The Role of Pathologist in Quality Assurance of Cancer Biomarking

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Home Town


Welcome to
Croeso i dref
PENARTH
Twinned with
Saint Pol de Leon, France
Wedi'i gefeillio â
Saint Pol de Leon, Ffrainc
PLEASE DRIVE CAREFULLY · GYRRWCH YN OFALUS


GARETH JOHNS
PHOTOGRAPHY

20th February 2015
Astronomical super-tide
48 ft rise in water
in just 4 hours

Cardiff University

Founded in 1883

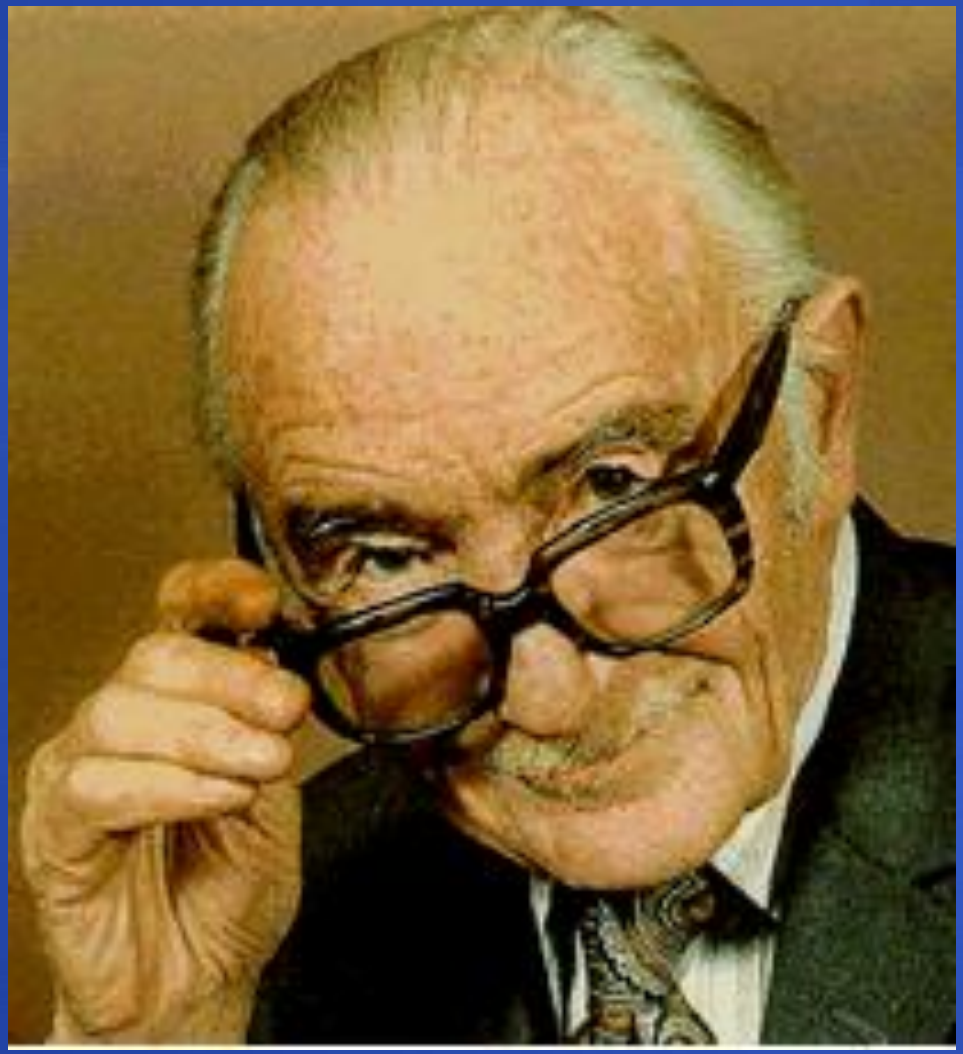
2015 Top five UK university for research excellence and impact

- 26,000 students, 6000 staff (12th largest)
- Member of the Russell Group

University Hospital of Wales

Medical School

University Main Building



Archibald Leman Cochrane
Born 12 January 1909
Galashiels, Scotland
Died 18 June 1988 (aged 79)
Nationality Scottish
Occupation Physician

https://en.wikipedia.org/wiki/Archie_Cochrane

Quality Assurance of Medical Evidence

Brief Outline

- Reflections on the past
  - Previous home and institution
  - Path to a clinical academic career in pathology
- Role of a pathologist in diagnostic medicine
- Cancer biomarking
  - Role in diagnostic pathology
  - Quality assurance
- Conclusion & Discussion

Career Path in Clinical Academic Pathology

- 2nd MB
  - Glasgow University, Scotland — 2 years
- BSc (Hons) Biochemistry
  - Glasgow University, Scotland — 2 years
- PhD in Immunology
  - Birmingham University, England — 3 years
- MBChB
  - Birmingham University, England — 4 years
- MRCPath
  - Cardiff University, Wales — 12 years

Role of Pathology in Diagnostic Medicine

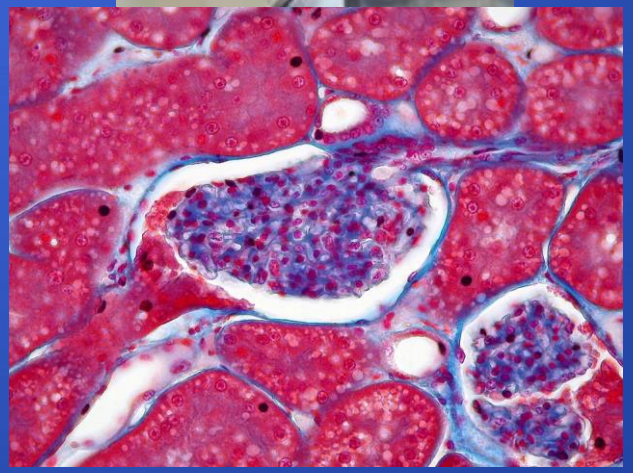
- Represent several different specialties
  - Cellular pathology, haematology, medical biochemistry & microbiology, clinical immunology
- Pathologists work in laboratories, in clinics and wards
- Millions of pathology tests /year
  - 14 tests for every man, woman and child in UK per year
- Many major advances depend on pathologists
  - Guidance to treatment of cancer & genetic disorders
  - ensuring safe blood transfusions
  - developing vaccines against infectious diseases
- Pathology is involved in 70% of all diagnoses and majority of the scientific advances made in Medicine

Role of a Pathologist in Cancer Medicine

- Diagnostic, prognostic & predictive analysis of disease as a guide for more precise & effective patient management
- Translational Research
- Quality assurance
  - Breast cancer as an example

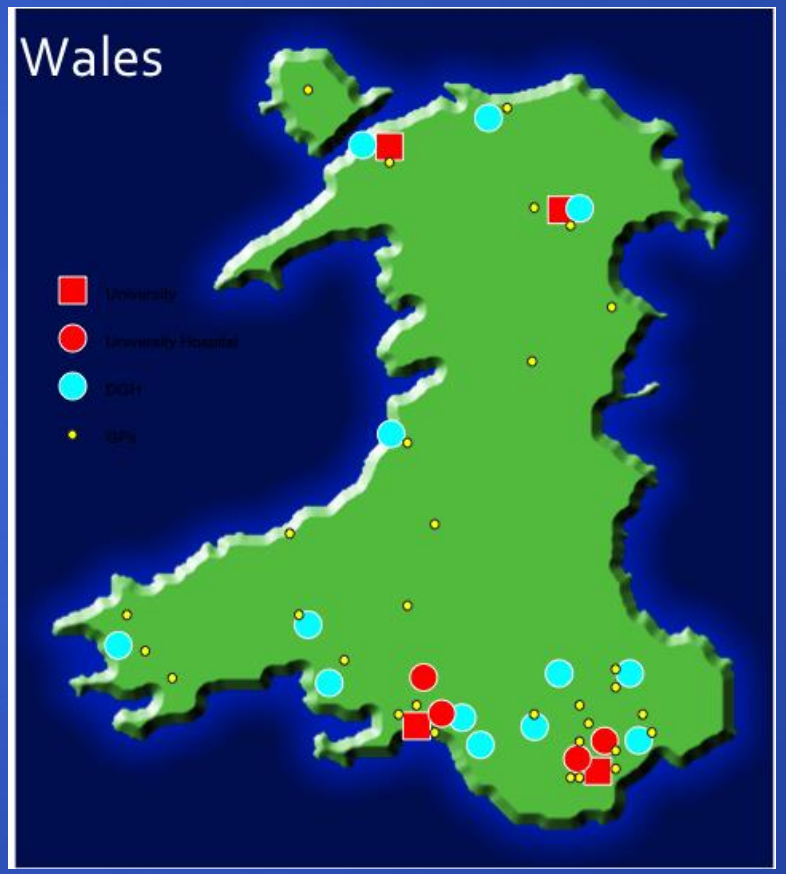
Tissue Based Analysis of Cancer

- Core & excision biopsies
- Macroscopic examination
- Microscopic examination
- Molecular analysis
  - Immunohistochemistry
  - In situ hybridisation
  - Genomic analysis

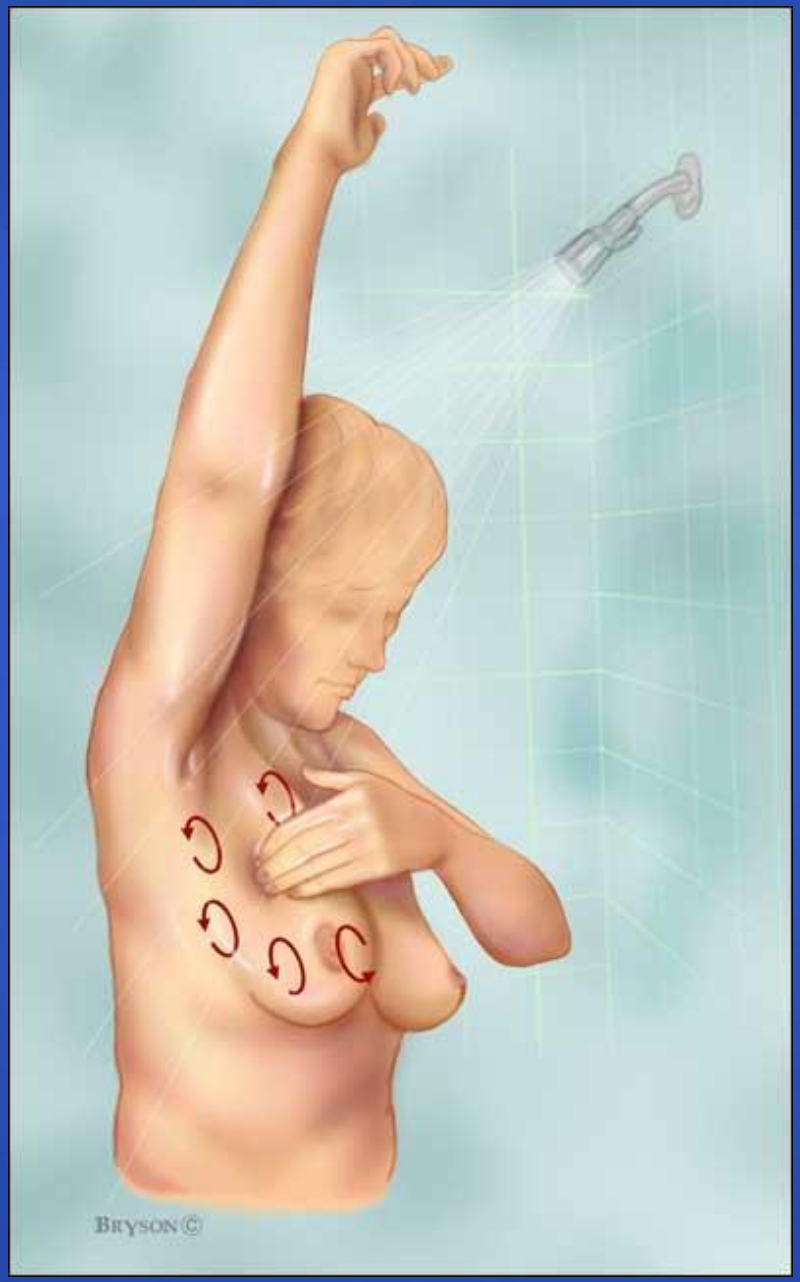


Breast Cancer Biomarking Workload: U.K. and Wales

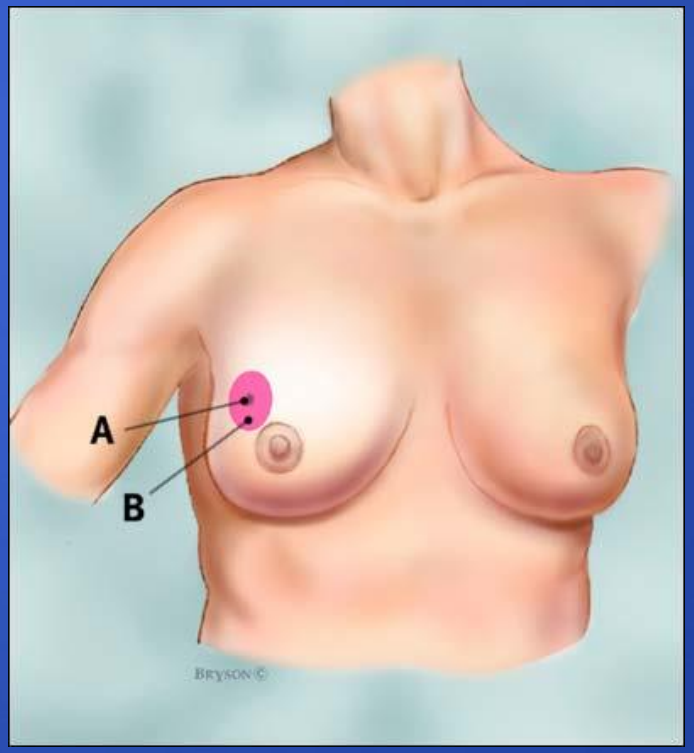
- UK
  - 50,000 new cases / year
- Wales
  - 2,500 new cases/year
- South East Wales Cancer Network
  - 1,500 new cases/year



