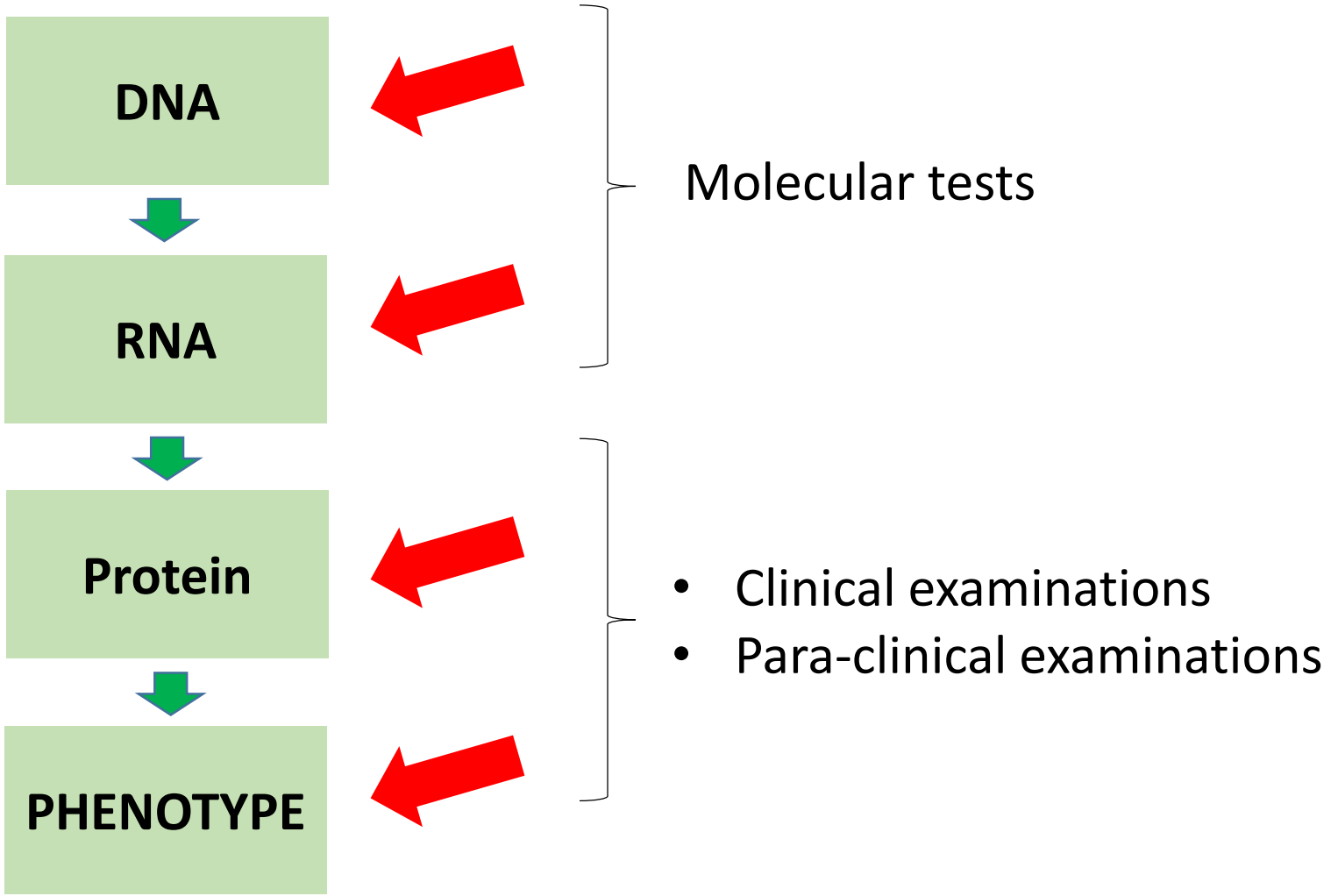
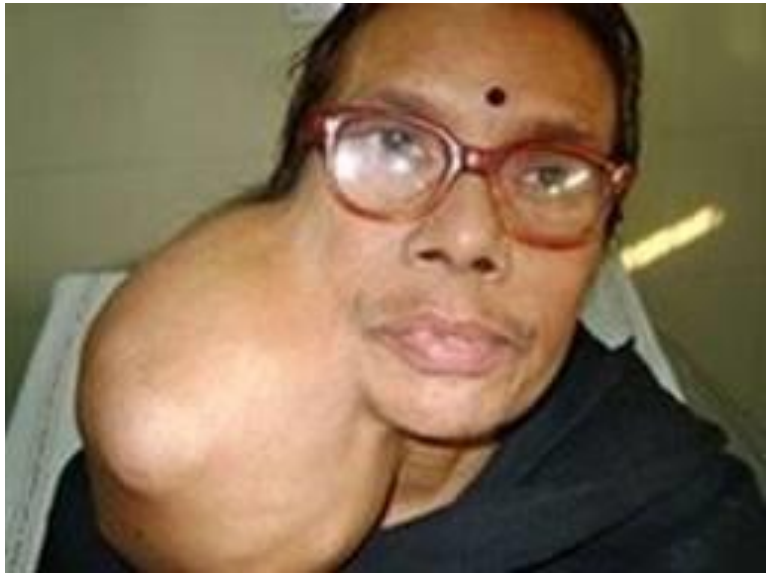
ỨNG DỤNG CHẨN ĐOÁN PHÂN TỬ TRONG NHÓM BỆNH UNG THƯ

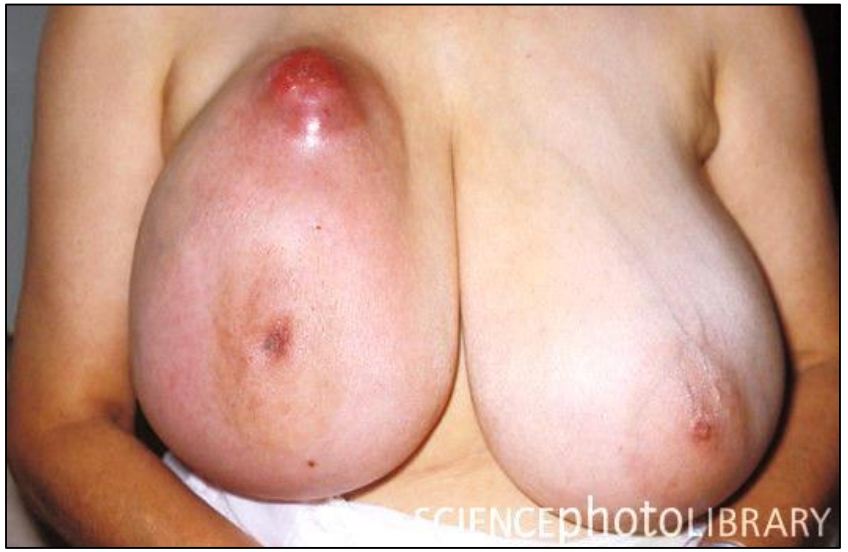
Ts Bs Nguyễn Hữu Ngọc Tuấn

APPROACH


DNA
RNA
Protein
PHENOTYPE
Molecular tests
• Clinical examinations
• Para-clinical examinations

At the body level



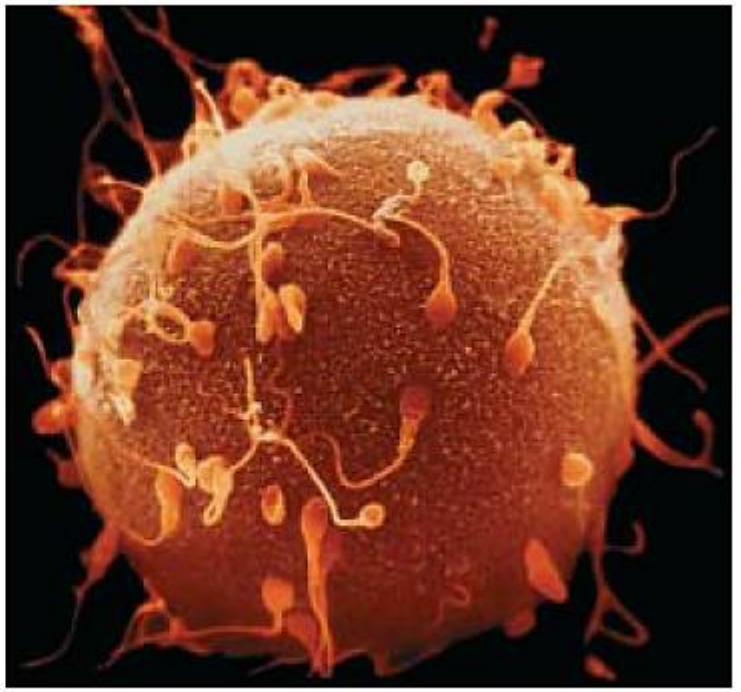


bert-firebert.blogspot.com
medicaljournalonline.blogspot.com
occupiedmedia.us
www.healthywomenlife.com
medicalxpress.com

How does life begin?

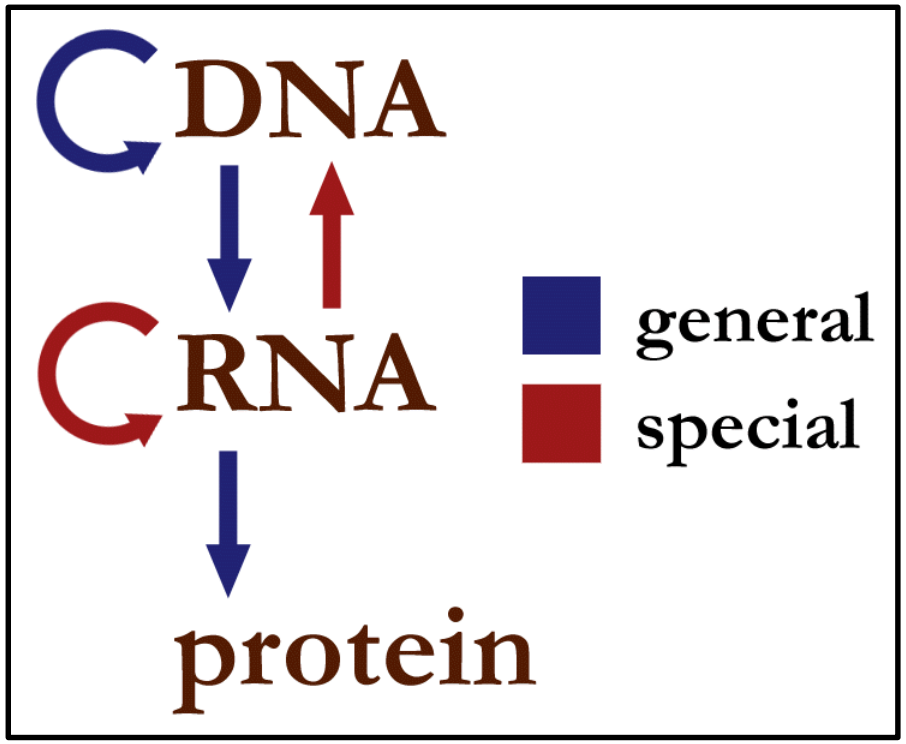
1

LIFE BEGINS WITH CELLS

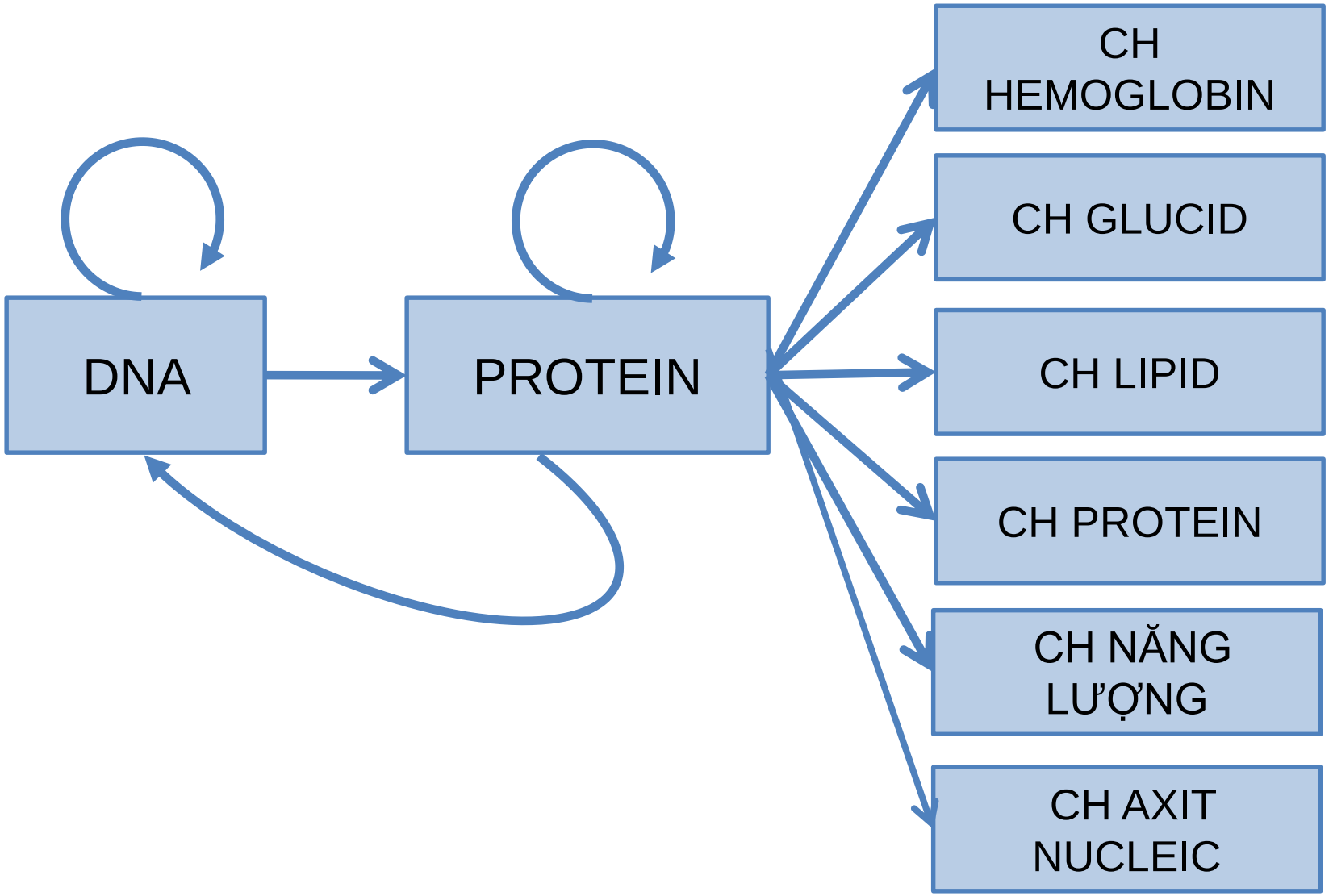


A single ~200 micrometer (μm) cell, the human egg, with sperm, which are also single cells. From the union of an egg and sperm will arise the 10 trillion cells of a human body.

