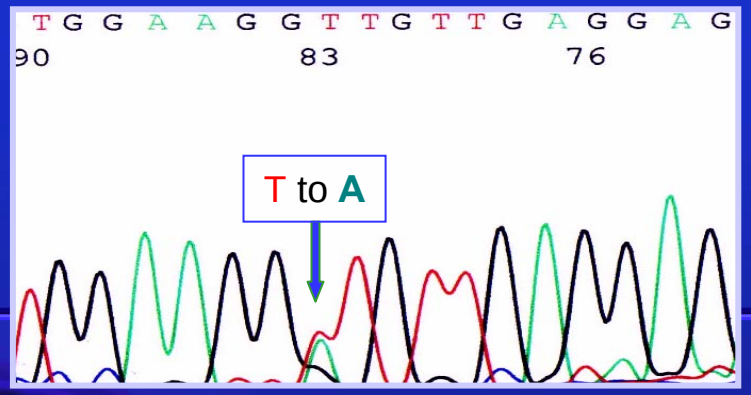
Amino Acid Sequence Alterations Encoded by cKIT Exon-11 in 23GISTs patients



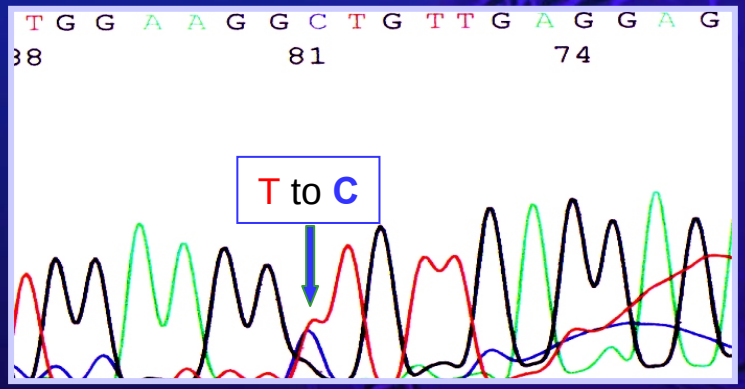
(A) Wild type



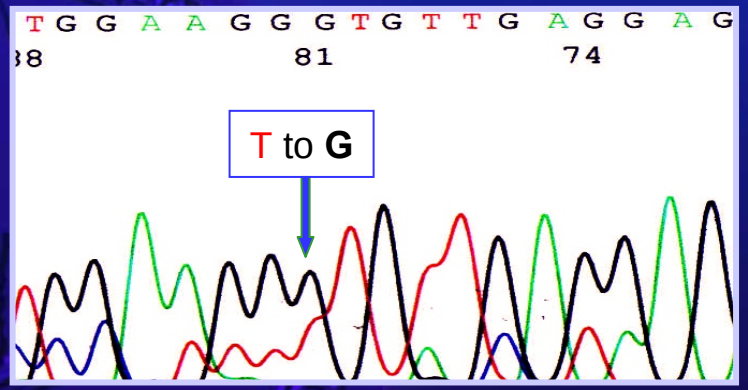
(B) V559D Mutation



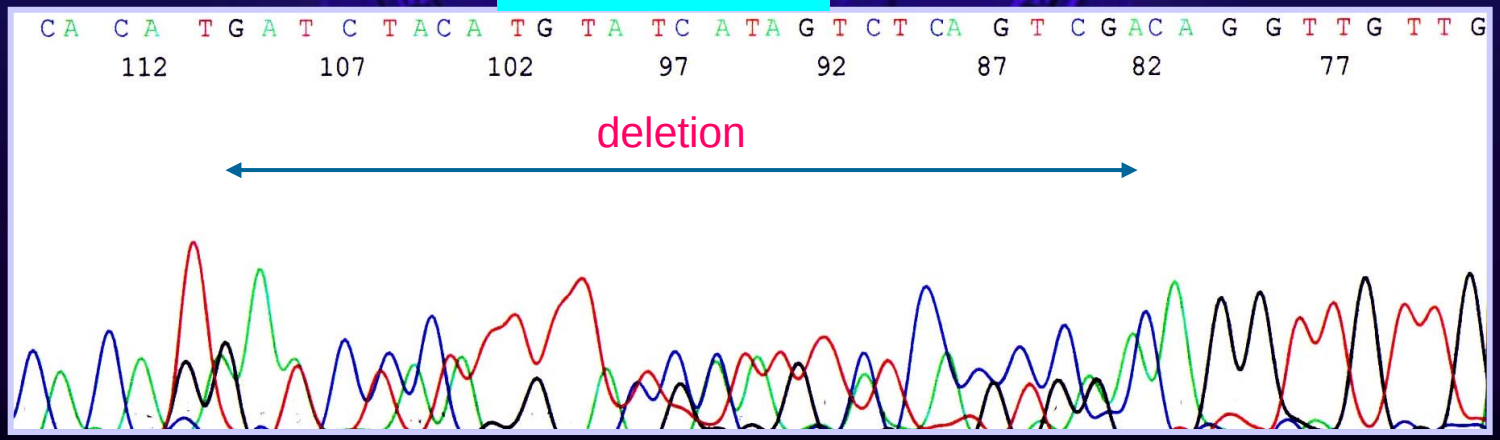
(C) V559A Mutation



(D) V559G Mutation



(E) 26 bp deletion



Changing Role for the Pathologist

- **Patients management plan**
  - **Immunohistochemistry**
  - **Flow cytometry**
  - **FISH**
  - **Mutation analysis**
  - **Microarry analysis**
- **Response to treatment**
  - **Quantitative measurement – methods as above**
- **Monitoring for resistance to treatment**
  - **Mutation analysis**