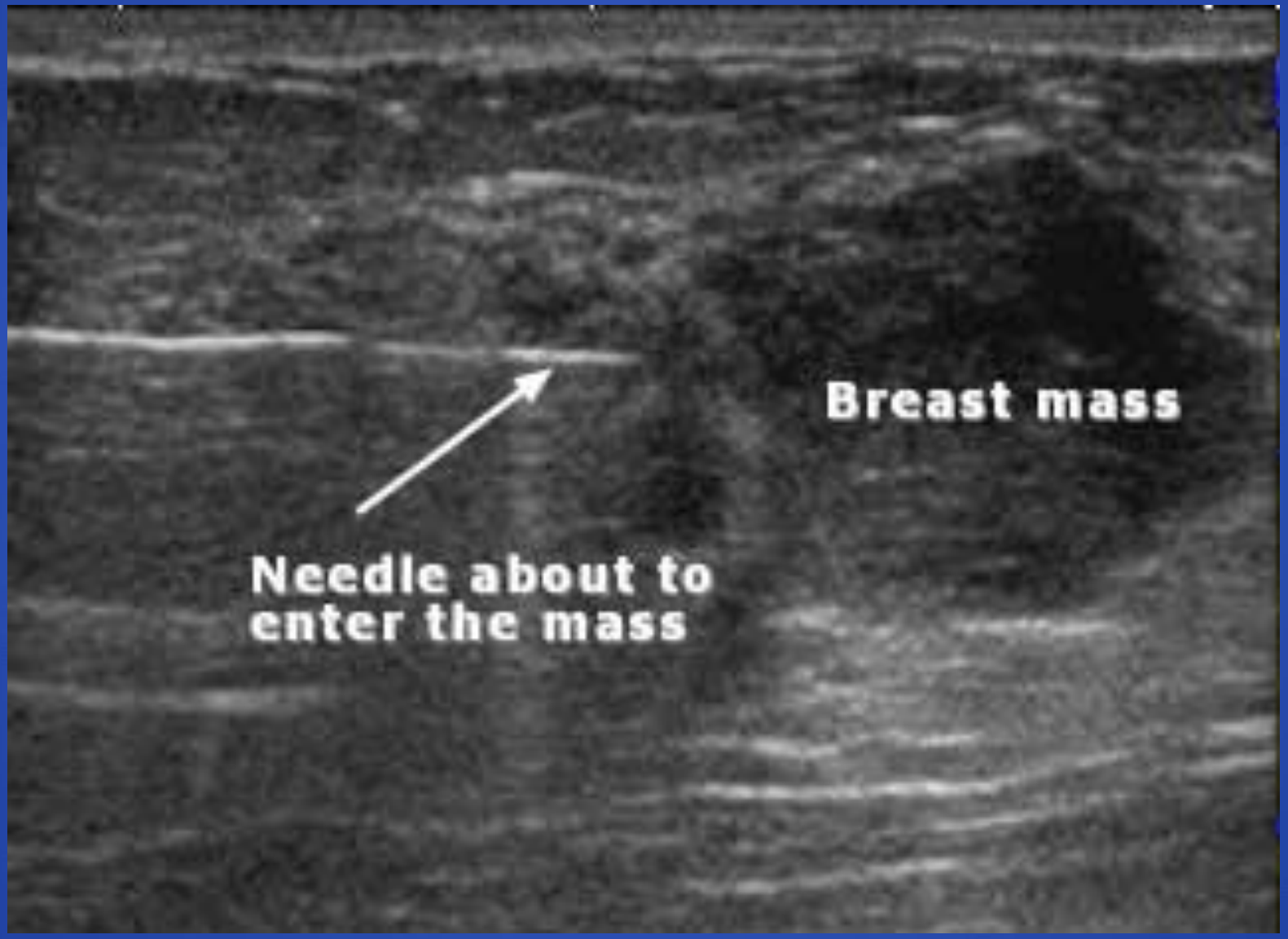
Symptomatic Diagnosis

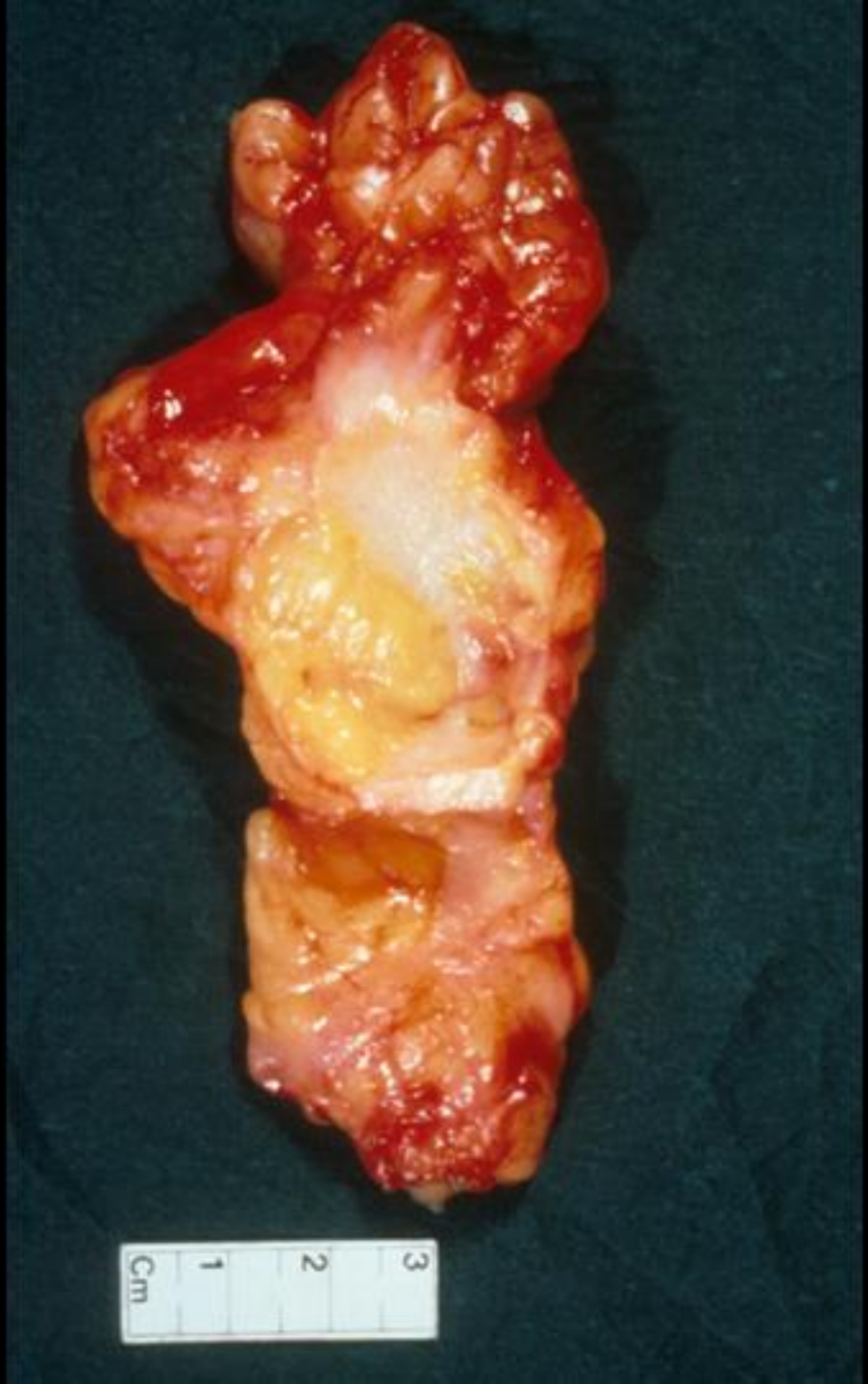


Clinical Diagnosis – Physical Examination

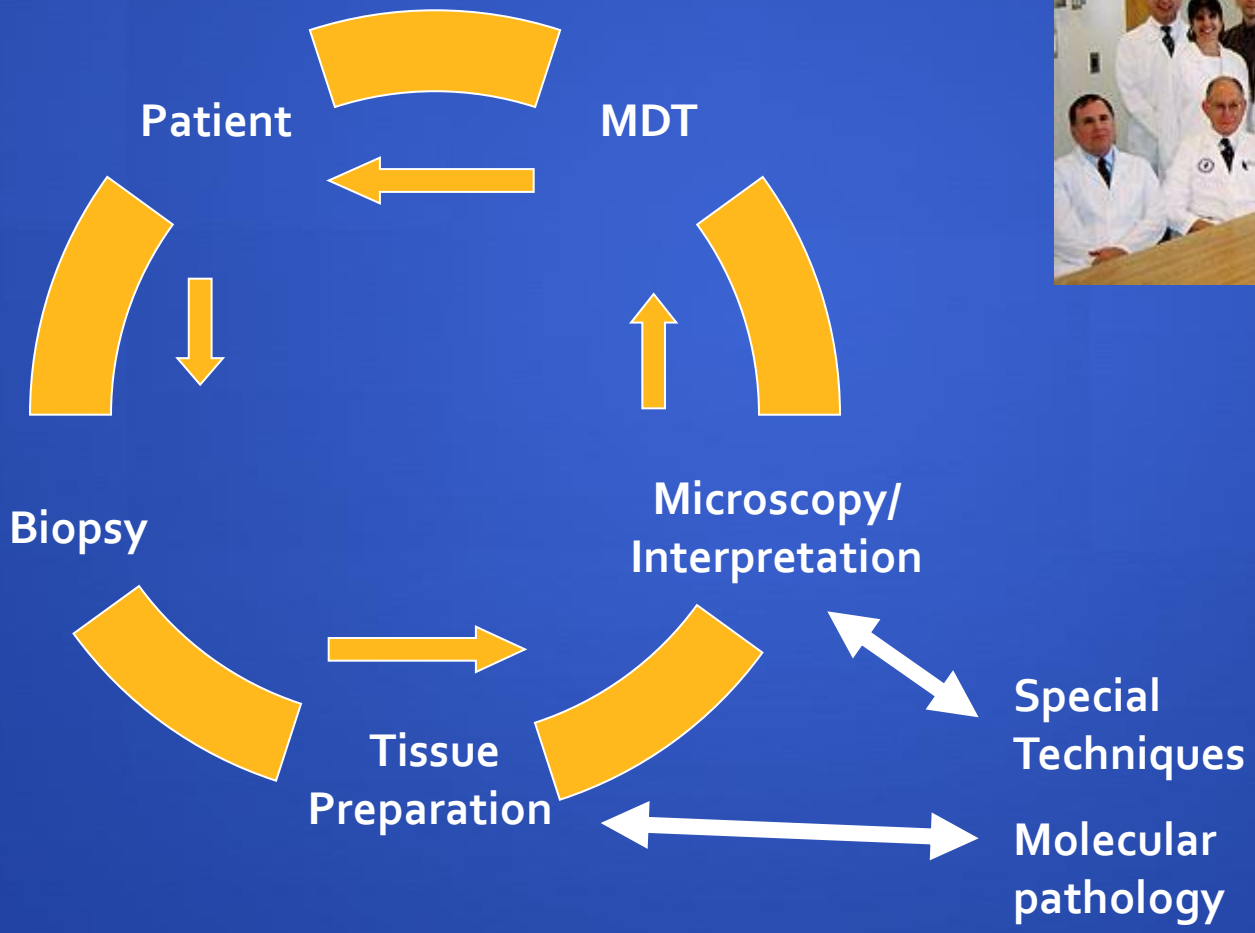


Triple Assessment Confirmation


Breast mass
Needle about to
enter the mass

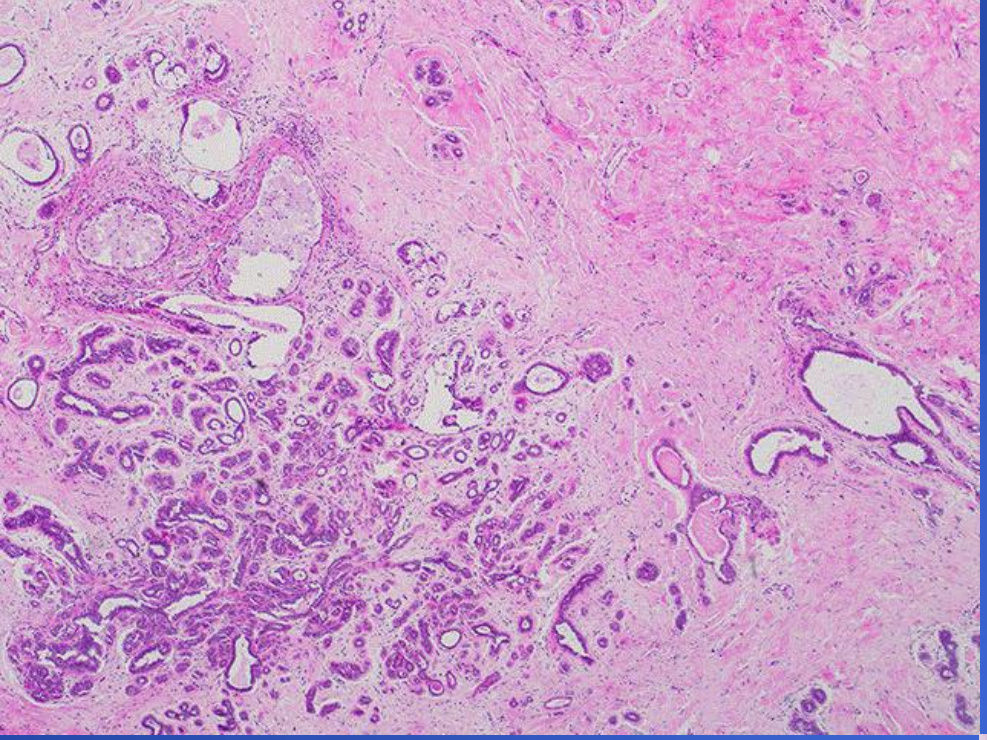

Cm
1
2
3

Breast Cancer Diagnostic Process



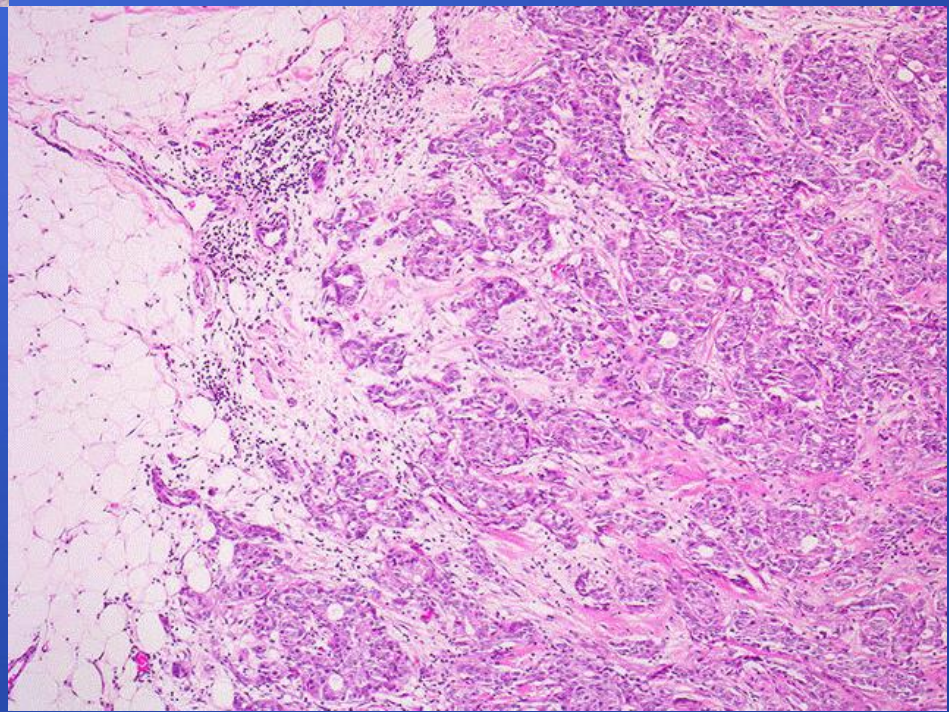
Biopsy Processing & Analysis

Macroscopic examination