The central dogma of biology



Relationship of molecules

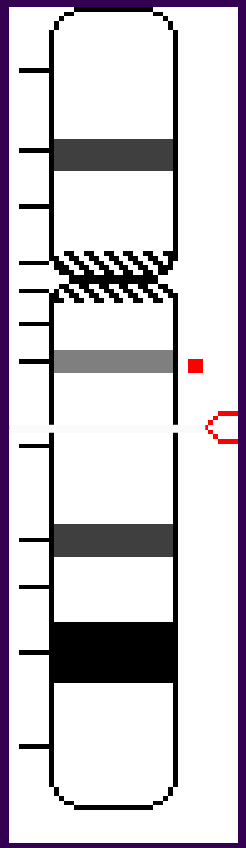


Cancer = Cellular Pathology

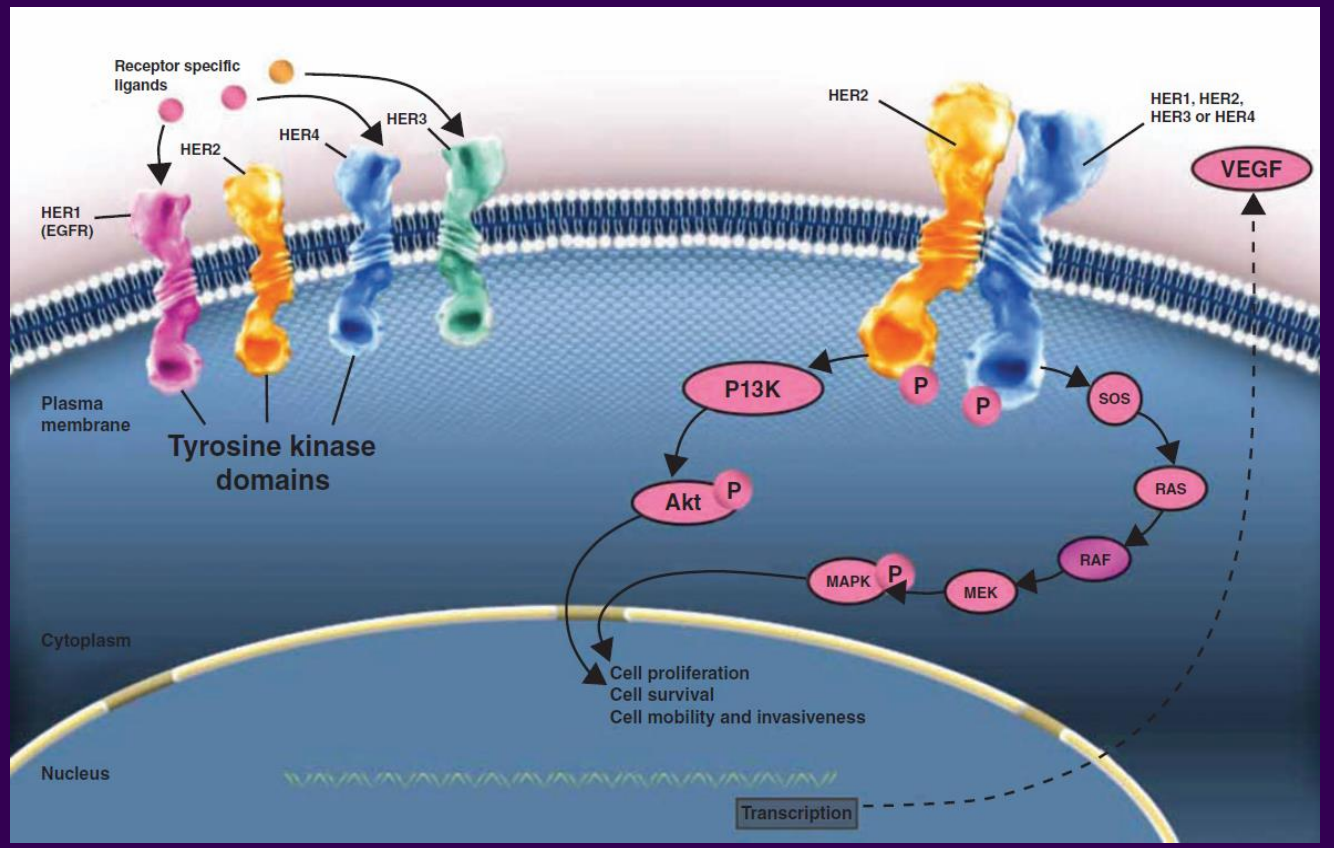
Cancer = Genomic Pathology

BREAST CANCER

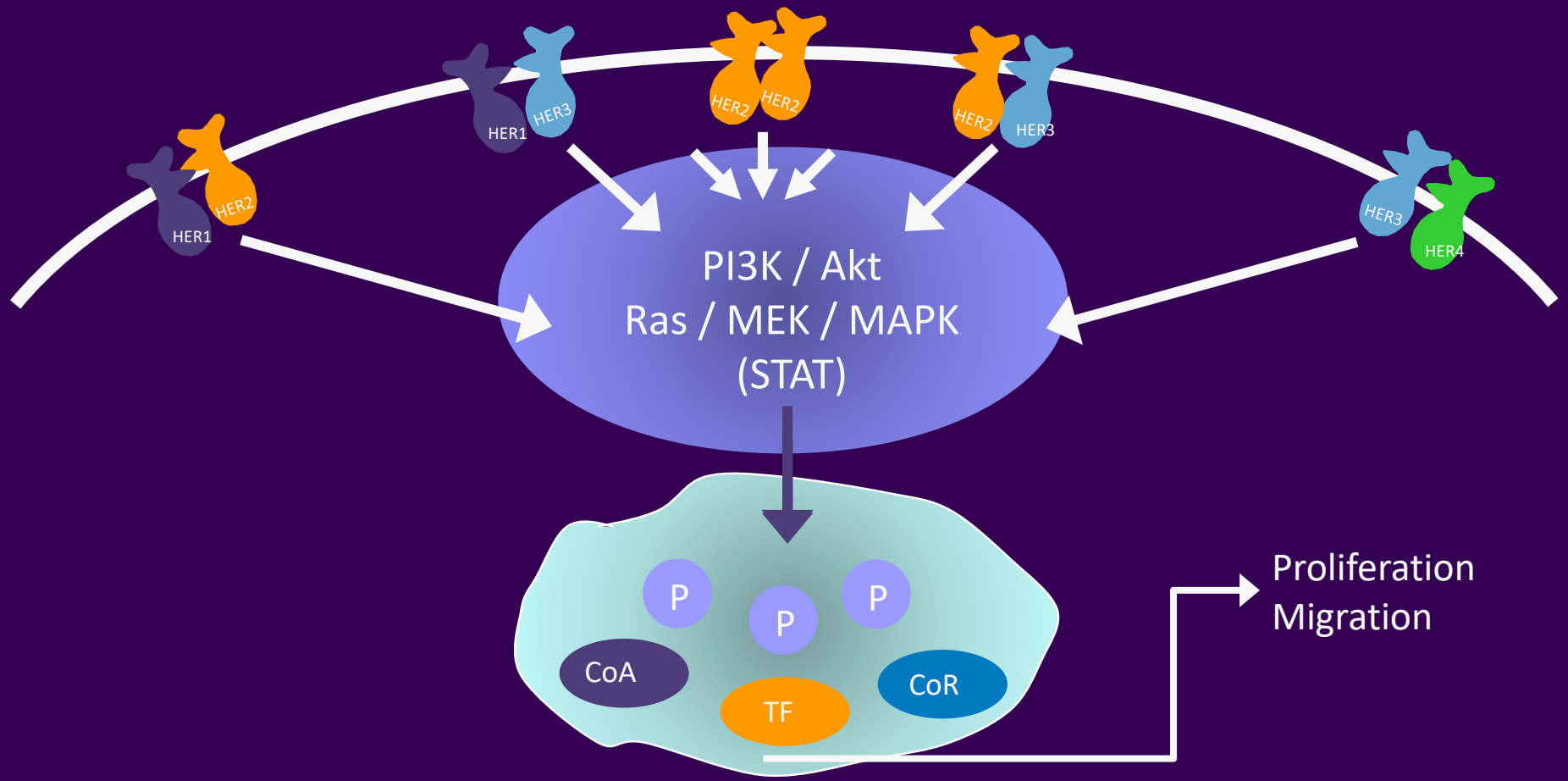
HER2 is required for normal cell development