Microscopic examination
Immunocytochemistry
Molecular analysis



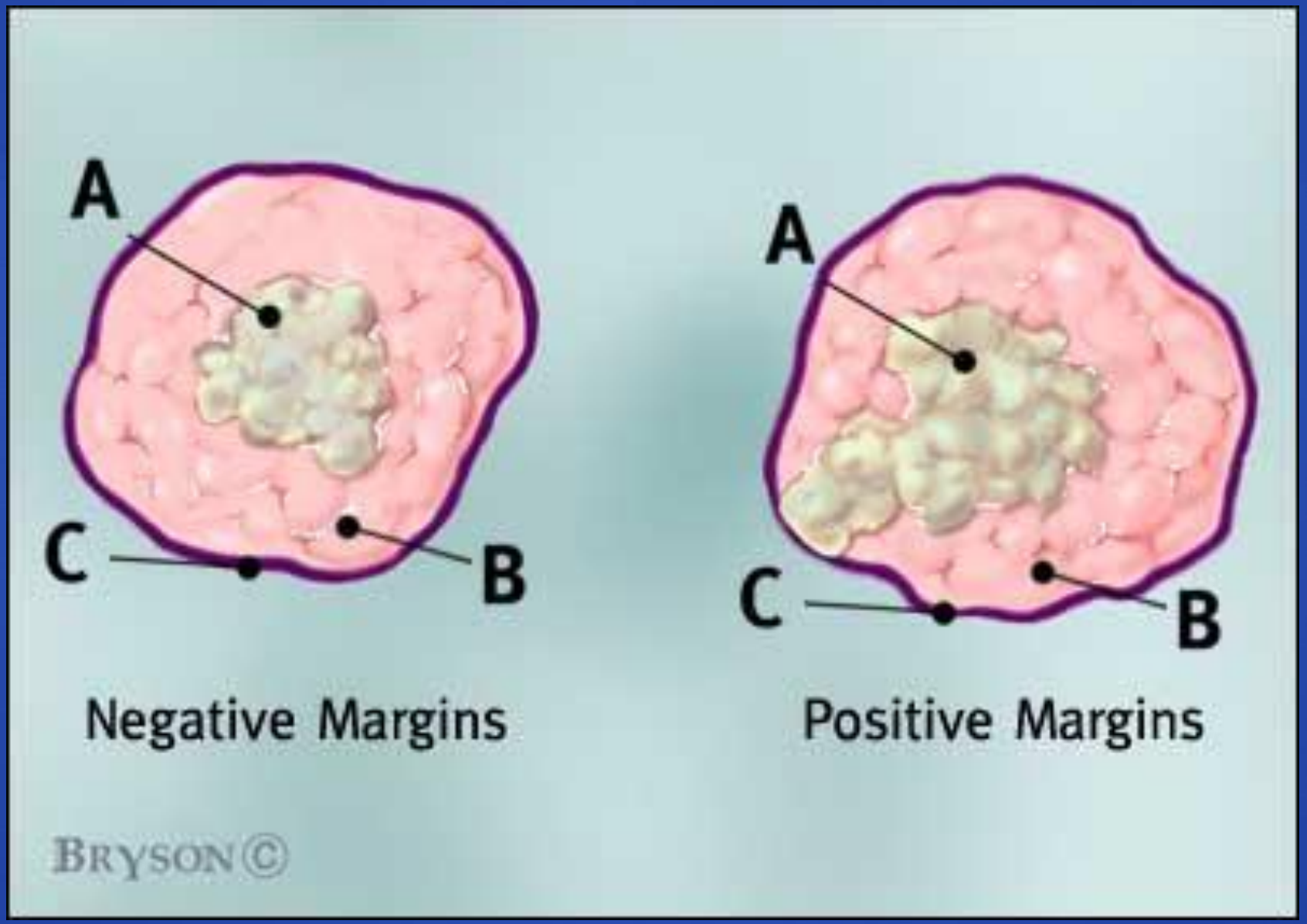
Benign lesion

Malignant lesion



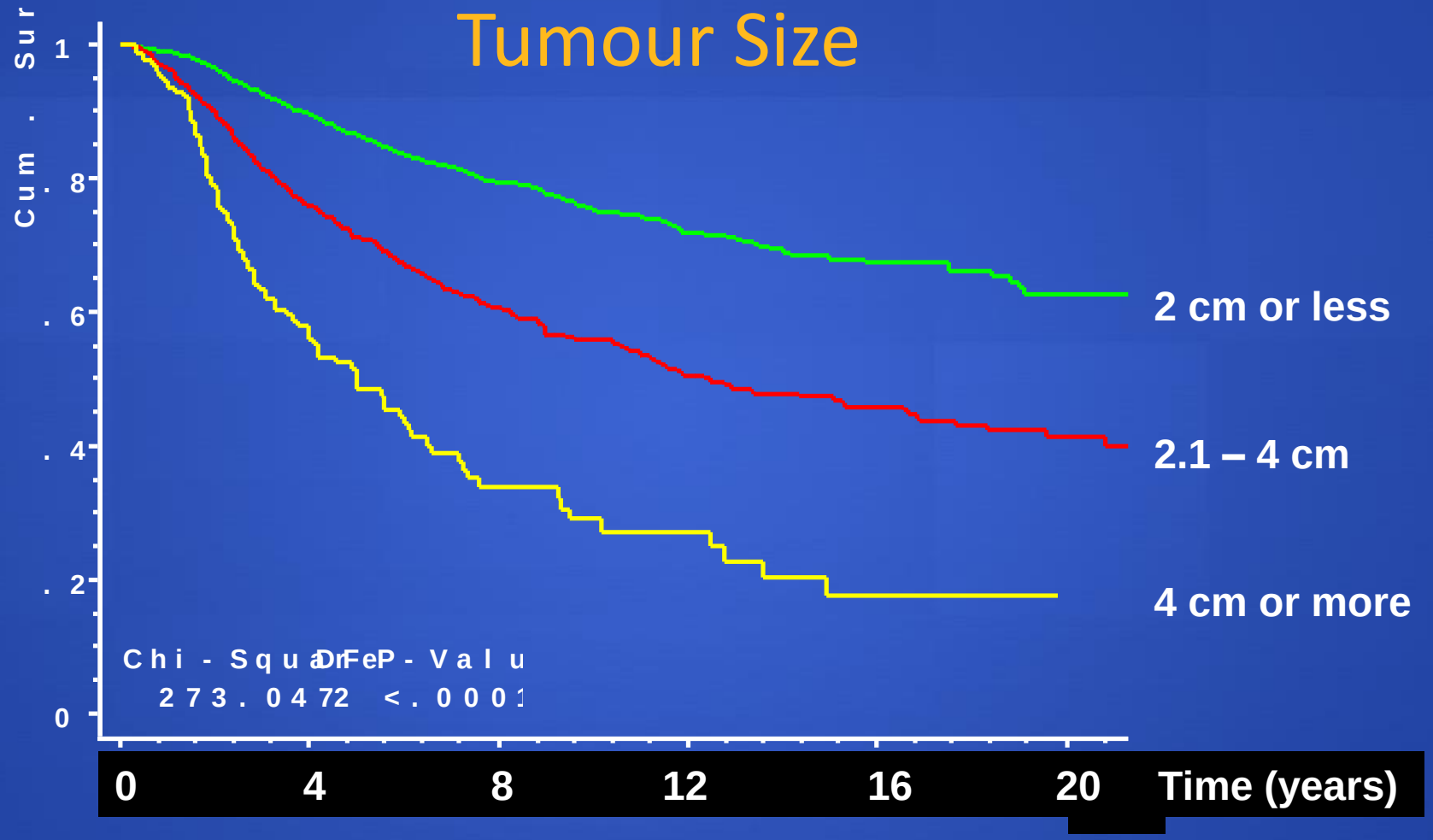
Prognostic Typing of Breast Cancer
Histopathology Minimum Data Set, UK

- Excision margins
- Tumour size
- Histological type
- Histological grade
- Lymph node stage
- Vascular invasion
- In situ component
- Hormone receptor status
- HER2 Status