ERBB2
17q21

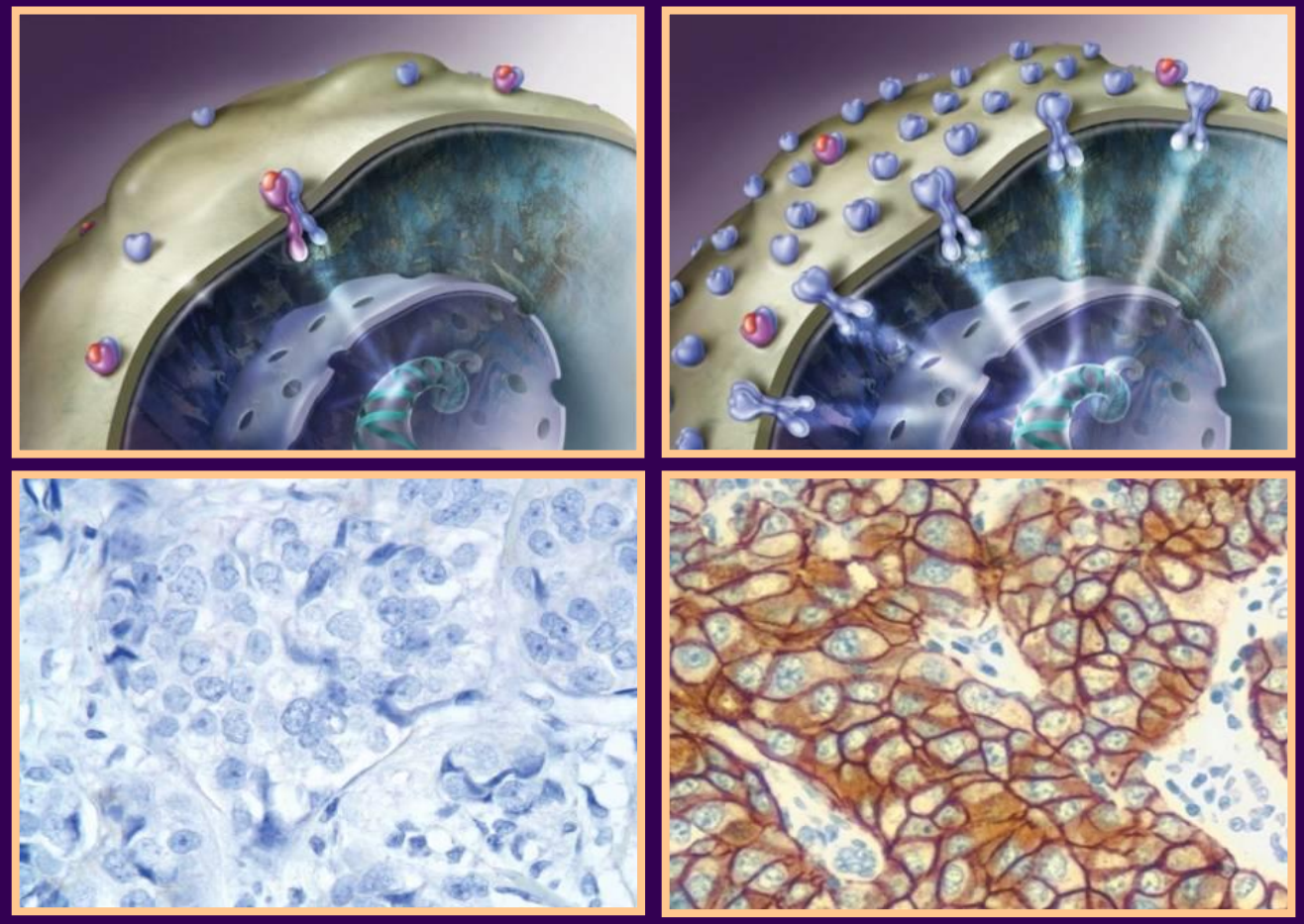


Overexpression of HER2 increases cellular proliferation and migration



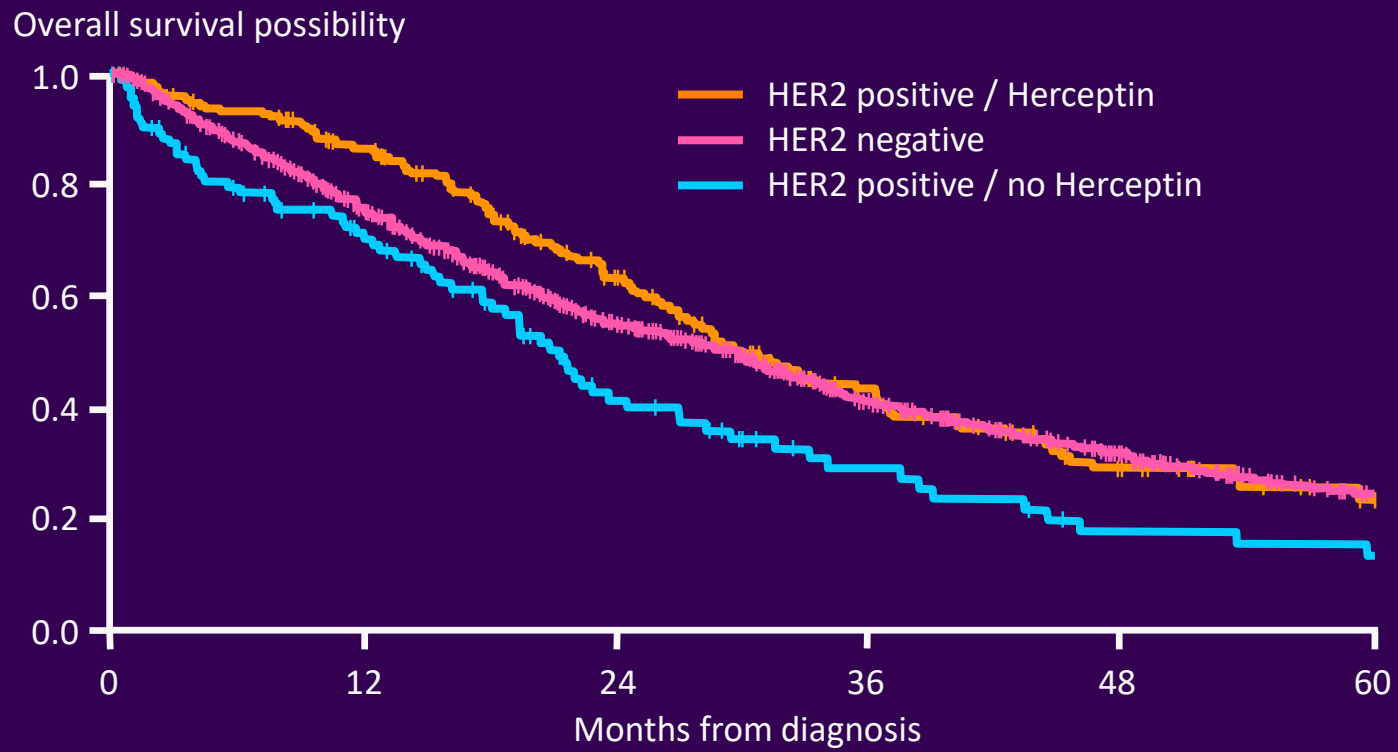
HER2, human epidermal growth factor receptor 2; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription; CoA, co-enzyme A; TF, tissue factor; CoR, co-repressor

Overexpression of HER2 in BC



Overexpression of HER2 is associated with reduced survival in patients with BC

- Herceptin improves the prognosis of patients with HER2-positive metastatic breast cancer

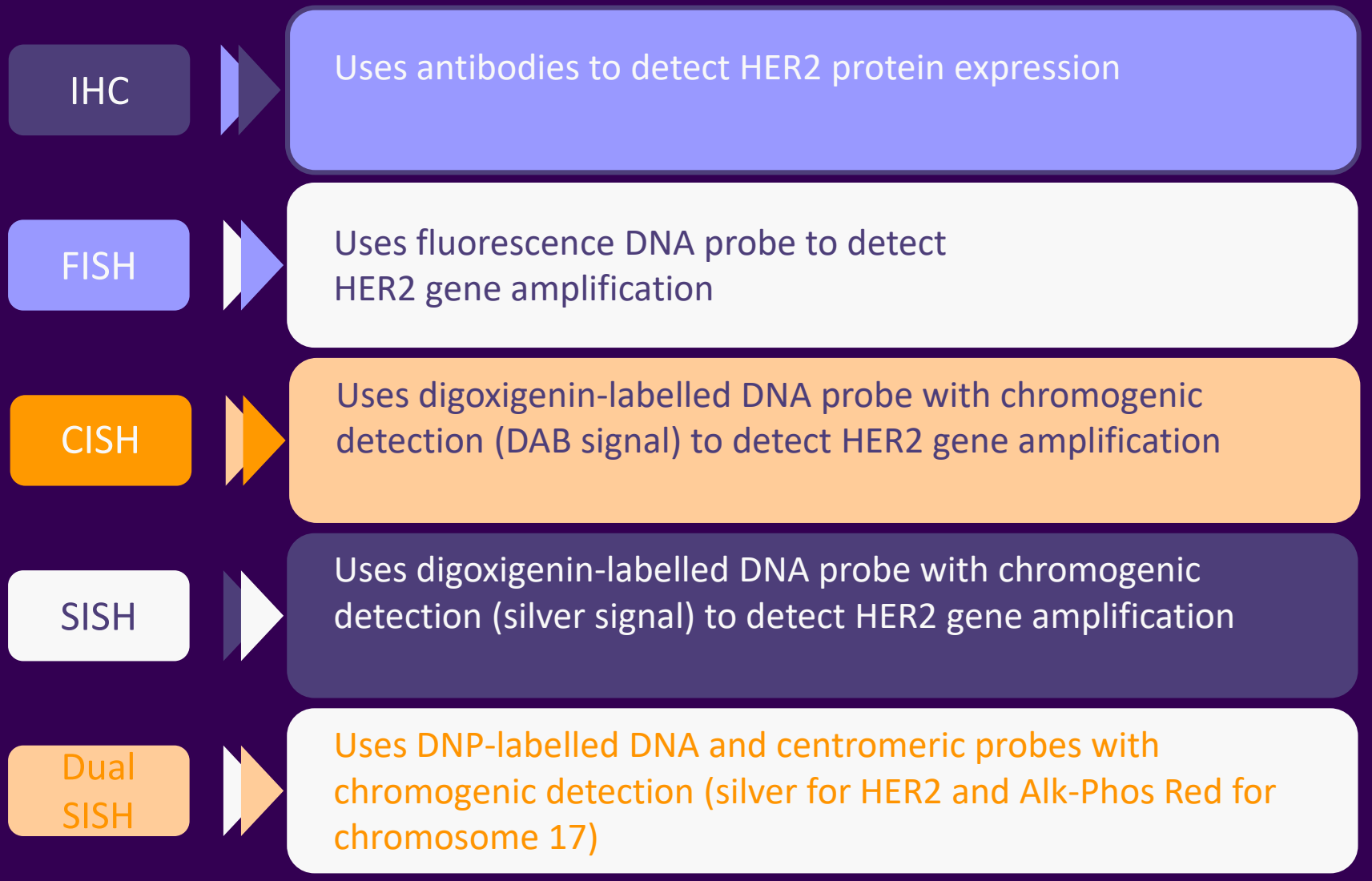


- In a retrospective analysis of database records, women with HER2-positive disease who received Herceptin had a 44% reduction in risk of death compared to women with HER2-negative disease (multivariate analysis adjusted for patient and tumour characteristics: HR 0.56; 95% CI 0.45, 0.69; p<0.0001)

HR, hazard ratio; CI, confidence interval

Dawood et al 2008

Summary of HER2-testing methods

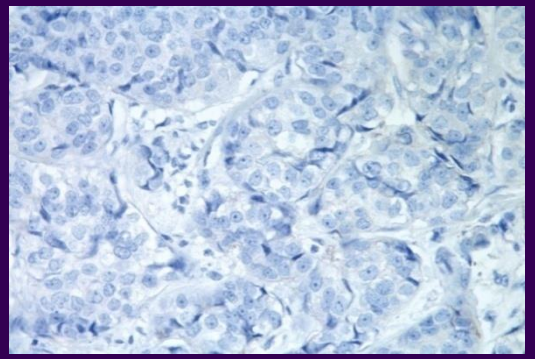


CISH, chromogenic ISH; SISH, silver-enhanced ISH

Advantages and disadvantages of HER2-testing methodologies (1)

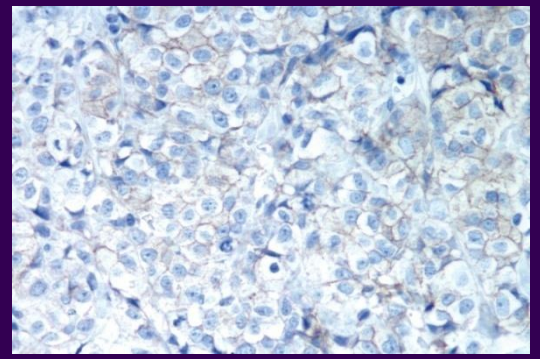
Method	Advantages	Disadvantages
IHC	Performed in majority of pathology laboratories Relatively easy, quick and cheap; can be automated IHC-stained slides can be stored and re-assessed Cell morphology can be seen in same section	Susceptible to variations in testing protocol Score interpretation subjective and semi-quantitative
FISH	Less affected by pre-analytical factors and handling than IHC Score interpretation more quantitative than for IHC Identifies HER2-positive tumours (gene amplified) within IHC 2+ cases Automation available	Costly (more expensive than IHC) Signal decays over time Areas of invasive carcinoma may be difficult to identify Few pathologists and technologists are trained in the methodology and its interpretation

Interpretation of IHC results



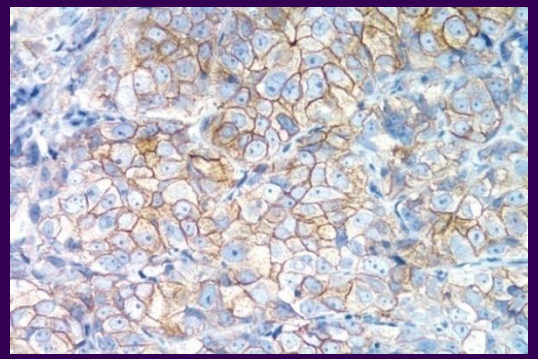
IHC 0

No staining or membrane staining in <10% of tumour cells



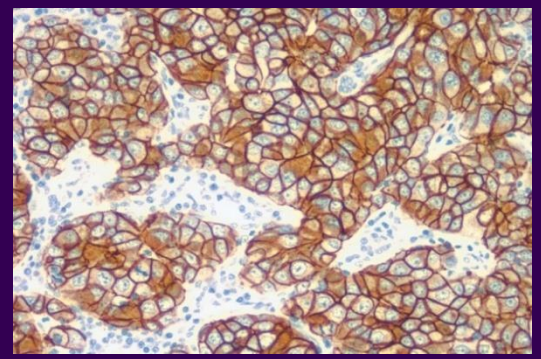
IHC 1+

Barely perceptible membrane staining in >10% of tumour cells;
cells only stained in part of membrane



IHC 2+

Weak / moderate complete membrane staining in >10% of tumour cells



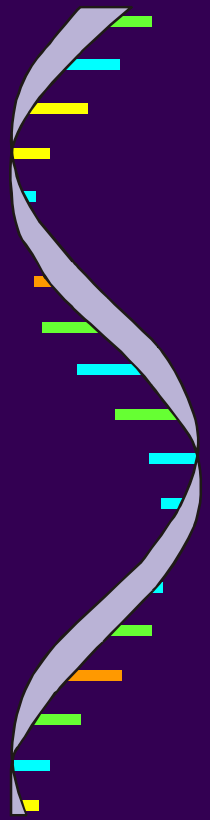
IHC 3+

Strong complete membrane staining in >10% of tumour cells

Images courtesy of Dako

Principles of ISH

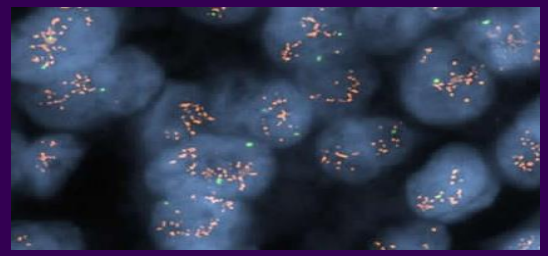
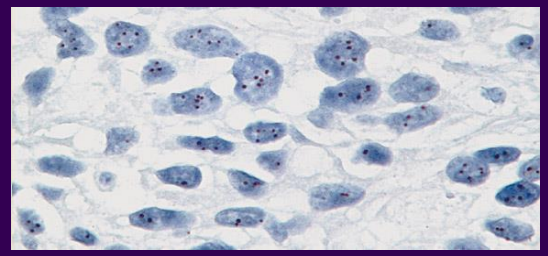
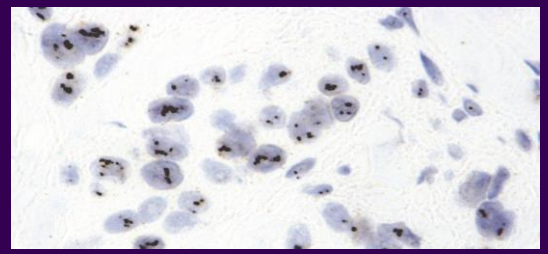
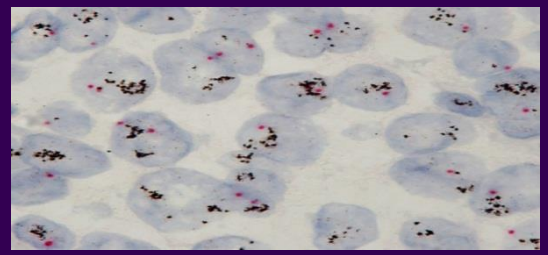
Prepare target DNA