A
C
B
Negative Margins
A
C
B
Positive Margins
BRYSON©

Nottingham Tenovus Primary Breast Cancer Study

Tumour Size

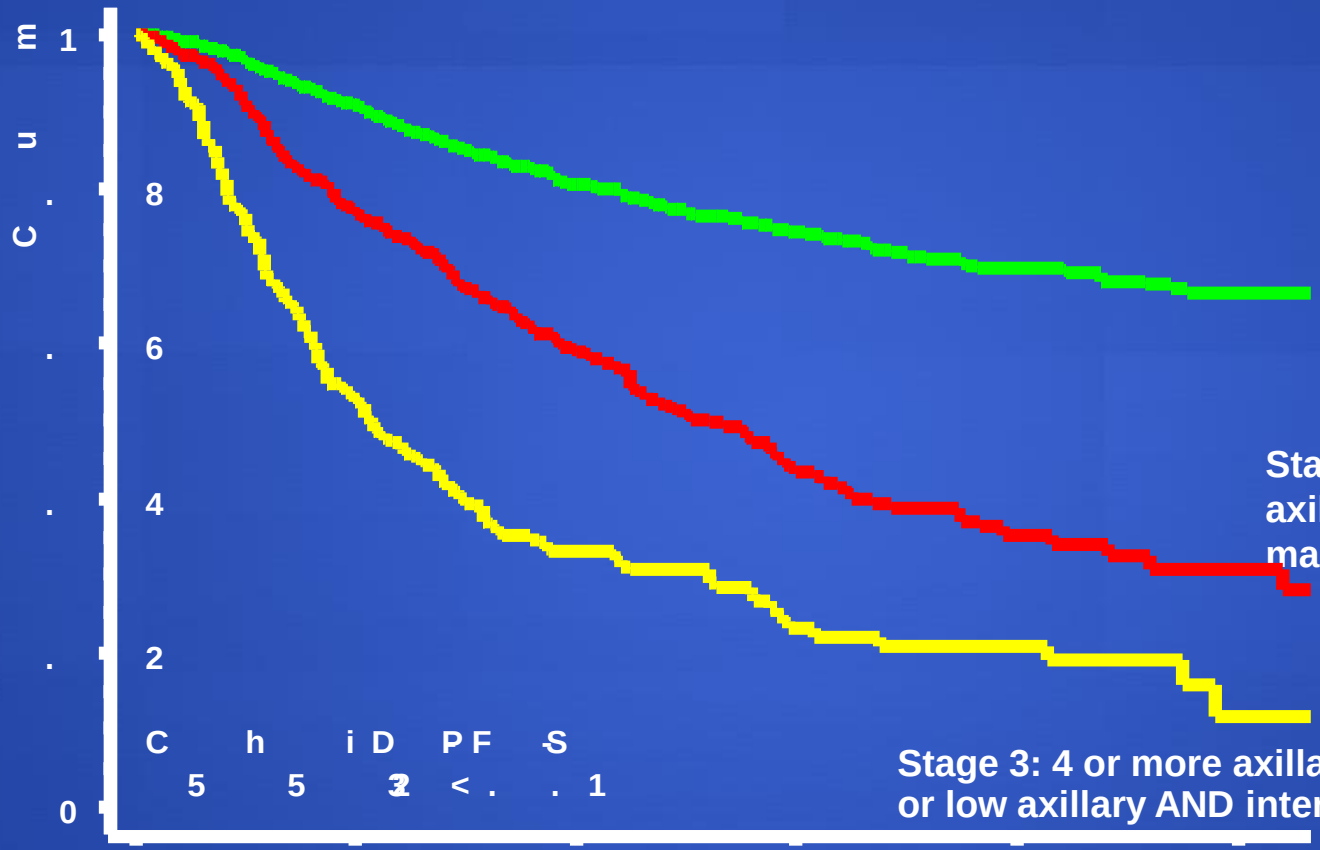


Nottingham Tenovus Primary Breast Cancer Study

Histological Grade

Nottingham Tenovus Primary Breast Cancer Study

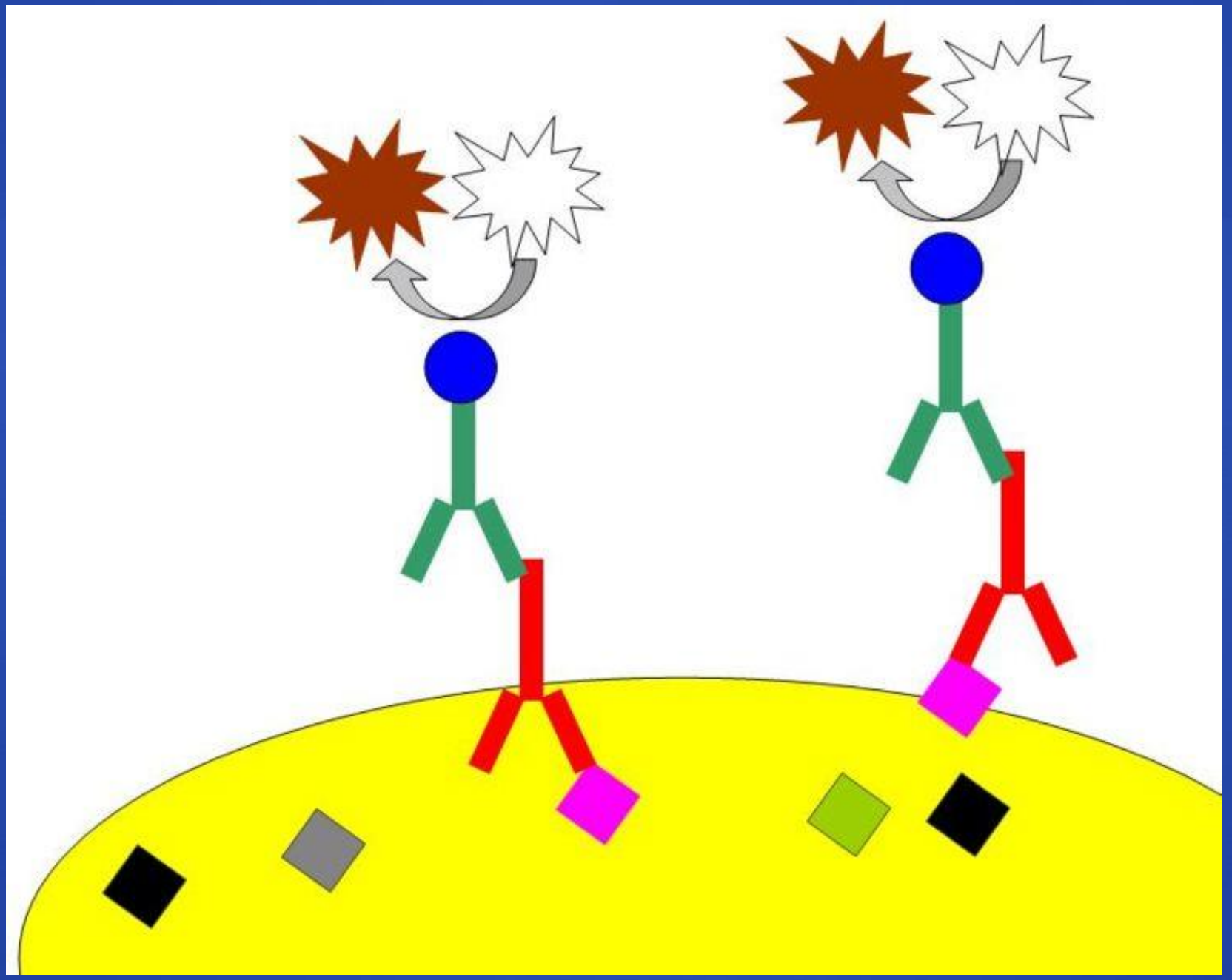
Lymph Node Stage

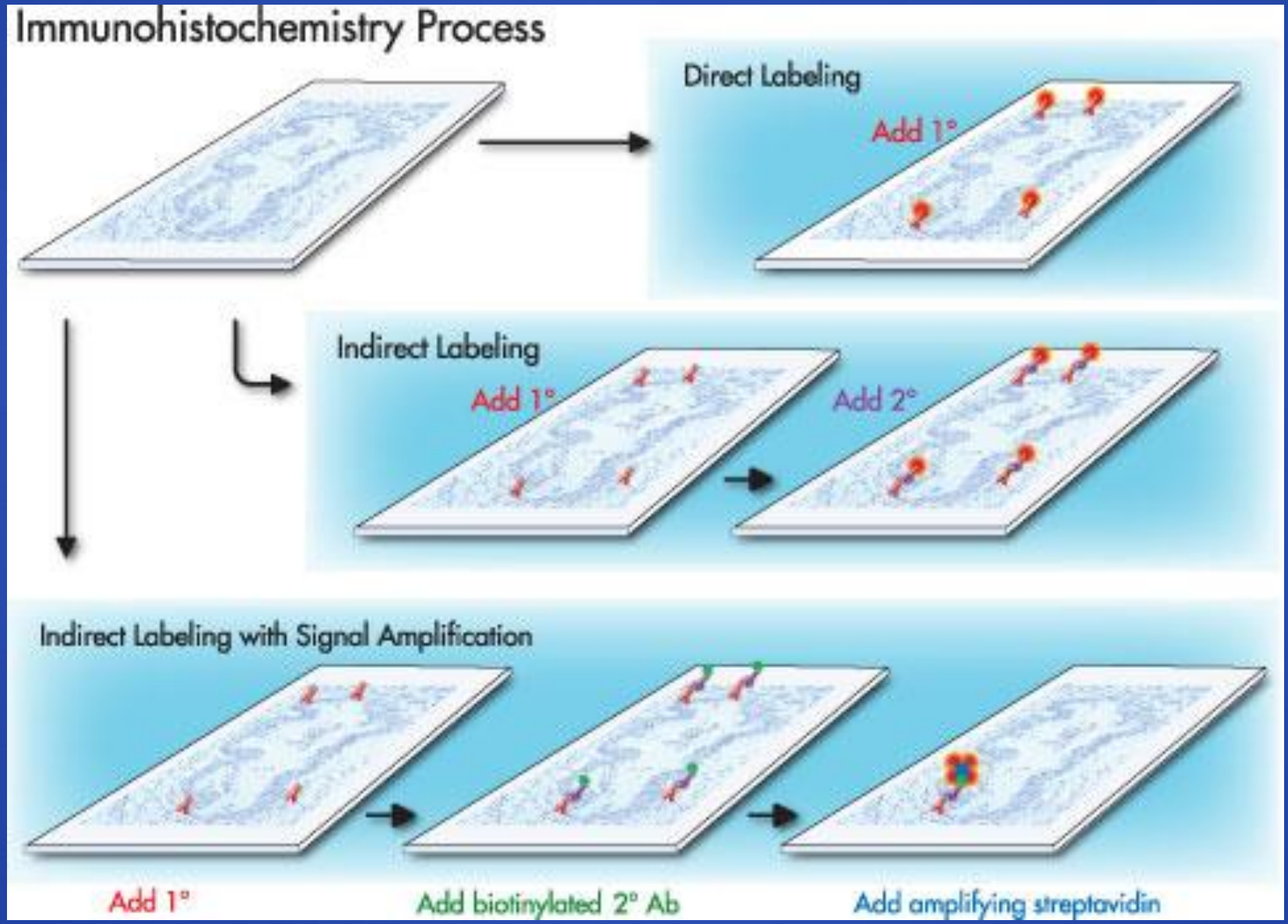


0	4	8	12	16	20	Time (years)
2360		909		207		Stage 1
973		192		34		Stage 2
385		56		13		Stage 3

Predictive Analysis

- Immunohistochemistry
  - ER & HER2
- In Situ Hybridisation
  - HER2 FISH
- Genomic Analysis
  - 21 gene OncoTypeDx assay




Immunohistochemistry Process
Direct Labeling
Add 1°
Indirect Labeling
Add 1°
Add 2°
Indirect Labeling with Signal Amplification
Add 1°
Add biotinylated 2° Ab
Add amplifying streptavidin