Denature target DNA

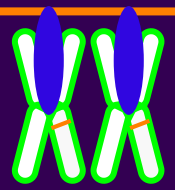
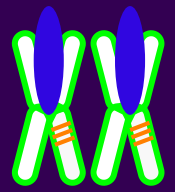
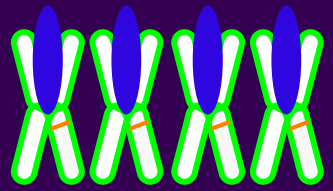
Probe binds to denatured DNA and emits a signal

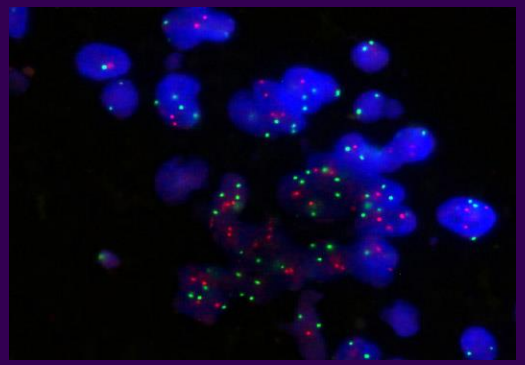
ISH: HER2 gene-amplification detection mechanisms

FISH positive[a]		Fluorescence DNA probe
CISH positive[b]		Digoxigenin-labelled DNA probe with chromogenic detection (DAB signal)
SISH positive[c]		Dinitrophenol-labelled DNA probe with chromogenic detection (silver signal)
Dual SISH / Red Amplified HER2[c]		Dinitrophenol-labelled DNA and centromeric probes with chromogenic detection (silver and Alk-Phos Red)

[a]Image courtesy of W Hanna;
[b]image from Invitrogen; [c]image from Ventana

Polysomy

Gene amplification vs polysomy[a]	
	Normal 2 CEP17 2 HER2 genes
	Gene amplification 2 CEP17 >2 HER2 genes
	Polysomy >2 CEP17 >2 HER2 genes



The CEP17 probe identifies the centromere of chromosome 17

Polysomy means there are >2 CEP17 signals (green) and in consequence >2 HER2 gene signals (orange) detected per nucleus

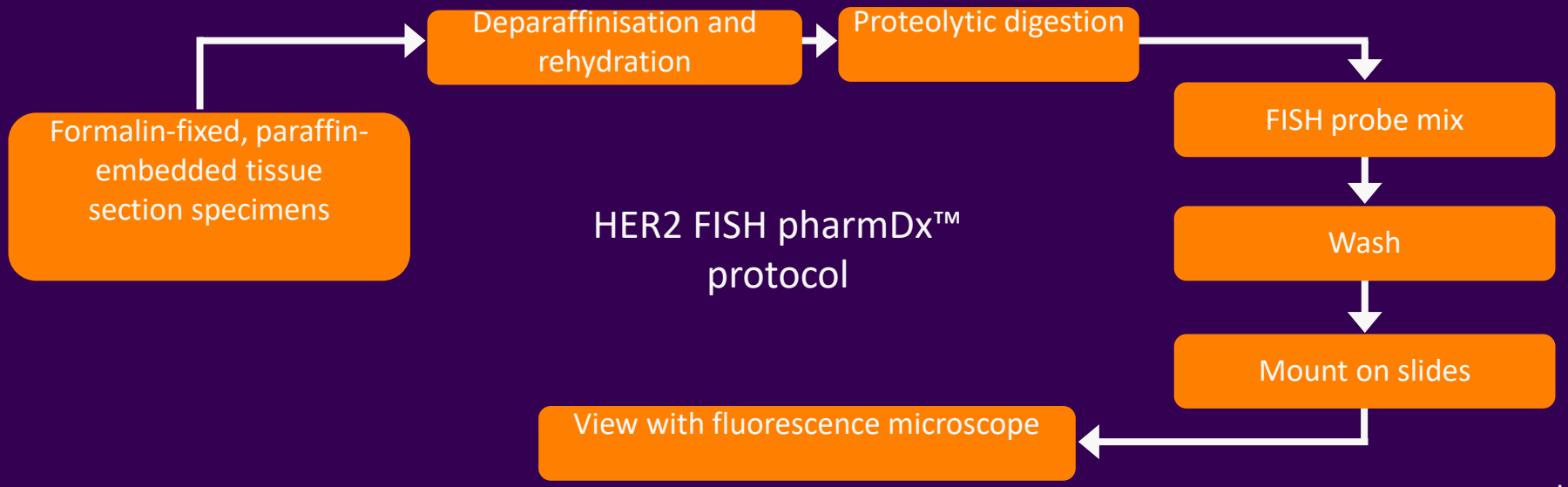
This can result in false-negative interpretation of ISH

[a]Data are signals per nucleus

Image courtesy of J Rüschoff and M Hofmann

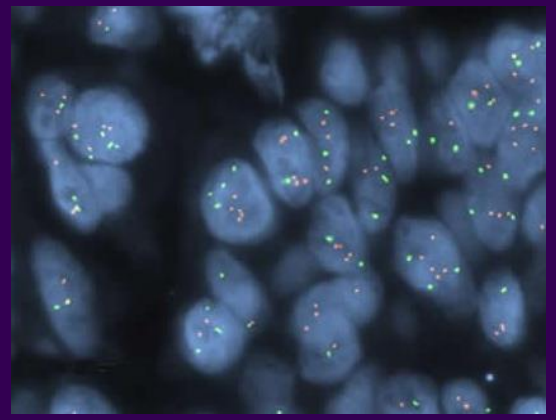
FISH methodologies

	PathVysion™ (Abbott) and HER2 FISH pharmDx™ (Dako)	INFORM® (Ventana)
No. of probes	2 (1 for HER2 gene, 1 for CEP17)	1 for HER2 gene
Definition of ISH positive	HER2:CEP17 ratio ≥2	HER2 signals >4
Polysomic case	Is recognised but can be scored as FISH negative (due to scoring ratio), should be retested with IHC	Is not recognised Is scored as FISH positive



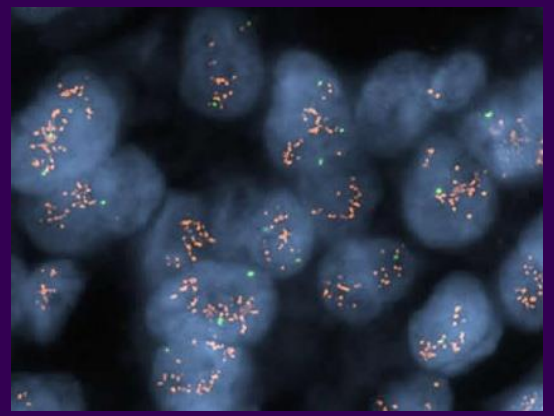
Hofmann et al 2008

FISH interpretation



FISH negative (no amplification)

Ratio of HER2 gene (orange) to CEP17 (green) signals is <2.0

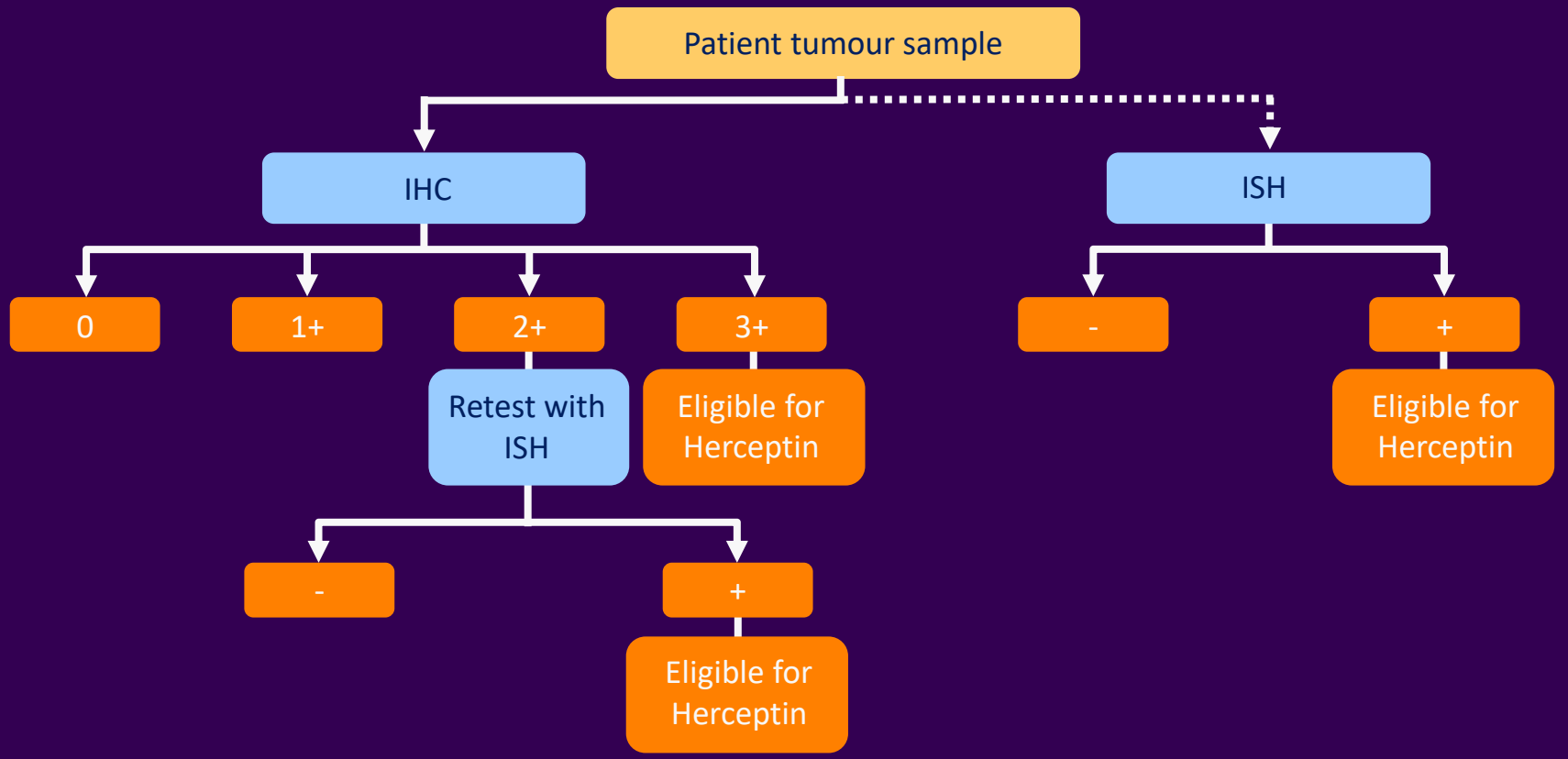


FISH positive

Ratio of orange to green signals is ≥2.0

Images courtesy of W Hanna using PathVysion

The HER2-testing algorithm



- If primary ISH testing is used, patients whose tumours overexpress HER2 (ie IHC 3+) may not be identified due to the HER2:FISH ratio being <2.0 (eg chromosome 17 polysomic cases, Hofmann et al 2007)
- ISH-detection mechanism can be fluorescent, chromogenic or silver