Immunohistochemistry

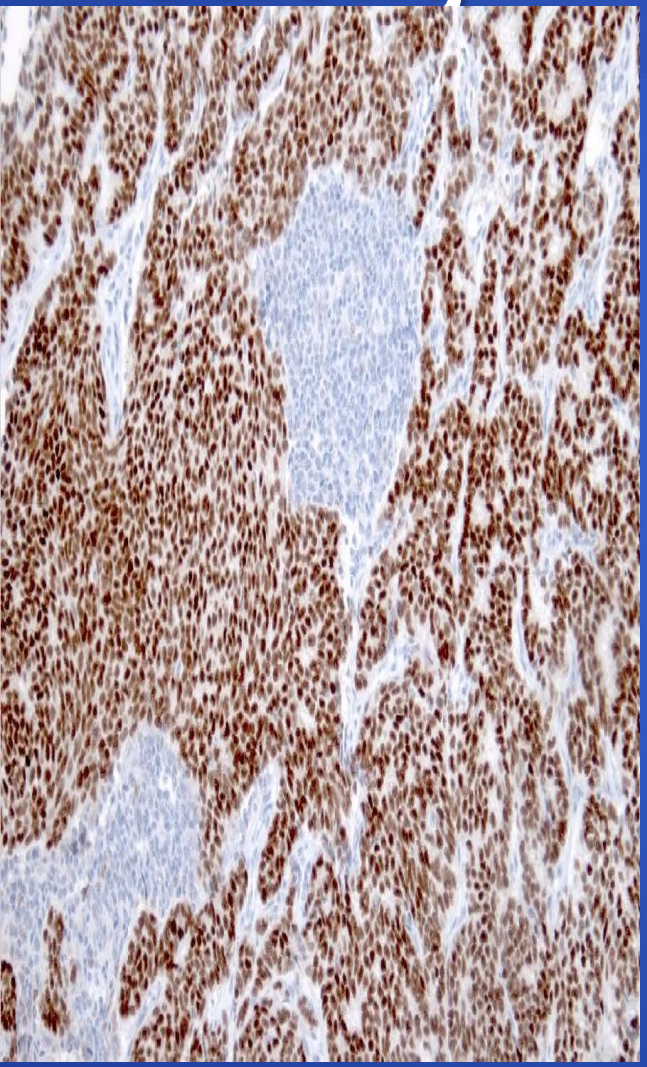
In Situ Hybridisation

Microscopic Assessment

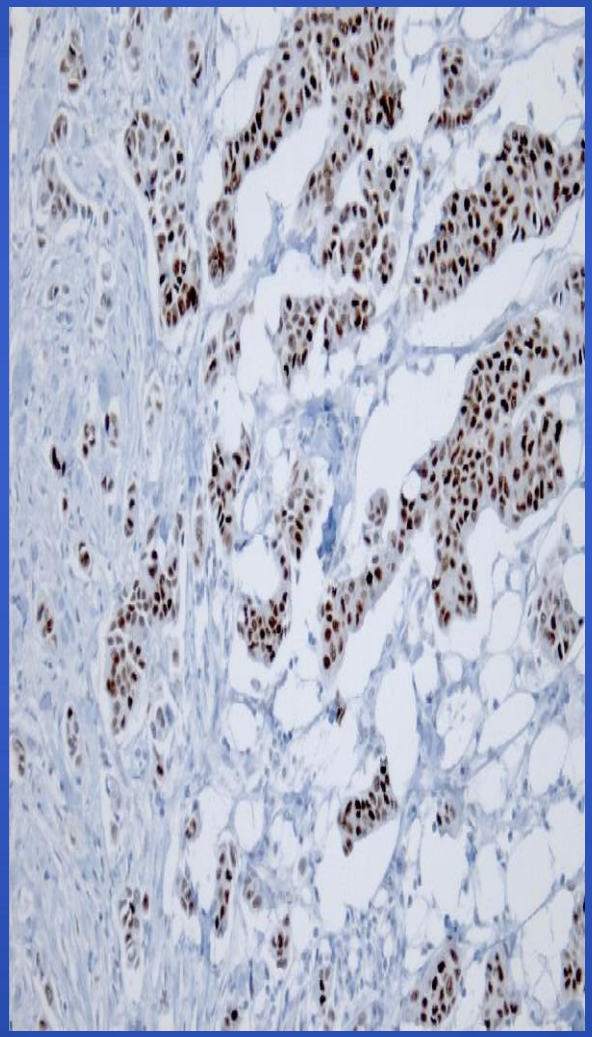
Reporting of Results

ER

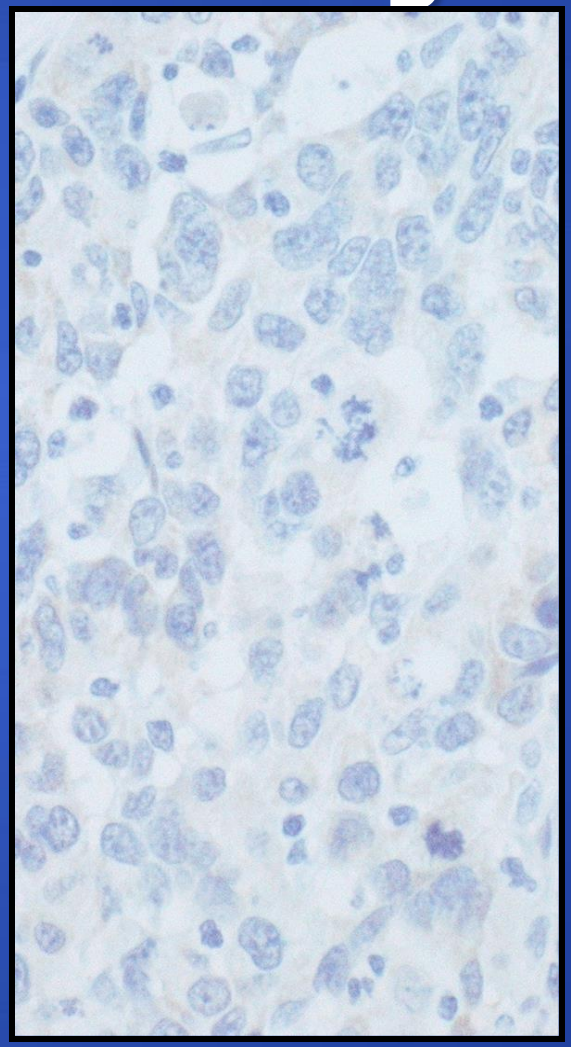
Strong



Medium



Neg



ER BY IHC IN BREAST CANCER

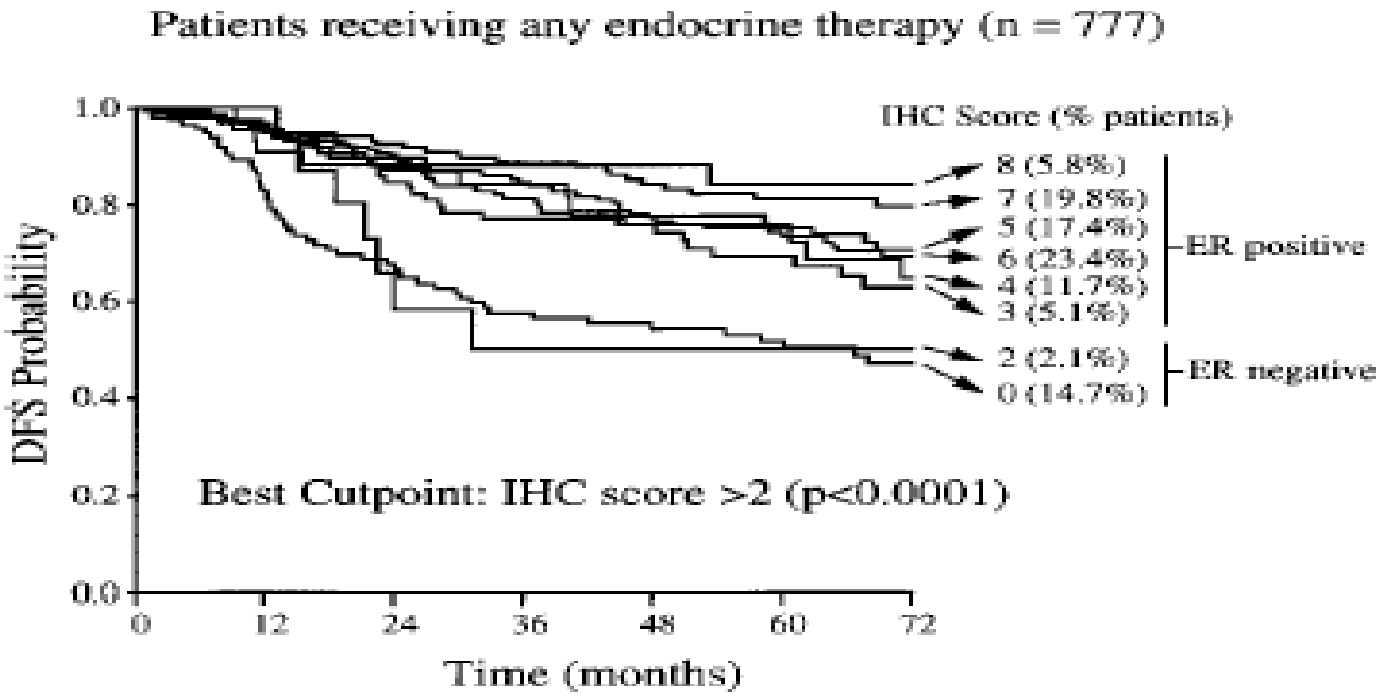
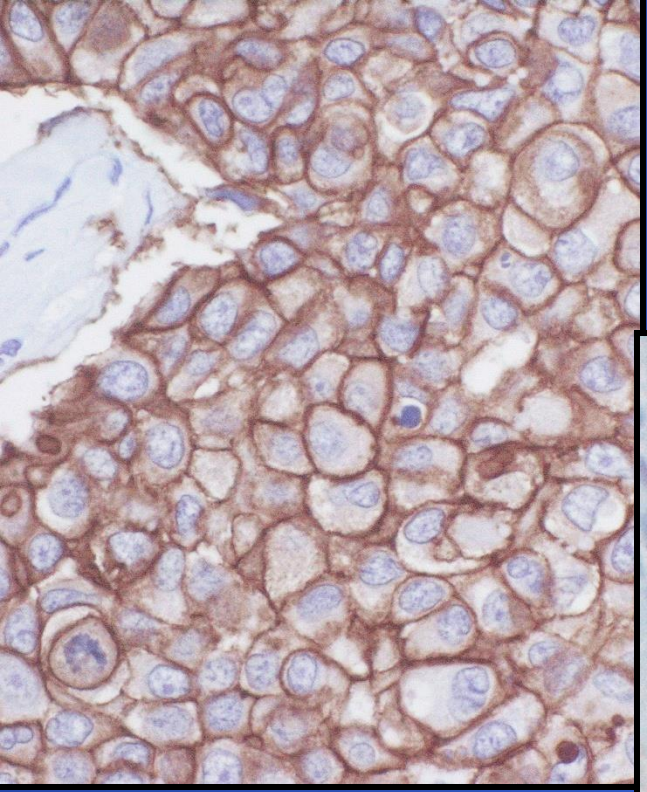
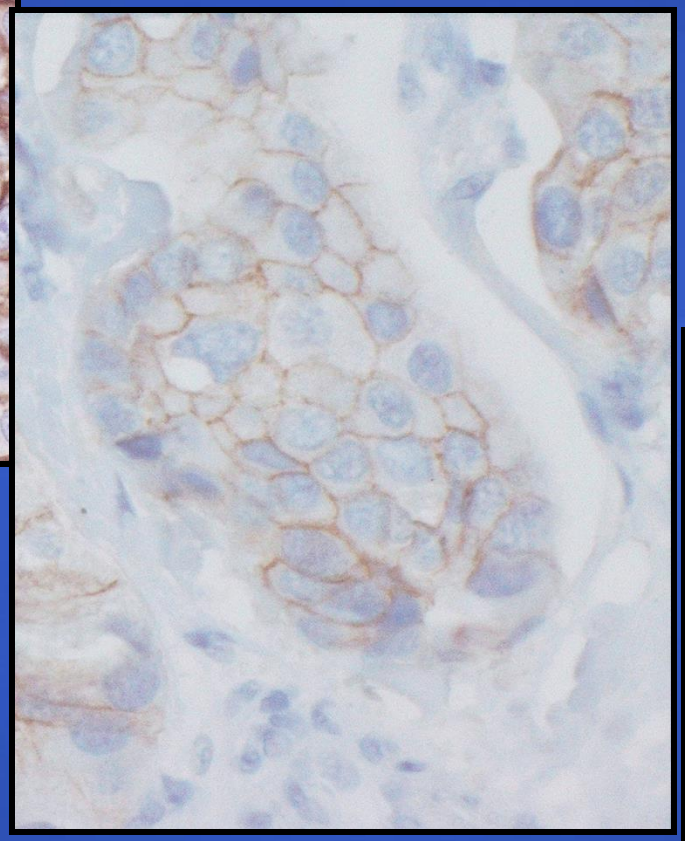


Fig 2. Univariate DFS curves for all possible total IHC scores in patients receiving any adjuvant endocrine therapy (almost always tamoxifen). An IHC score > 2 was the optimal cut point for predicting significantly improved outcome ($P < .0001$), and this value was used to define ER positivity throughout the study.

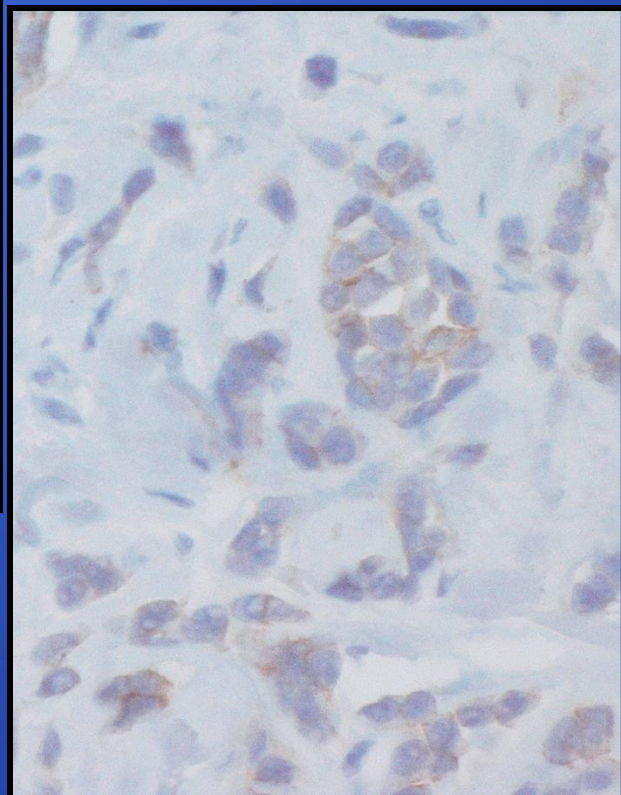


HER2 Positive

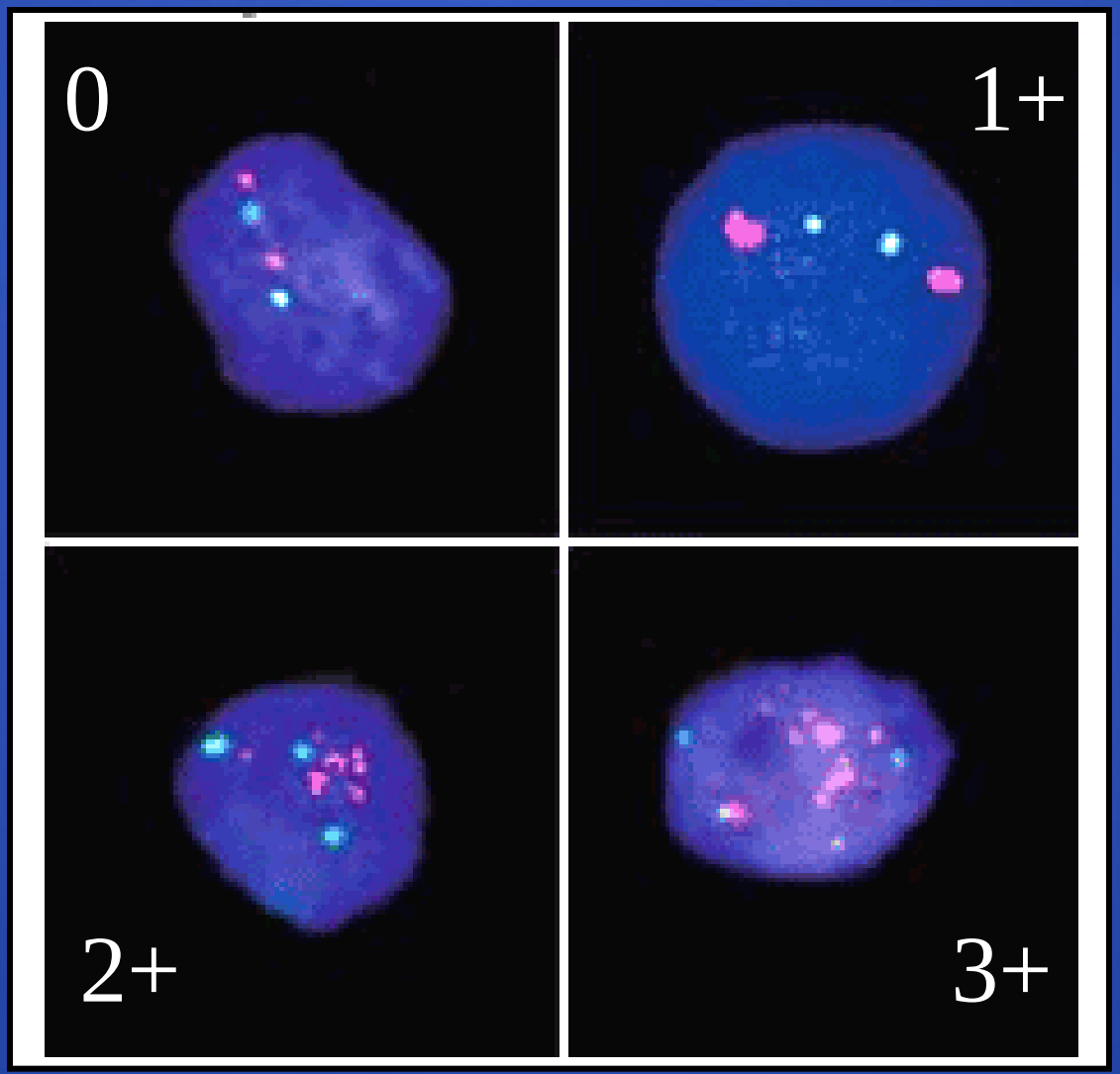


HER2 Negative

HER2 Negative

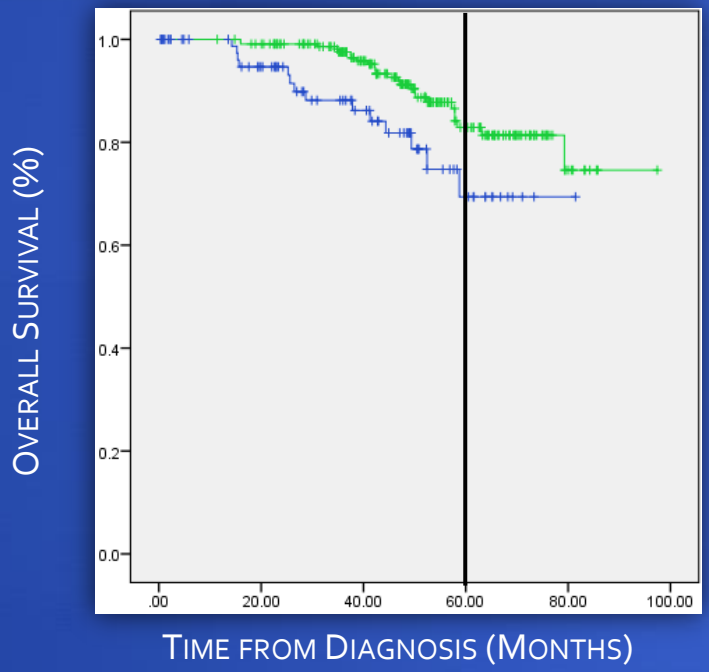


FISH for HER2

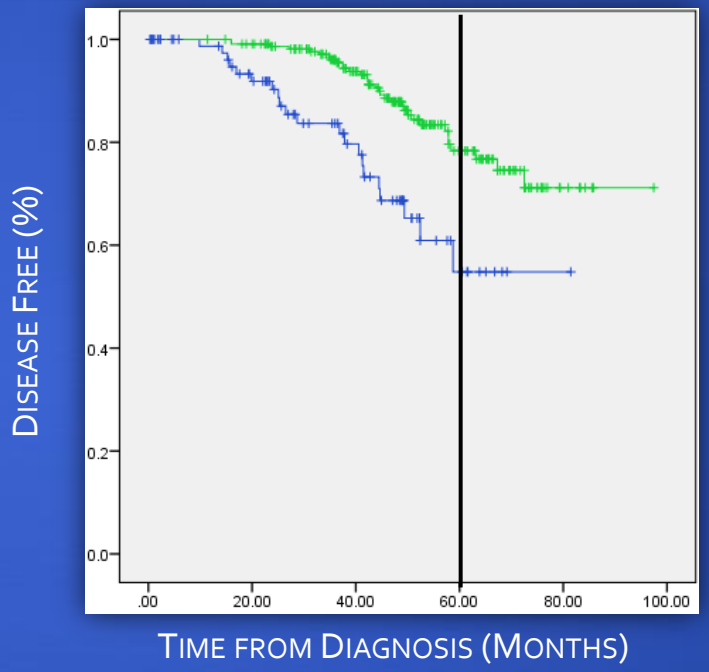


Response to Herceptin of HER2+ Cancer Cardiff Data: 2005-2008

Overall Survival from Diagnosis



Time to Recurrence from Diagnosis



Herceptin

No Herceptin

Oncotype DX® 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

INVASION
Stromelysin 3
Cathepsin L2

CD68

HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

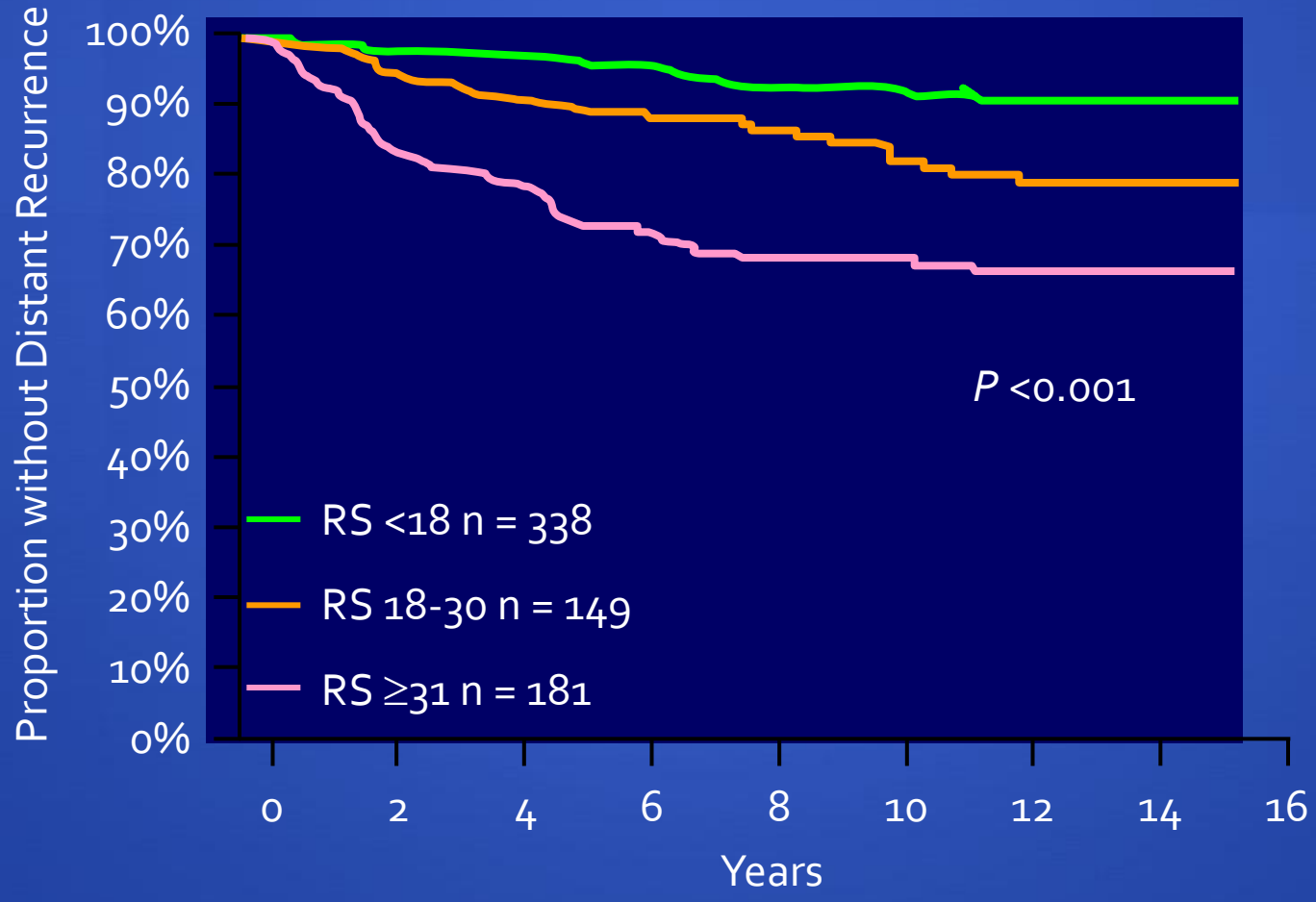
RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Category	RS (0 -100)
Low risk	RS <18
Int risk	RS 18 - 30
High risk	RS ≥ 31

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX® Clinical Validation: B-14 Results – Distant Recurrence

Distant Recurrence for the three distinct cohorts identified



Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Quality Assurance of Diagnostic & Prognostic Cancer Biomarking

- Tumour size
- Histological type
- Histological grade
- Lymph node stage
- Vascular invasion
- Excision margins
- In situ component
- Analysis performed and results reported by pathologists

Quality Assurance of Diagnostic & Prognostic Cancer Biomarking: Probable Error Rate

Susan G. Komen for the Cure White Paper: June 2006

- **While it is exceedingly difficult to determine the incidence of incorrect breast cancer diagnoses in the United States, our consultants estimate that the error rate could be as high as 2% to 4%.**
- **If accurate, as many as 5,000 to 10,000 patients diagnosed with invasive or in-situ breast cancer each year may have been misdiagnosed and inappropriately treated (Appendix II).**
- **More than 90,000 people currently living with breast cancer may, in fact, be living (or dying) with an incorrect diagnosis (Appendix II).**

Quality Assurance of Diagnostic & Prognostic Cancer Biomarking

- Training in Pathology (Doctors & Biomedical Scientists)
  - Undergraduate
  - Postgraduate
    - General
    - Sub-specialist
- Continual Professional Development
- External Quality assurance
- Audit

Quality Assurance of Predictive Breast Cancer Cancer Analysis

- Hormone Receptor
- HER2 Receptor
  - Performed by biomedical scientists in hospital or private pathology laboratories
  - Results interpreted and reported by senior biomedical scientists and/or sub-specialist pathologists

Unrecognised Error Rate

- Hormone Receptor
- An official inquiry convened in July 2007
  - In Newfoundland and Labrador over 2,000 originally ER-negative cases were retested in another laboratory in Ontario, and nearly 40% were found to be ER-positive

Wall Street Journal – Jan 4 2008

- "We all make the assumption that every test is done well. It turns out that it's not a correct assumption"
  - Lee Newcomer, a senior cancer doctor

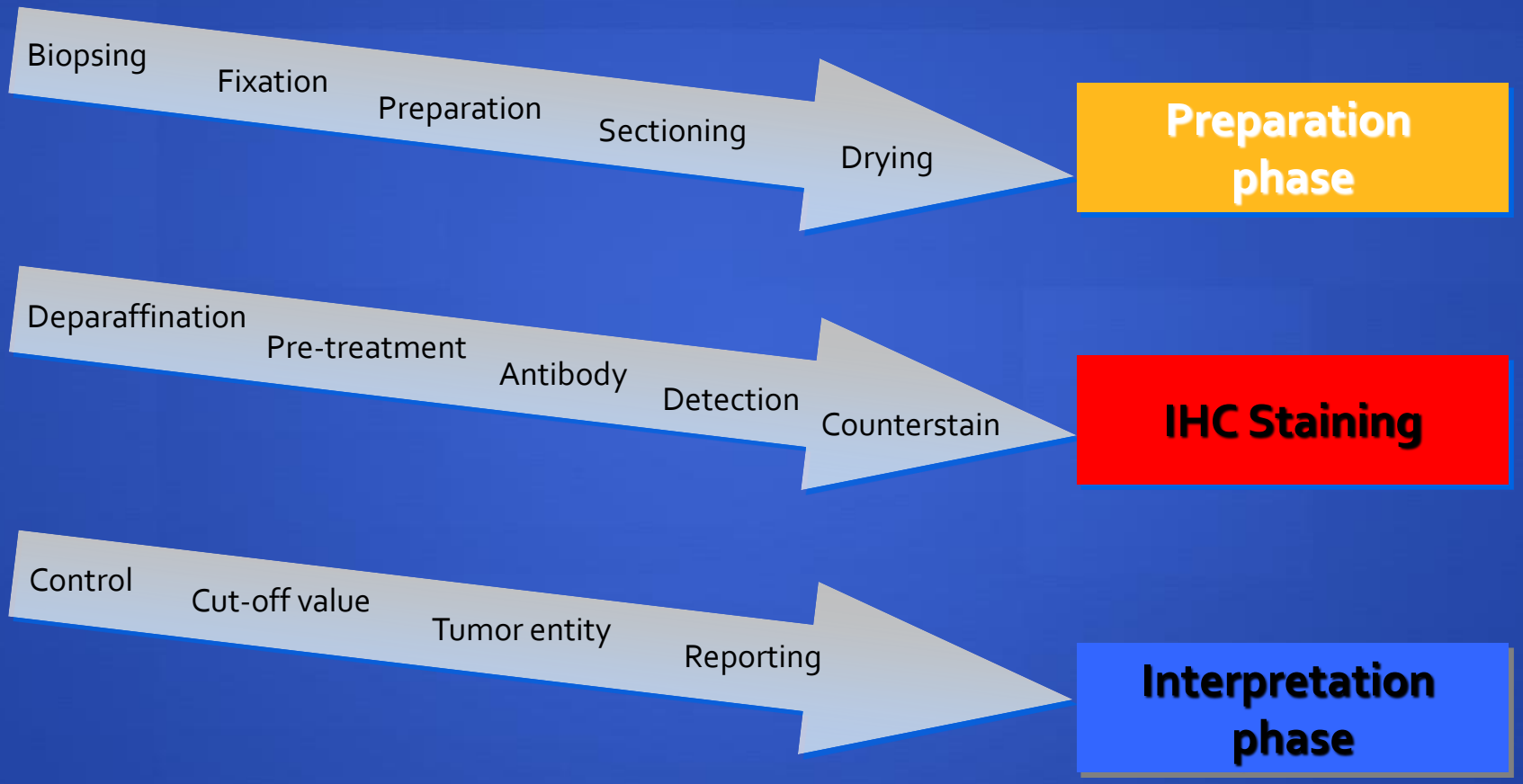
An Admission by an Expert

- "While far from being scientific, the false-negative rate of IHC testing for both receptors in my consulting practice over the past 10 years is about 30%, which is similar to that of other experienced consulting pathologists I have spoken with on this issue"
  - *D. Craig Allred. Commentary: Hormone Receptor Testing in Breast Cancer: A Distress Signal from Canada. The Oncologist 13: 1134-1136, 2008*

How Can We Improve Quality of Predictive Biomarking of Breast Cancer ?

Standardization?

4.8 million ways of doing the same thing….



3^{14} = 4.8 mio procedures (assuming 3 choices in 14 steps)

Optimisation of Methodology

- Pre-analytical
  - Tissue fixation
  - Antigen retrieval
- Analytical
  - Primary antibody specificity & sensitivity
  - Secondary detection system amplification
- Post-analytical
  - Interpretation & objective scoring & reporting

Challenges to Optimisation of Pre-Analytical Factors

- Quality of tissue preservation
  - Variable delay in fixation
  - Variable quality of fixative
  - Variable penetration of fixative
  - Variable duration of fixation
- Quality of tissue sample
  - Core biopsy vs resection specimen
- Quality of tissue sections
  - Variable and uneven section thickness
  - Variable drying temperature
  - Variable length of storage

Recommended Solutions

- Standardisation of Tissue Preservation
  - Avoidance of delay in fixation (<30 min)
  - Use of appropriate fixative
    - 4% buffered formalin (pH control)
  - Adequate penetration of fixative
    - Tissue slicing (5-10 mm)
  - Adequate duration of fixation
    - 6-48h at room temperature

Challenges to Optimisation of Analytical Factors

- Plethora of Analytical Reagents
  - Primary antibodies
  - Secondary Detection Agents & Systems
- Variety of Antigen Retrieval Methods
  - Types of antigen retrieval reagents
    - High pH, Low pH
  - Modes of antigen retrieval
    - Microwave ovens, pressure cookers, water baths, auto-stainer platforms

Table 1 Primary antibody (ER).

Antibody details	N	% (>10/20)
Biocarta CM 093C (clone 6F11)	1	100
Dakocytomation M7047 ER (clone 1D5)	63	65
Incomplete data	11	36
Lab vision RM 91015 (clone SP1)	31	58
Novocastra NCL-ER 6F11 ER (clone 6F11)	125	77
Neomarkers RM 9101 (clone SP1)	29	76
Microm RM 9101 (clone SP1)	1	100
Ventana 760-2132 ER (clone 6F11)	26	73
Vector VP E614 (clone 6F11)	34	92
Zymed 08 1149 (Clone 1D5)	3	33

Table 2 Pre-treatment (antigen retrieval).