ISH, *in situ* hybridisation

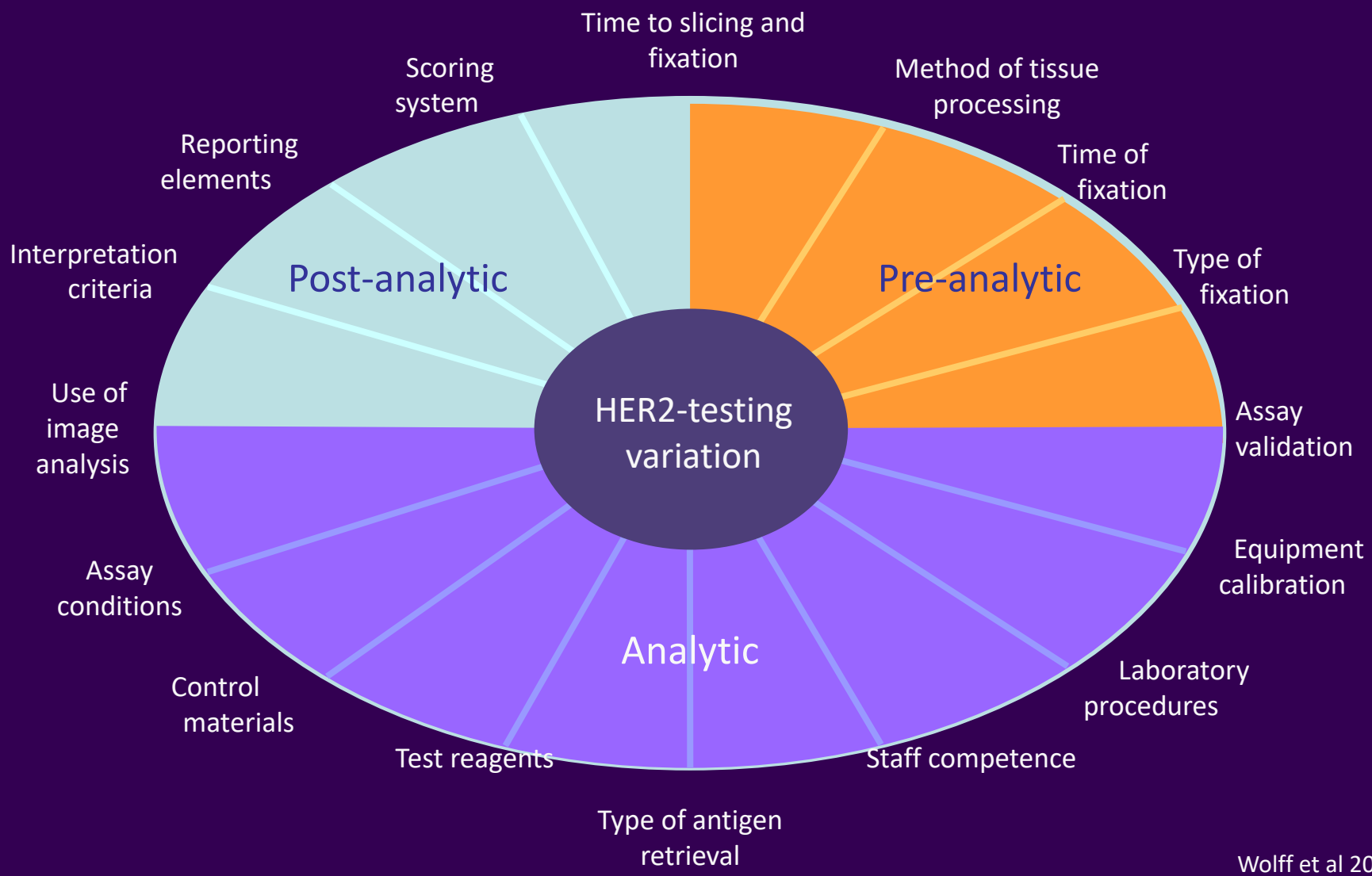
Hanna & Kwok 2006

Concordance between IHC and FISH is 75-100%

Study	No. of cases	Overall concordance,[a] %
Di Palma et al 2007	161	93
Ricardo et al 2007	161	83, 82[b]
van de Vijver et al 2007	209	81
Vocaturo et al 2006	111	76
Sapino et al 2003	106	85, 80[b]
Dandachi et al 2002	171	92
Tanner et al 2000	157	98

[a]For IHC results, 3+ scores are considered positive; [b]study used different antibodies for IHC; therefore, concordance data are presented per antibody
Only studies with >100 cases are shown

Sources of variation in HER2 testing

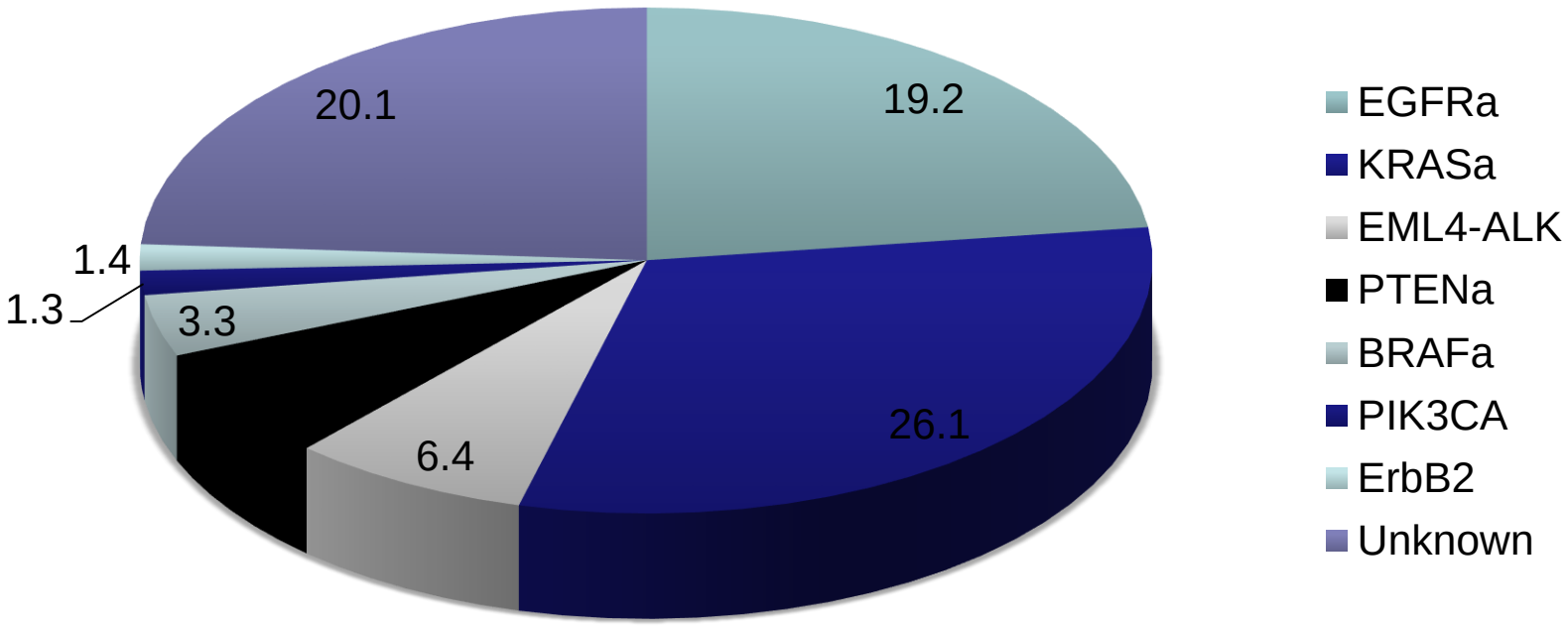


Wolff et al 2007

NON SMALL CELL LUNG CANCER

Lung cancer genetics – increasing complexity

Incidence of individual mutations for western NSCLC (adenocarcinoma)

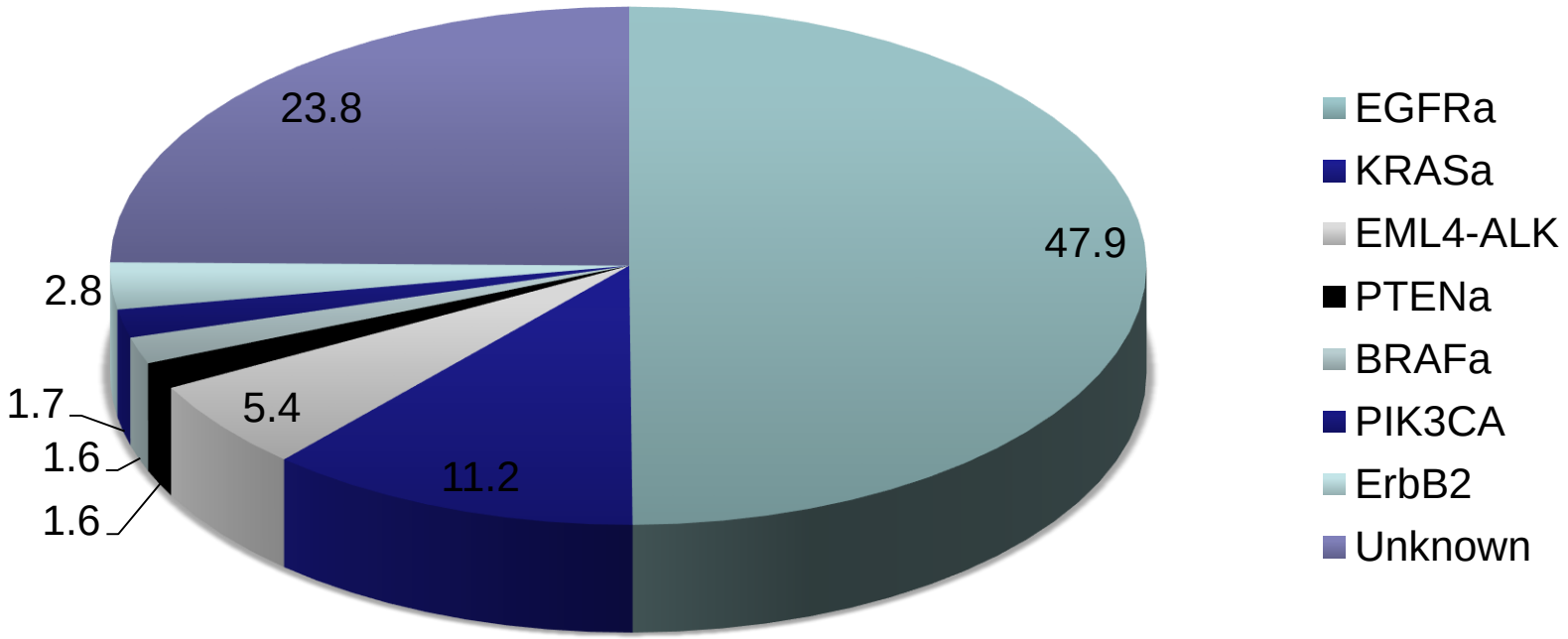


After Dearden et al., Ann Oncol 2013.

Warwick Medical School

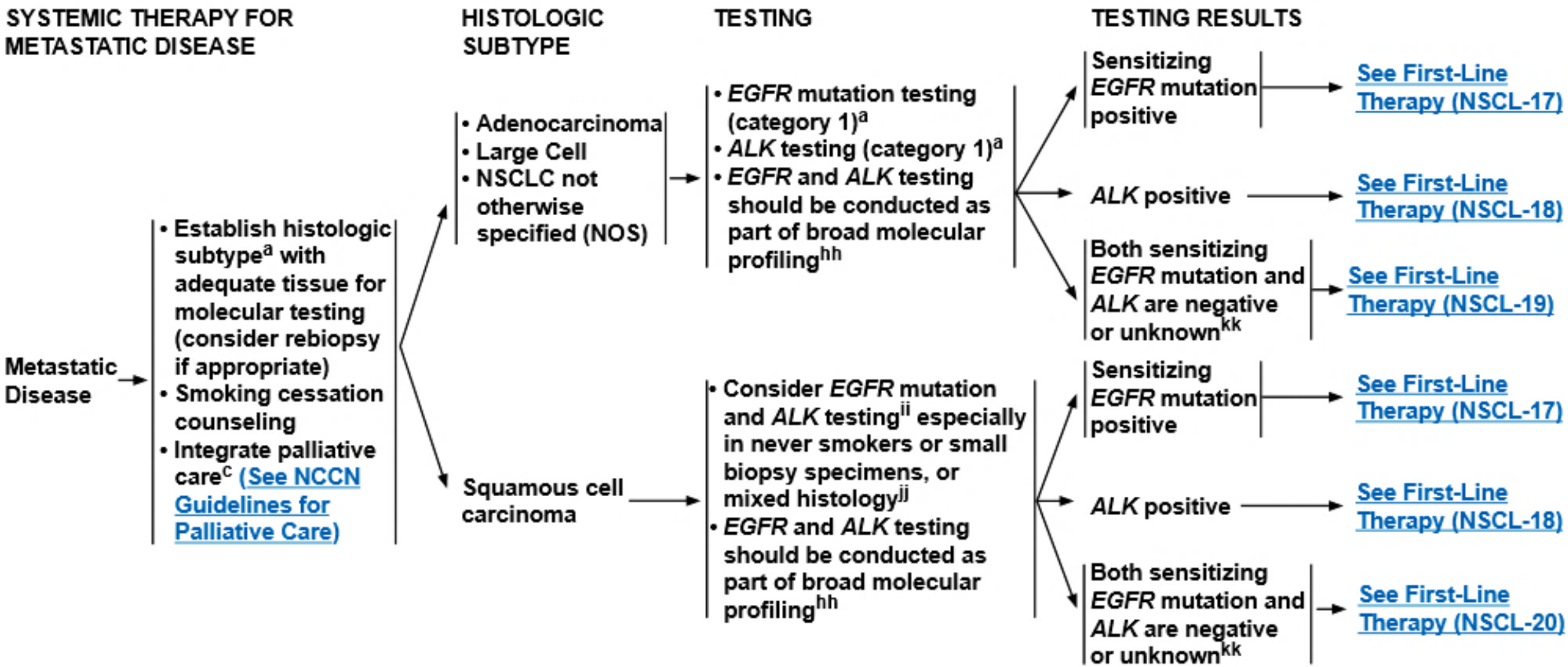
Lung cancer genetics – increasing complexity

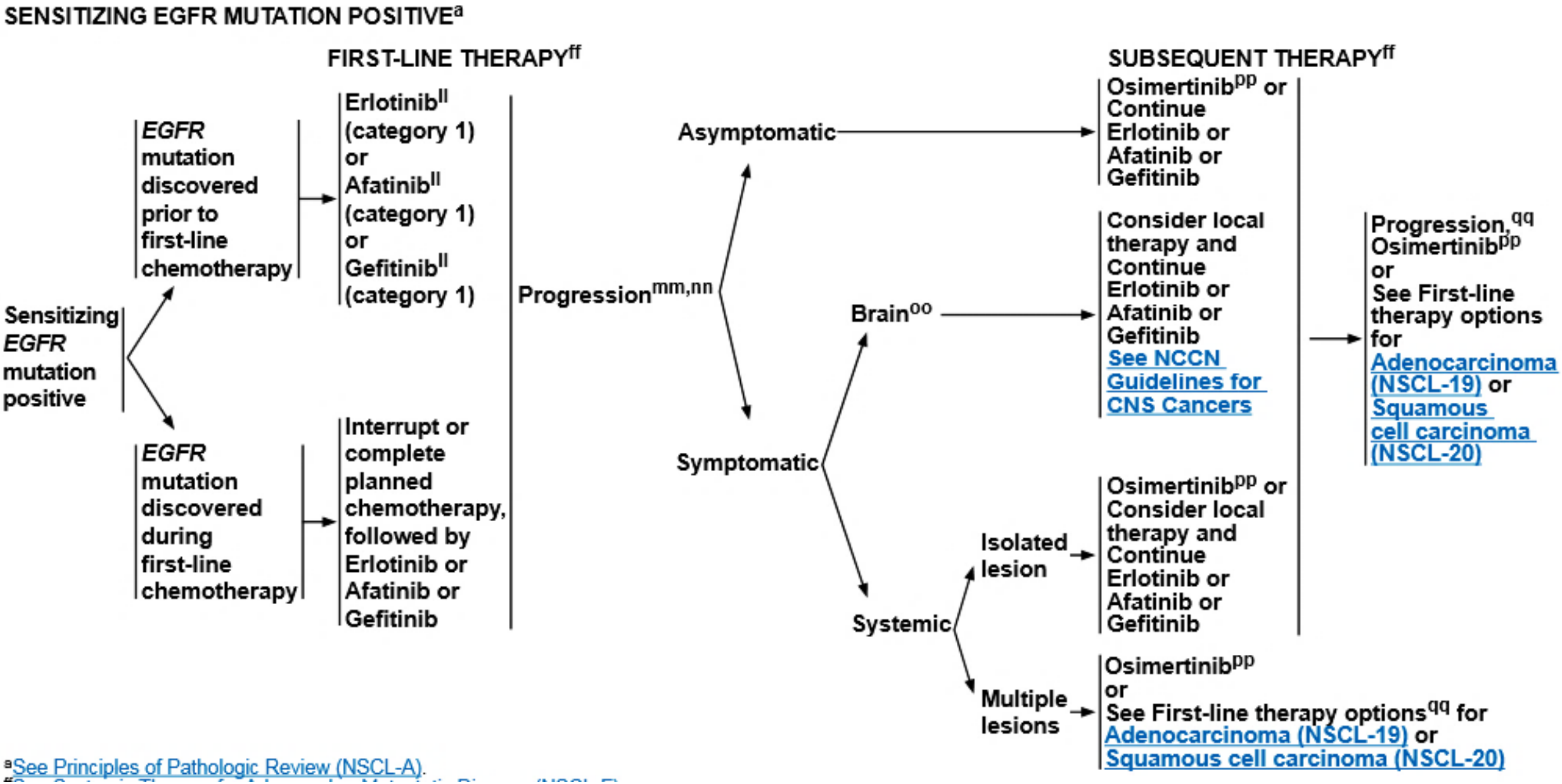
Incidence of individual mutations for asian NSCLC (adenocarcinoma)



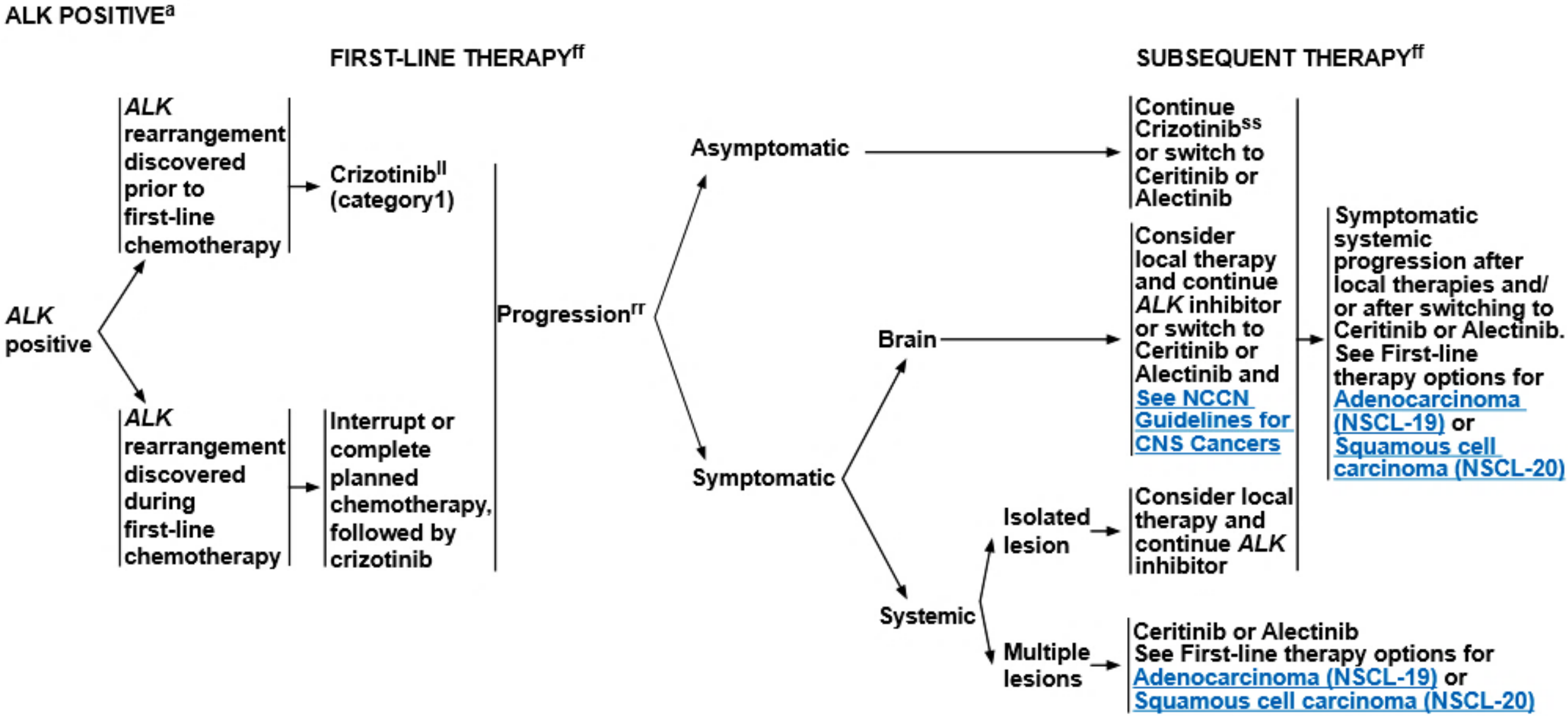
After Dearden et al., Ann Oncol 2013.

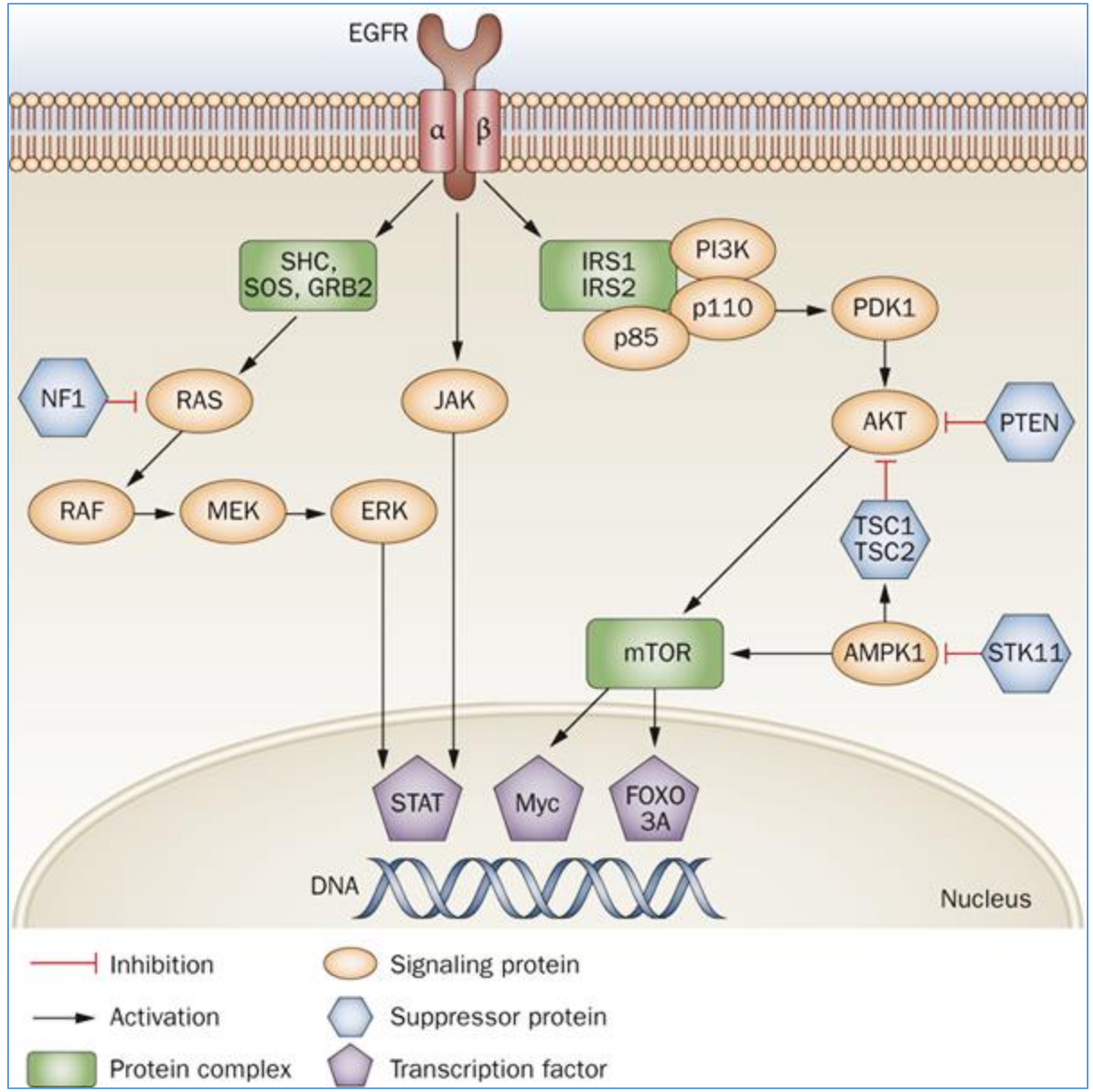
Warwick Medical School

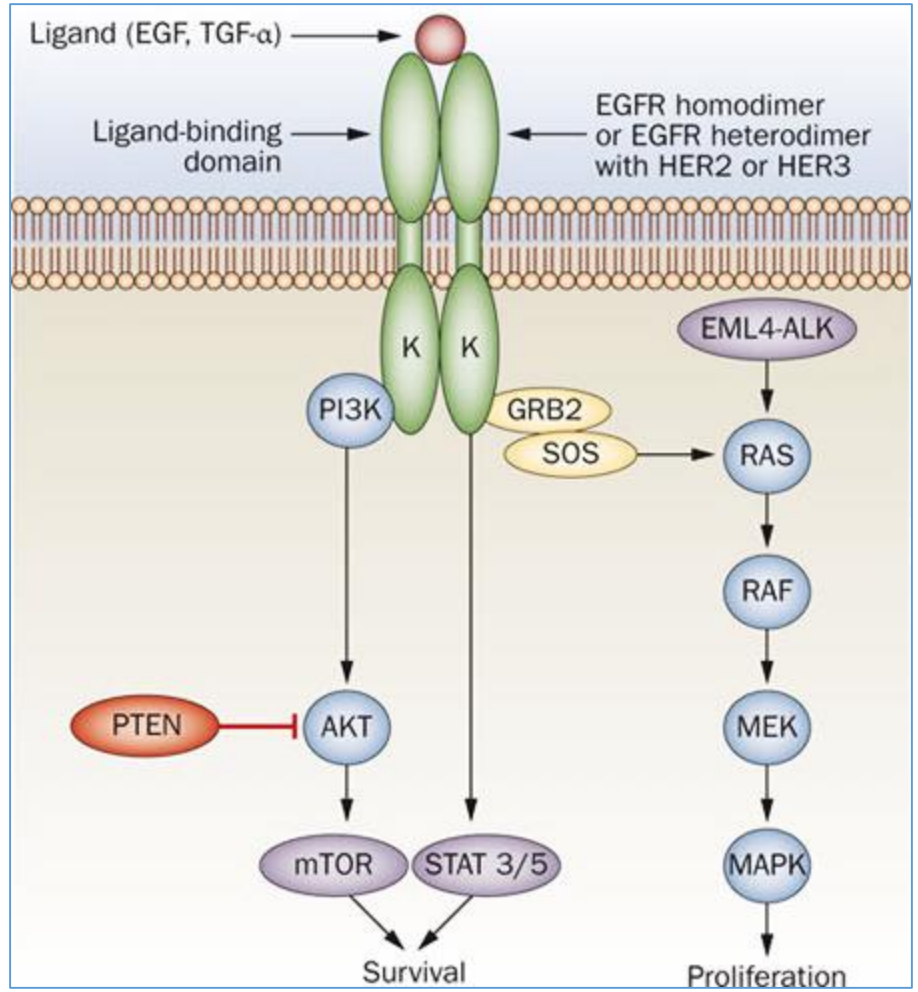

SYSTEMIC THERAPY FOR METASTATIC DISEASE
HISTOLOGIC SUBTYPE
TESTING
TESTING RESULTS
Metastatic Disease
• Establish histologic subtype[a] with adequate tissue for molecular testing (consider rebiopsy if appropriate)
• Smoking cessation counseling
• Integrate palliative care[C] (See NCCN Guidelines for Palliative Care)
• Adenocarcinoma
• Large Cell
• NSCLC not otherwise specified (NOS)
• *EGFR* mutation testing (category 1)[a]
• *ALK* testing (category 1)[a]
• *EGFR* and *ALK* testing should be conducted as part of broad molecular profiling[hh]
Sensitizing *EGFR* mutation positive
See First-Line Therapy (NSCL-17)
ALK positive
See First-Line Therapy (NSCL-18)
Both sensitizing *EGFR* mutation and *ALK* are negative or unknown[kk]
See First-Line Therapy (NSCL-19)
Squamous cell carcinoma
• Consider *EGFR* mutation and *ALK* testing[ii] especially in never smokers or small biopsy specimens, or mixed histology[jj]
• *EGFR* and *ALK* testing should be conducted as part of broad molecular profiling[hh]
Sensitizing *EGFR* mutation positive
See First-Line Therapy (NSCL-17)
ALK positive
See First-Line Therapy (NSCL-18)
Both sensitizing *EGFR* mutation and *ALK* are negative or unknown[kk]
See First-Line Therapy (NSCL-20)