Pre-treatment	N	%
Incomplete data	12	50
Microwave oven	64	72
Other	1	100
Pressure cooker inside microwave oven	38	74
Pressure cooker	128	77
Water bath, 95–98 °C	24	42
Decloaking chamber	4	75
LabVision pre-treatment module	2	100
Vision Biosystems Protease K	1	100
Vision Biosystems+ER 1	3	67
Vision Biosystems+ER 2	3	100
Vision Biosystems+Dako S3307 buffer	1	0
Ventana benchmark XT	2	100
Ventana benchmark	41	73

Table 3 Detection system.

Type, supplier and product code	N	%
Biocare STUHRP700L10	1	100
Biogenex LP000-UL	11	91
Biogenex HK 519–06 K HRP	3	100
Biogenex QD420	1	0
Biogenex QD430	5	80
BiocarterBCA HP504 US	1	100
Chemicom HP 1000	2	100
Dakocytomation ChemMate L.St.Av/HRP K5001	51	75
Dakocytomation ChemMate L.St.Av/alk phos K5005	2	50
Dakocytomation ChemMate Envision K5007 DAB	52	79
Dakocytomation Duet St. ABC K0492	4	75
Dakocytomation Envision K0675	28	71
Dakocytomation St.ABC/HRP K0377	4	75
Incomplete data	22	45
Others	5	40
LabVision TS 125 HR	13	54
Vector Elite ABC PK6100	3	100
Vector Elite ABC PK6102	1	0
Vector Elite Universal ABC PK6200	19	89
Vector Elite ABC PK7200	11	64
Ventana iView System	52	69
Ventana basic System 250–2001	16	75
Vision Biosystems DS9404	1	100
Vision Biosystems DS9713	10	70
Power Vision DPVP999 HRP	1	0
Zymed 85 9043	1	0
Zymed 87 8143	1	100

Table 4 Chromogen.

Chromogen and supplier	N	%
Biogenex HK-153-5 K DAB	10	90
Biogenex HK-124-9 K DAB	6	100
Biogenex QD430 DAB	3	67
Dakocytomation K3466 DAB	5	80
Dakocytomation K3468 DAB	26	77
Dakocytomation K3465 DAB	4	75
Dakocytomation K5001 DAB	51	75
Dakocytomation K5005 Alk Phos	1	0
Dakocytomation S3000 DAB	1	100
Dakocytomation K5007 DAB	53	79
Dakocytomation Envision	21	62
HD Supplies -4170 DAB	4	75
Other	12	67
Incomplete data	21	48
LabVision TS 125 HR	7	57
Merck New Fuchsin	1	100
Sigma D5637 DAB	7	43
Sigma D5905 DAB	3	67
Vector SK4100 DAB	2	100
Ventana DAB	67	72
Vision Biosystems DAB	10	70
Zymed kits DAB	2	50

Table 6 Primary antibody (HER-2/neu).

Antibody details	N	%
Amenarini MU 1344 UC (Clone CB11)	2	0
Biogenex MS 730p	1	0
Dakocytomation HerCep Kit K5204	39	92
Dakocytomation HerCep Kit K5205	12	83
Dakocytomation HerCep Kit K5206	10	100
Dakocytomation HerCep Kit K5207	22	82
Dakocytomation A0485 Cerb B2 (Polyclonal)	36	53
Neomarkers RM 9103 (Clone SP3)	1	0
Novocastra NCL-CB11 (Clone CB11)	15	47
Incomplete data	12	83
Ventana 760 2694 CB11(Pathway)	14	64
Zymed 28 003 or 18-107	4	75

Table 7 Pre-treatment (antigen retrieval).

Pre-treatment	N	%
Water bath, 95–98 °C for 40 min with 20 min cooling	92	85
Incomplete data	13	92
Microwave oven	18	33
Other	1	100
Pressure cooker	19	58
Biocarta Decloaker	1	0
None	2	0
Vision Biosystems ER1	2	50
Ventana Benchmark XT	9	67
Ventana Benchmark	11	64

Table 8 Detection system.

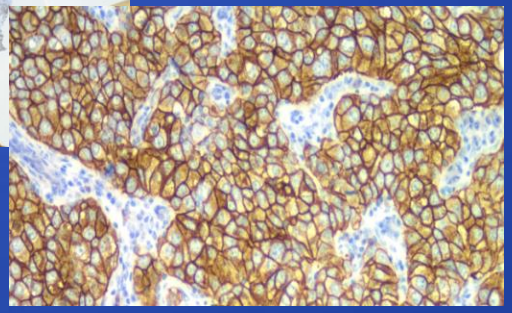
Type, supplier and product code	N	%
Biogenex Super Sentitive Multi link/HRP LP000-UL	1	100
Biogenex HK519–06 K HRP	1	0
Dakocytomation HerCep Test K5204	40	90
Dakocytomation HerCep Test K5205	9	100
Dakocytomation HerCep Test K5206	10	100
Dakocytomation HerCep Test K5207	21	76
Dakocytomation ChemMate L.St.Av/HRP K5001	9	11
Dakocytomation Envision Plus	8	75
Dakocytomation ChemMate Envision K5007	21	76
Dakocytomation K5005	1	100
Dakocytomation LSAB Kit/HRP K0675	1	100
Dakocytomation K0690	1	100
Incomplete data	17	65
LabVision TA125ML	5	20
Others	2	100
Power Vision DPVB999	2	100
Vector Elite Universal ABC PK6200	2	50
Vector PK7200 (ready to use)	1	0
Ventana IView System	14	64
Ventana PATHWAY	14	64
Vision Biosystems	3	33
Zymed 87 8143	1	100

Recommended Solutions

- Use of High Quality Kit Based Reagents
  - Highest specificity primary antibodies
  - Highest sensitivity secondary detection systems
- Use of Standardised Antigen Retrieval Platforms
  - Reliable consistent quality reproducible antigen retrieval
- Use of semi-automation (e.g. Dako PT-Link) or full automation (e.g. Ventana Benchmark)

Use of clinically validated assay systems

- HercepTest and HER2 FISH pharmDx was used in clinical trials for use of Herceptin in breast cancer.
- 5+ million tests performed worldwide since launch in 1998
- HercepTest has also been used in gastric cancer clinical trial (ToGA)



3+ HercepTest result

Challenges to Optimisation of Post- Analytical Factors

- Variation in approach to microscopic examination
  - Use of different objective lens power
  - 'hot' spot vs random vs total tumour area analysis
- Variation in method of scoring
  - H-Score vs Quick Score
- Variation in thresholds for negative results
  - <1% vs <10%; Allred 0-1 vs 0-2

Recommended Solutions: I

- Use of Optimised Protocols
  - Microscopic examination
  - Interpretation
  - Scoring
  - Reporting
- Evidence based consensus guidelines for scoring
- Clinically validated thresholds for reporting positive and negative results

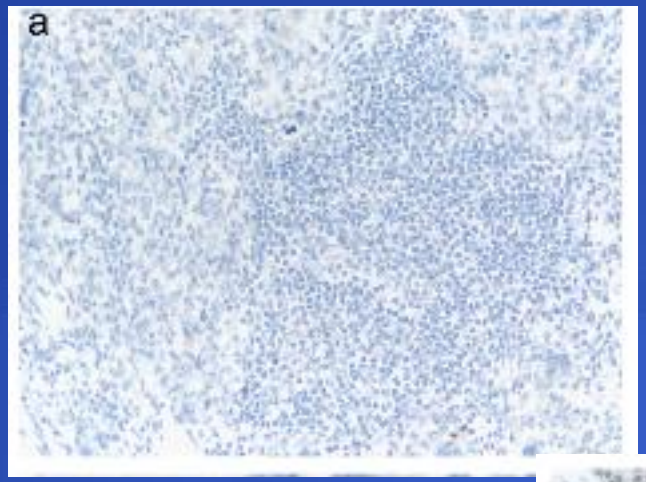
Recommended Solutions: II

Frequent Effective Evaluation of the Performance via Participation in External Quality Assurance Schemes

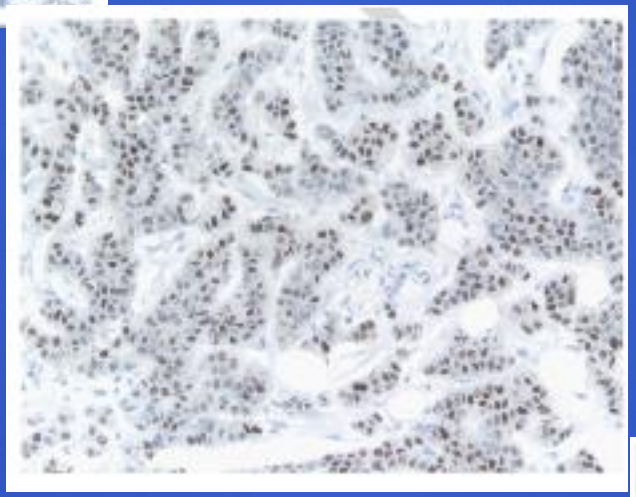
Participation in External Quality Assurance Scheme: UK National External Quality Assurance Scheme (UKNEQAS)

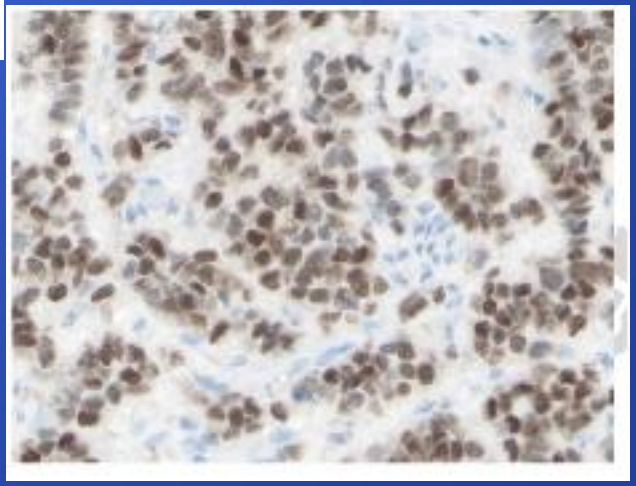
Headquarters in London

- >5000 Slides per run
- 4 Weeks of assessments
- 1-2 days depending on module
- 4 assessors & 1 driver

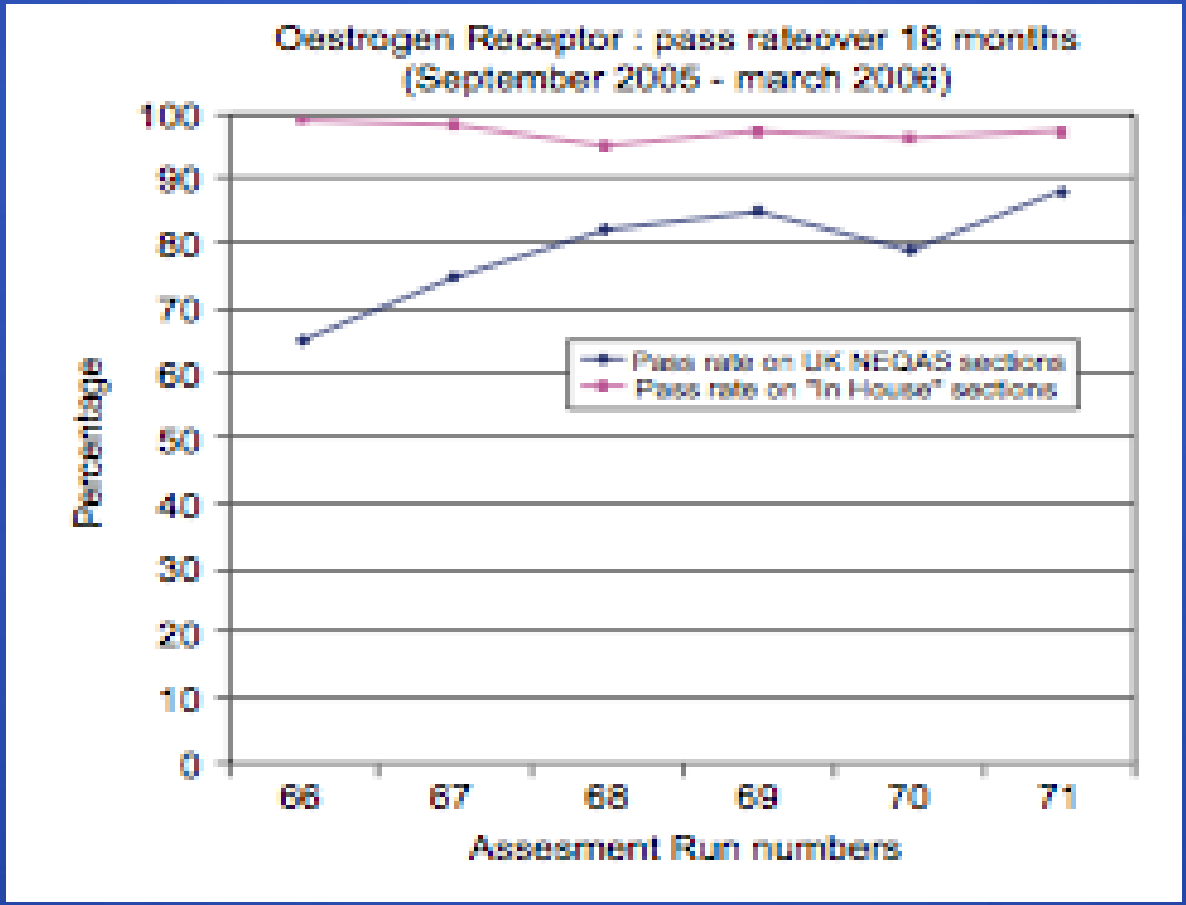


UKNEQAS Tissue Section Controls for ER





Improvement in Performance on External Control Samples



UK NEQAS HER-2 CELL LINES

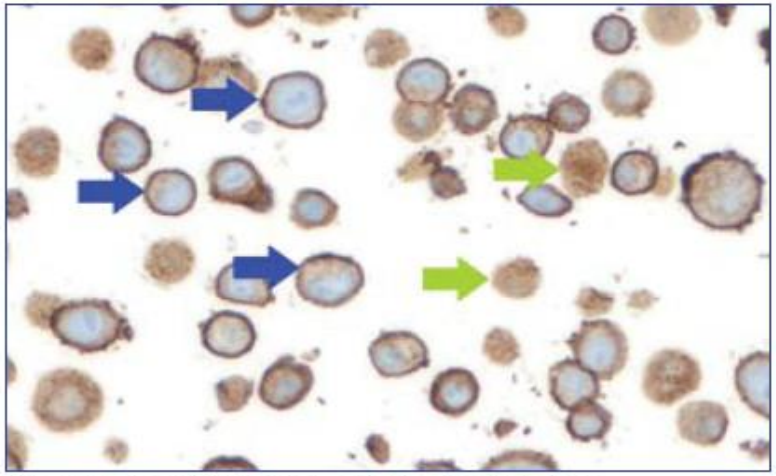


Plate 14. UK NEQAS-ICC cell line SK-BR3 (3+).