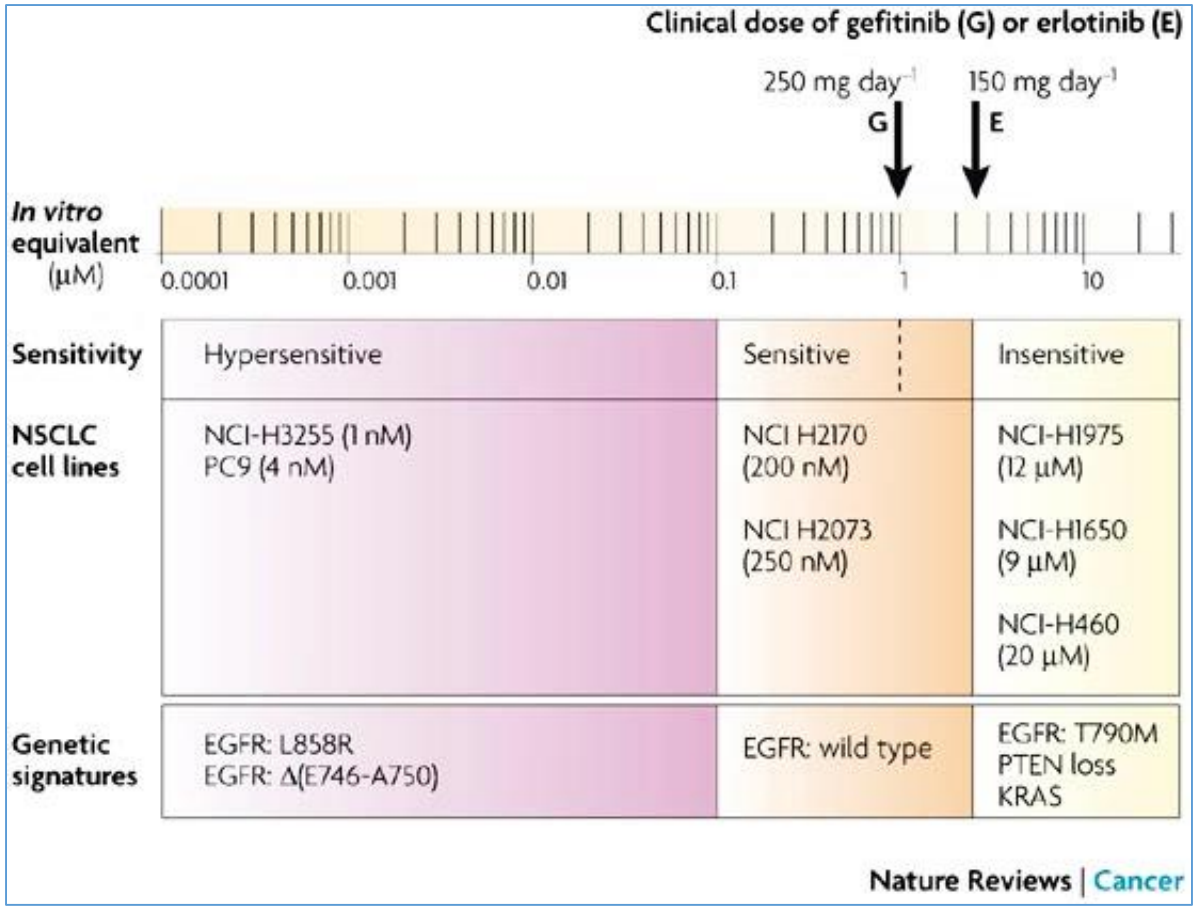


[a] See Principles of Pathologic Review (NSCL-A).


ALK POSITIVE[a]
FIRST-LINE THERAPY[ff]
SUBSEQUENT THERAPY[ff]
ALK positive
ALK rearrangement discovered prior to first-line chemotherapy
Crizotinib[ll] (category1)
ALK rearrangement discovered during first-line chemotherapy
Interrupt or complete planned chemotherapy, followed by crizotinib
Progression[rr]
Asymptomatic
Continue Crizotinib[ss] or switch to Ceritinib or Alectinib
Symptomatic
Brain
Consider local therapy and continue ALK inhibitor or switch to Ceritinib or Alectinib and See NCCN Guidelines for CNS Cancers
Systemic
Isolated lesion
Consider local therapy and continue ALK inhibitor
Multiple lesions
Ceritinib or Alectinib
See First-line therapy options for Adenocarcinoma (NSCL-19) or Squamous cell carcinoma (NSCL-20)
Symptomatic systemic progression after local therapies and/or after switching to Ceritinib or Alectinib. See First-line therapy options for Adenocarcinoma (NSCL-19) or Squamous cell carcinoma (NSCL-20)

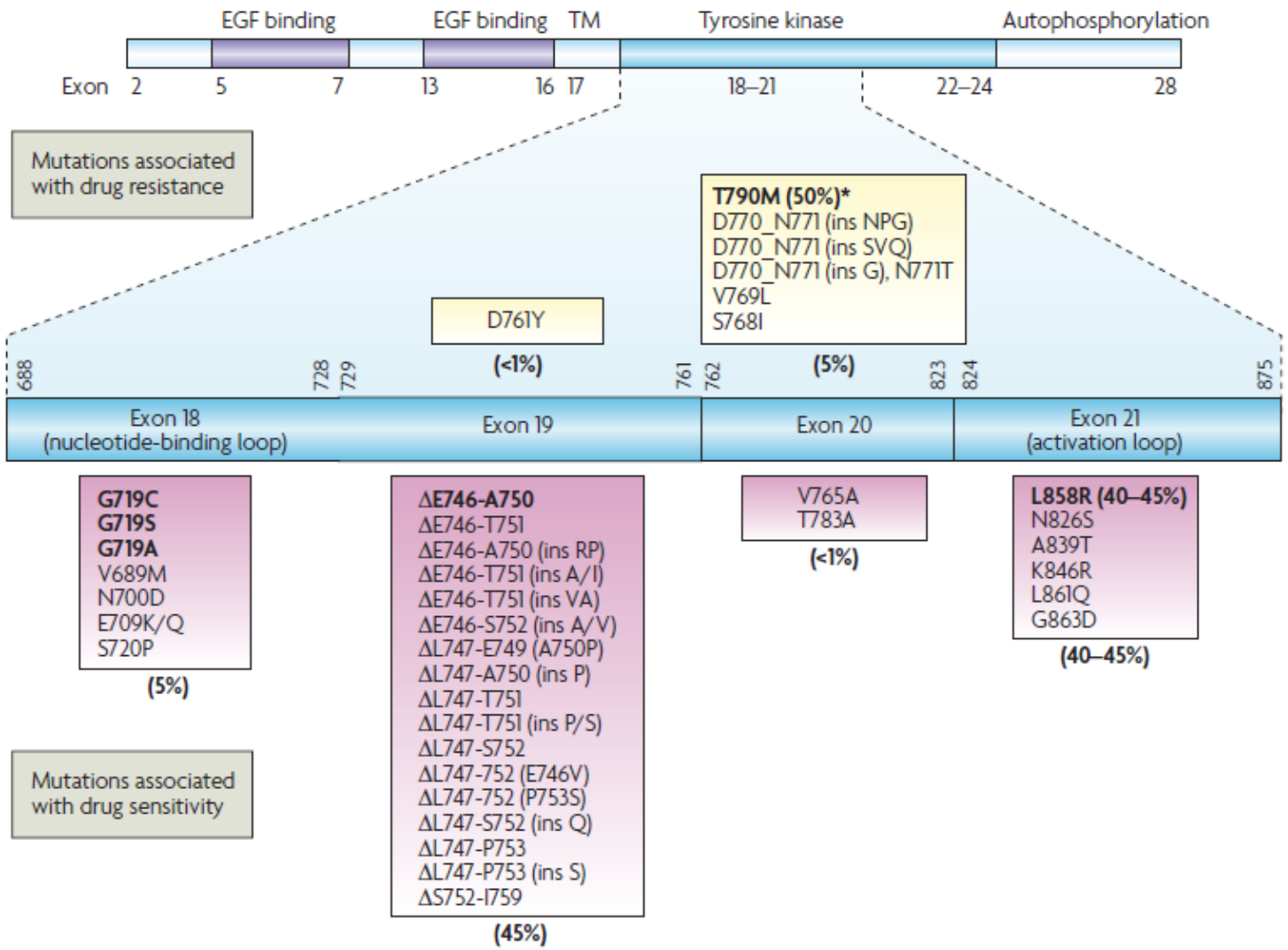

EGFR
α
β
SHC,
SOS, GRB2
IRS1
IRS2
PI3K
p110
p85
PDK1
NF1
RAS
JAK
AKT
PTEN
RAF
MEK
ERK
TSC1
TSC2
mTOR
AMPK1
STK11
STAT
Myc
FOXO
3A
DNA
Nucleus
Inhibition
Activation
Protein complex
Signaling protein
Suppressor protein
Transcription factor


Ligand (EGF, TGF-α)
Ligand-binding domain
EGFR homodimer or EGFR heterodimer with HER2 or HER3
K
K
EML4-ALK
PI3K
GRB2
SOS
RAS
RAF
MEK
MAPK
PTEN
AKT
mTOR
STAT 3/5
Survival
Proliferation



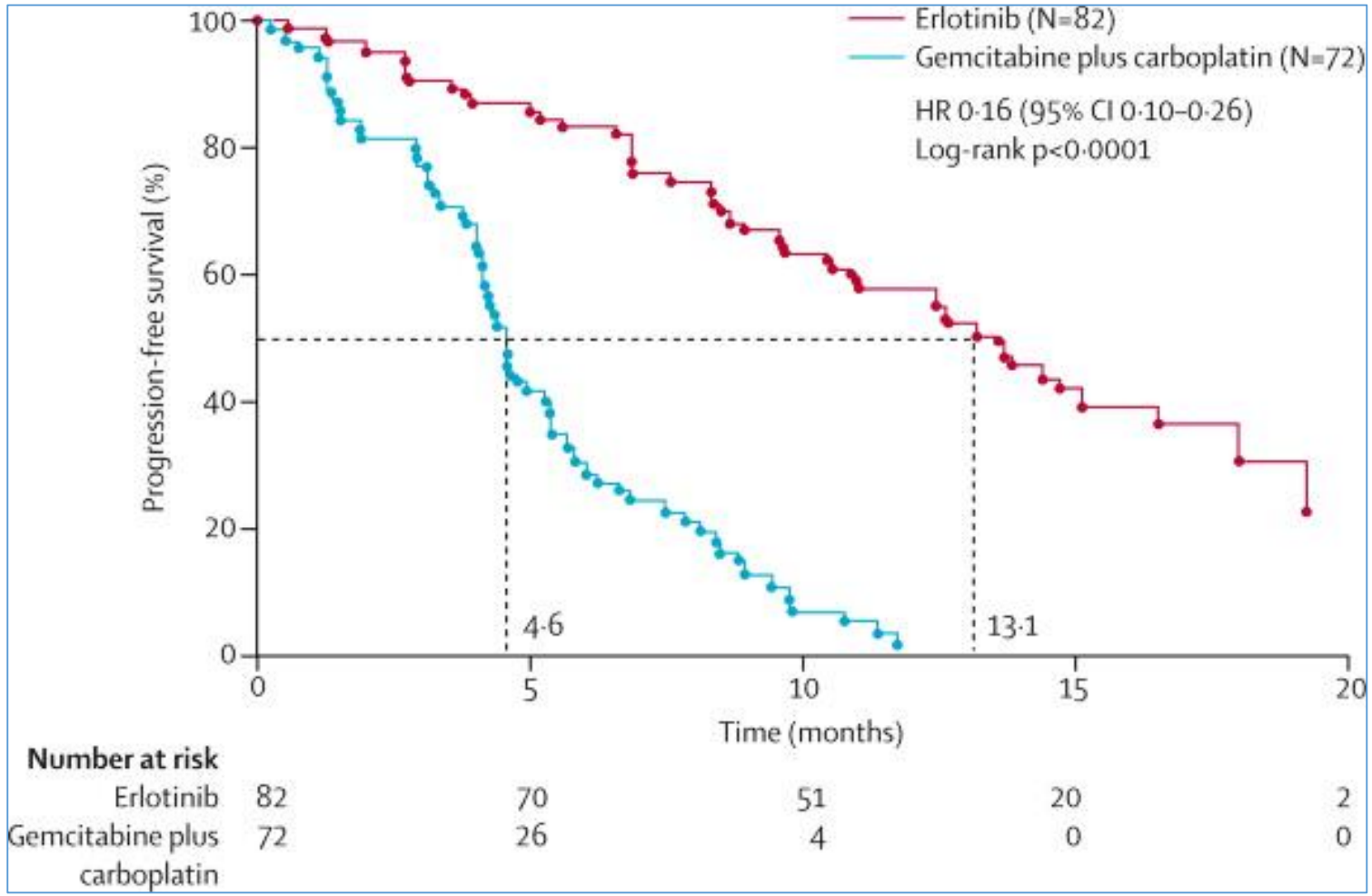
Sharma *et al. Nature Reviews Cancer* **7**, 169–181 (March 2007) | doi:10.1038/nrc2088

nature REVIEWS CANCER



EGFR mutations

Nat Rev Cancer (2007)7:169



OPTIMAL trial

cobas® EGFR Mutation Test v2 (IVD)

- *The very first FDA-approved liquid biopsy assay, expanding targeted therapies to patients unable to contribute a tissue biopsy*

INTENDED USE

The **cobas® EGFR Mutation Test v2** is a real-time PCR test for *the in vitro* qualitative detection and identification of mutations in exons 18, 19, 20, and 21 of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPET) **tumor tissue and/or plasma** from non-small cell lung cancer (NSCLC) patients.

The **cobas®** EGFR Mutation Test v2 for use with plasma is a real-time PCR test for the ***in vitro* qualitative and semi-quantitative** measurement of mutations in exons 18, 19, 20, and 21 of the EGFR gene in human plasma. The EGFR test is further indicated for **serial measurement of EGFR mutation status** as an aid in the management of NSCLC cancer patients.

FFPET specimens are processed using the cobas® DNA Sample Preparation Kit and plasma specimens are processed using the **cobas® cfDNA Sample Preparation Kit**. The **cobas®** EGFR Mutation Test v2 and cobas z 480 analyzer are used for automated amplification and detection.

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MUTATIONS DETECTED

AMPLIFICATION MMX	TARGET
MMX1	EX19Del; S768I; EX28/IC
MMX2	L858R; T790M; EX28/IC
MMX3 v.2	L861Q; G719A/C/S; EX20Ins; EX28/IC

Semi Quantitative Index

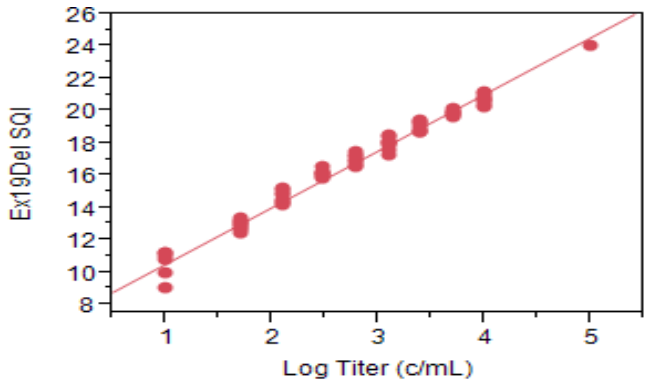
- *New reporting tool for management of NSCLC patients*

What is a Semi Quantitative Index (SQI)?

The SQI is a semi-quantitative measure of the amount of mutant cfDNA in a sample that can be used to measure the presence of EGFR mutations over time

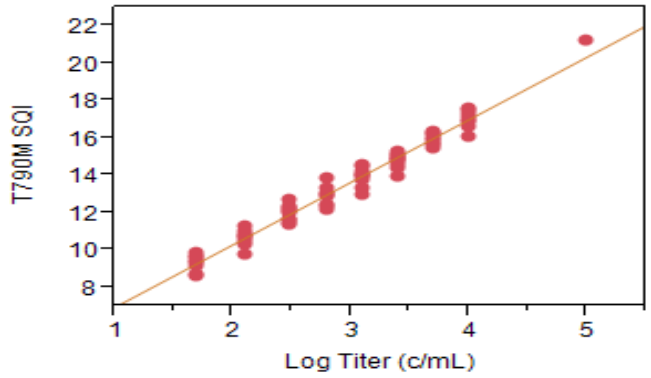
SQI ≈ **MUTANT cfDNA TREND OVER TIME**

Linearity of mutant DNA in K2 EDTA Plasma: Ex19 Del cell line DNA

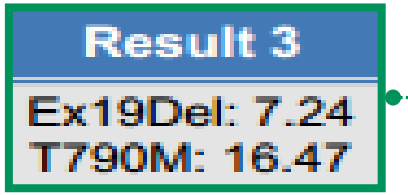


SI = 7.042 + 3.507 * Log Copies per mL
R^2 = 0.981

Linearity of mutant DNA in K2 EDTA Plasma: T790M cell line DNA



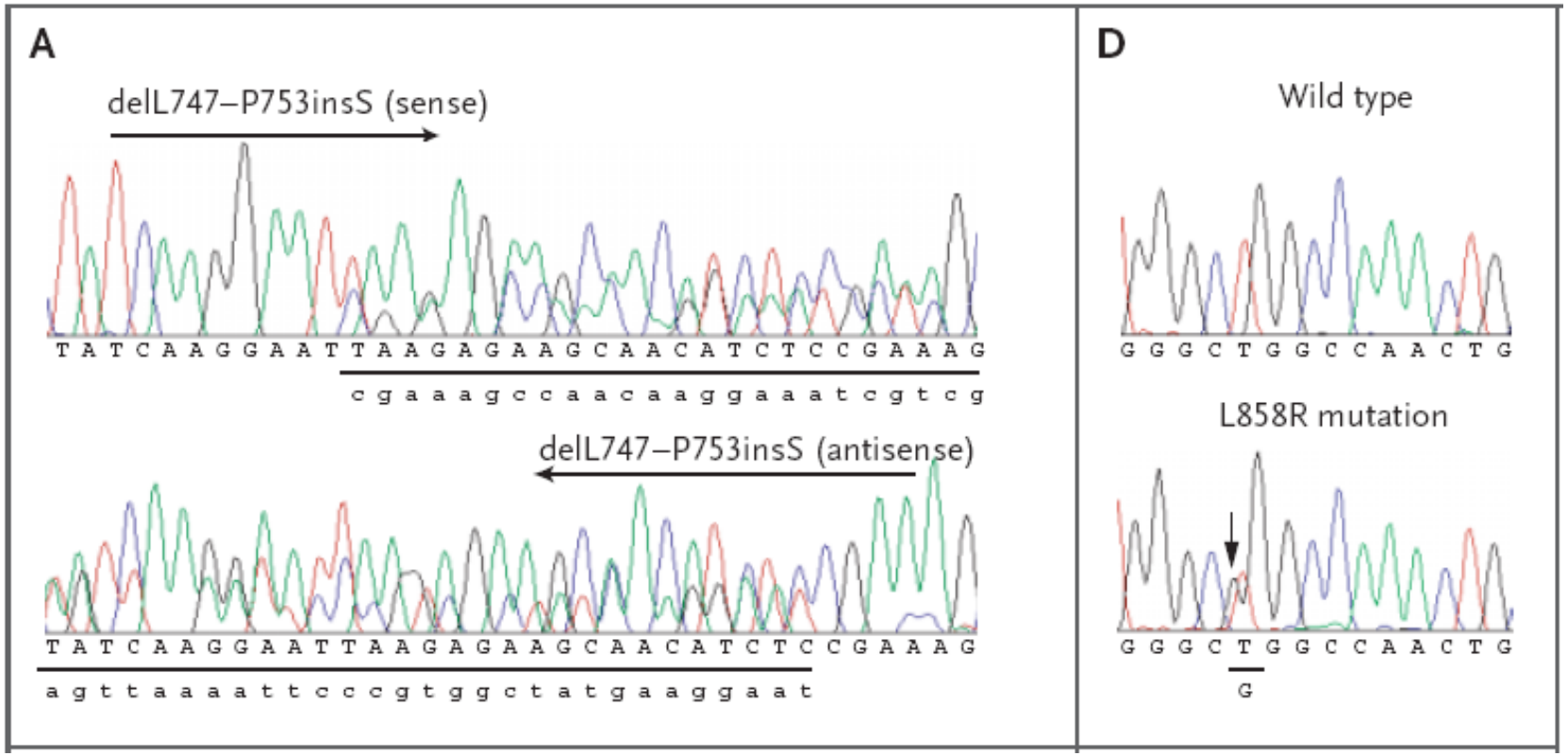
SQ = 3.593 + 3.352 *Log Copies per mL
R^2 = 0.973



SQI TREND
Designed to reflect a change in the amount of mutant cfDNA over time per corresponding target mutation within a patient

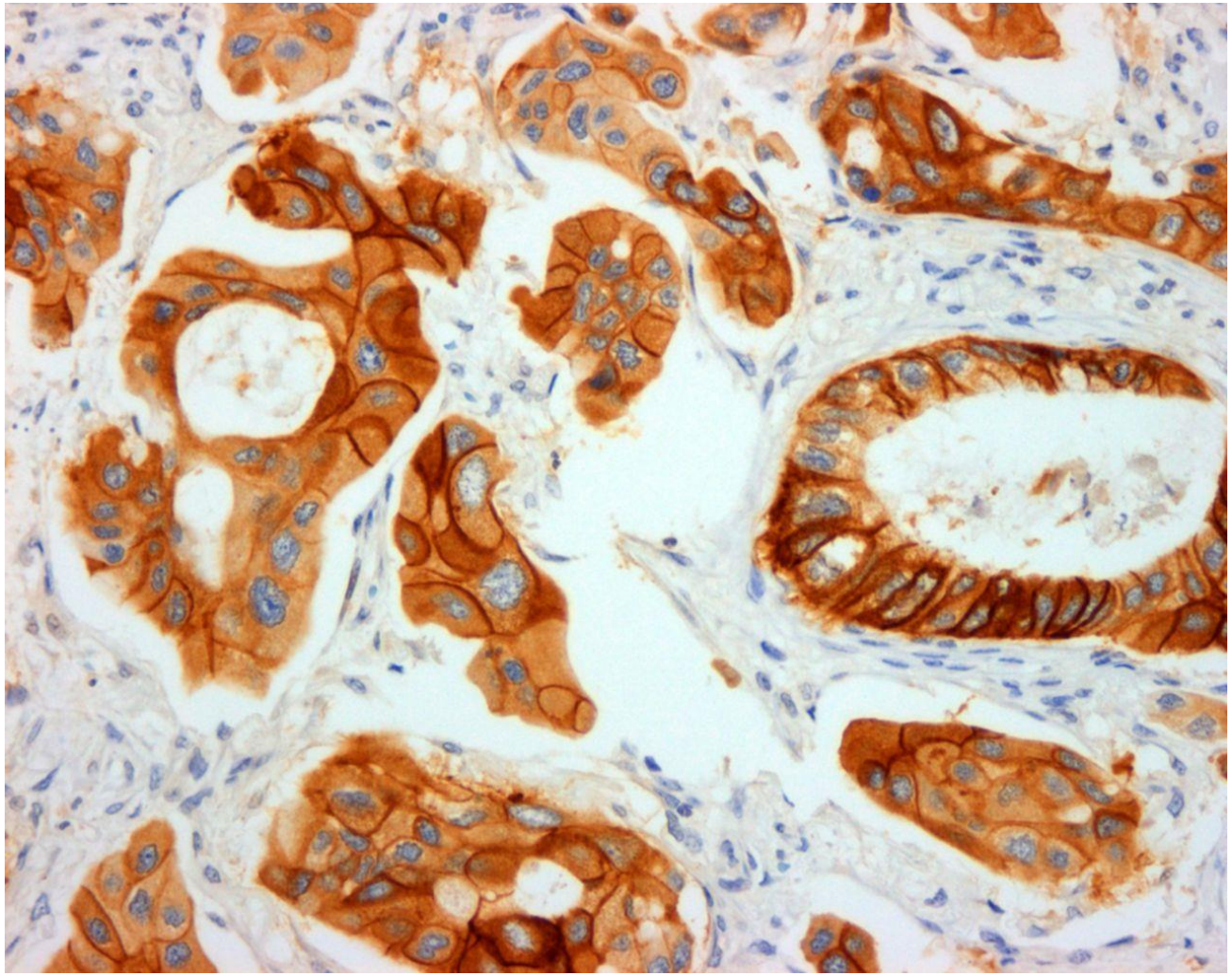
cobas® EGFR Test v2 CE-IVD package insert

Direct sequencing of EGFR mutations



NEJM (2004)350:2129

Adenocarcinoma with positive staining for EGFR *exon 21 L858R mutation-specific antibody (x200)*



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