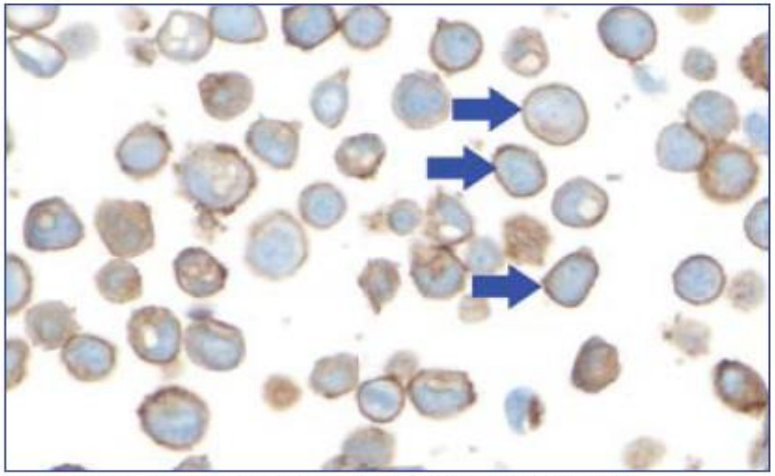


Plate 15. UK NEQAS-ICC cell line MDA-MB-453 (2+).

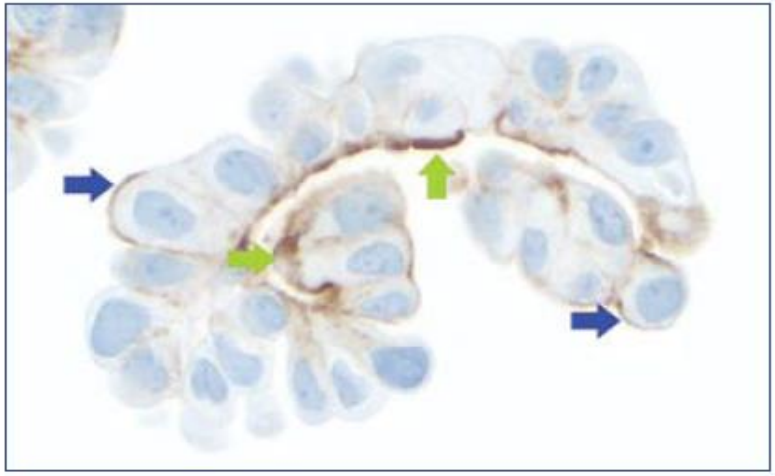


Plate 16. UK NEQAS-ICC cell line MDA-MB-175 (1+).

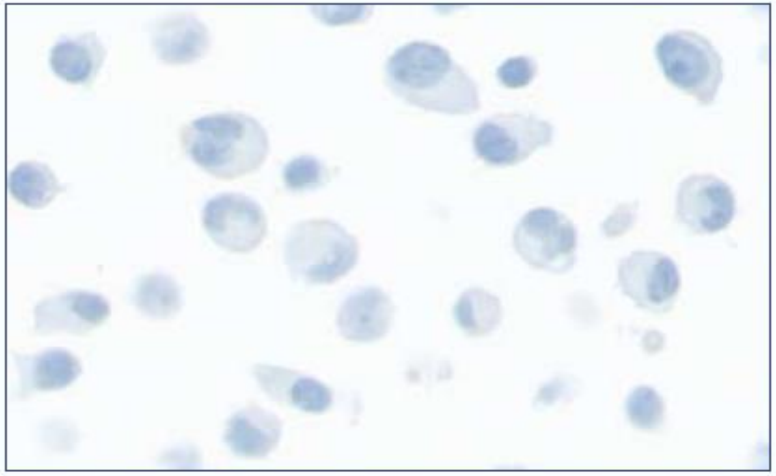
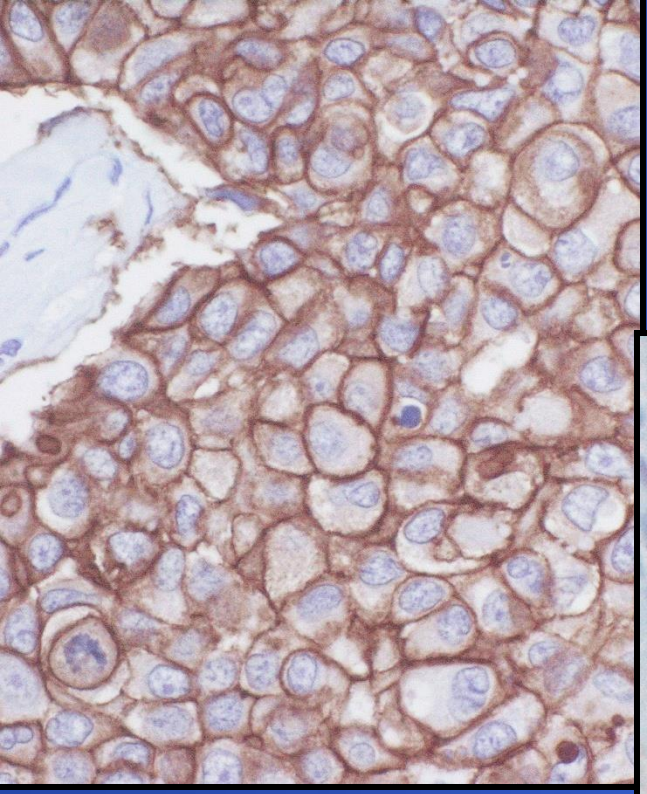
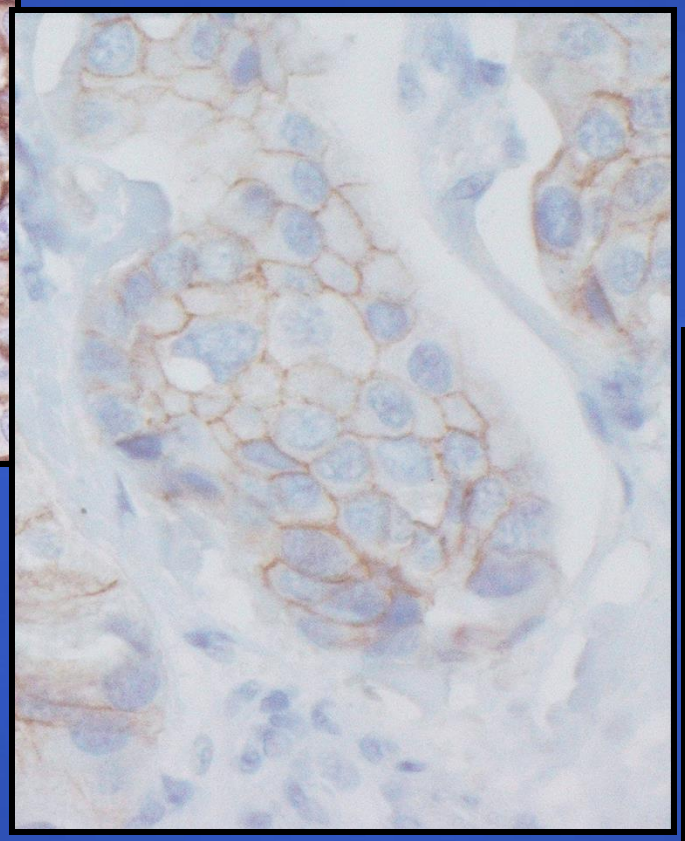
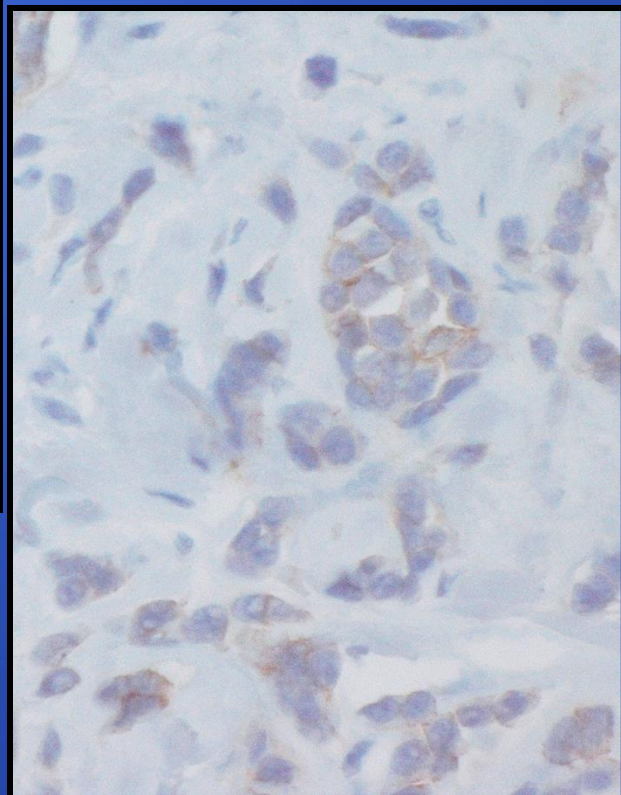


Plate 17. UK NEQAS-ICC cell line MDA-MB-231 (0).

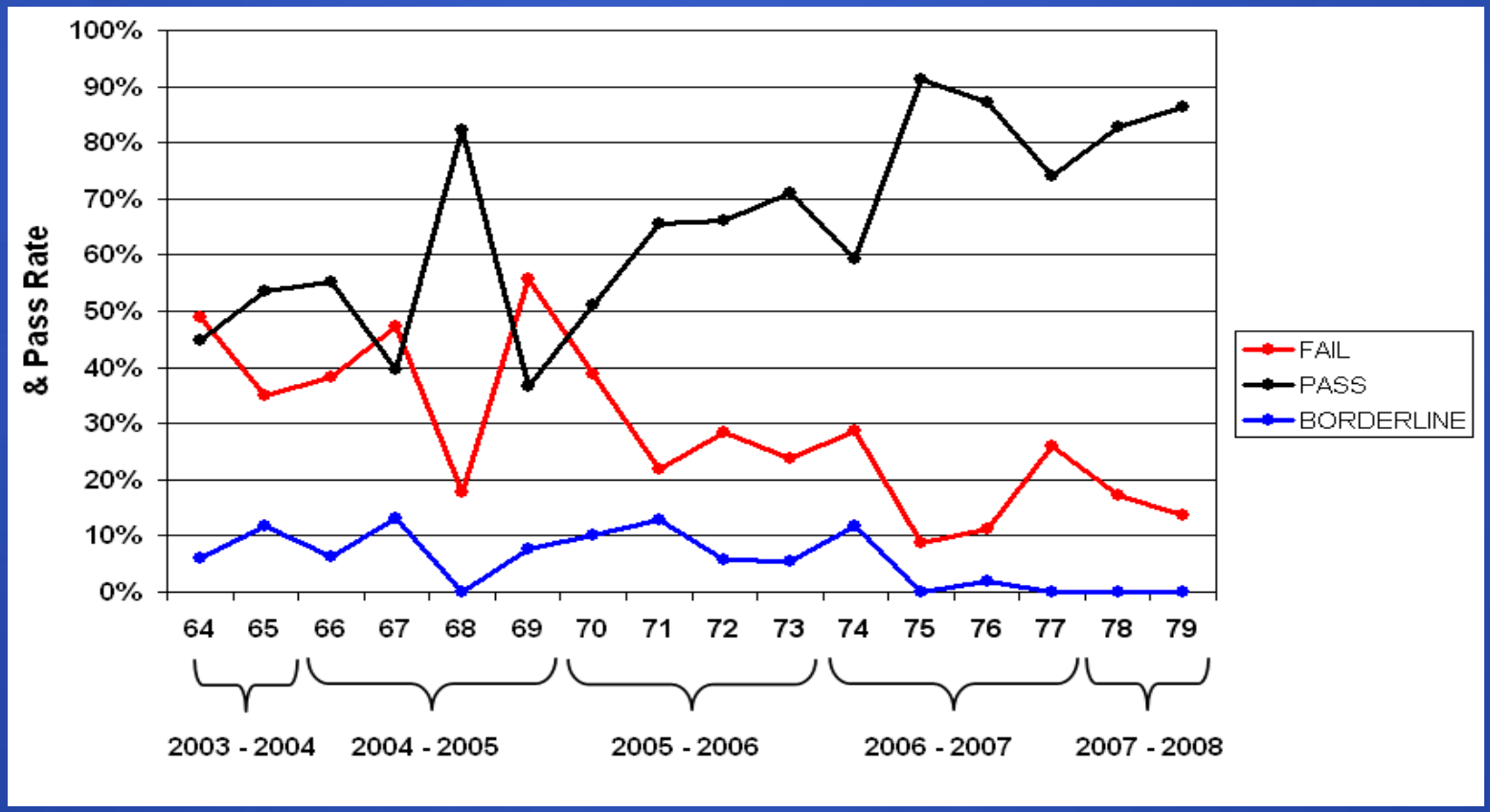
jasani@cf.ac.uk





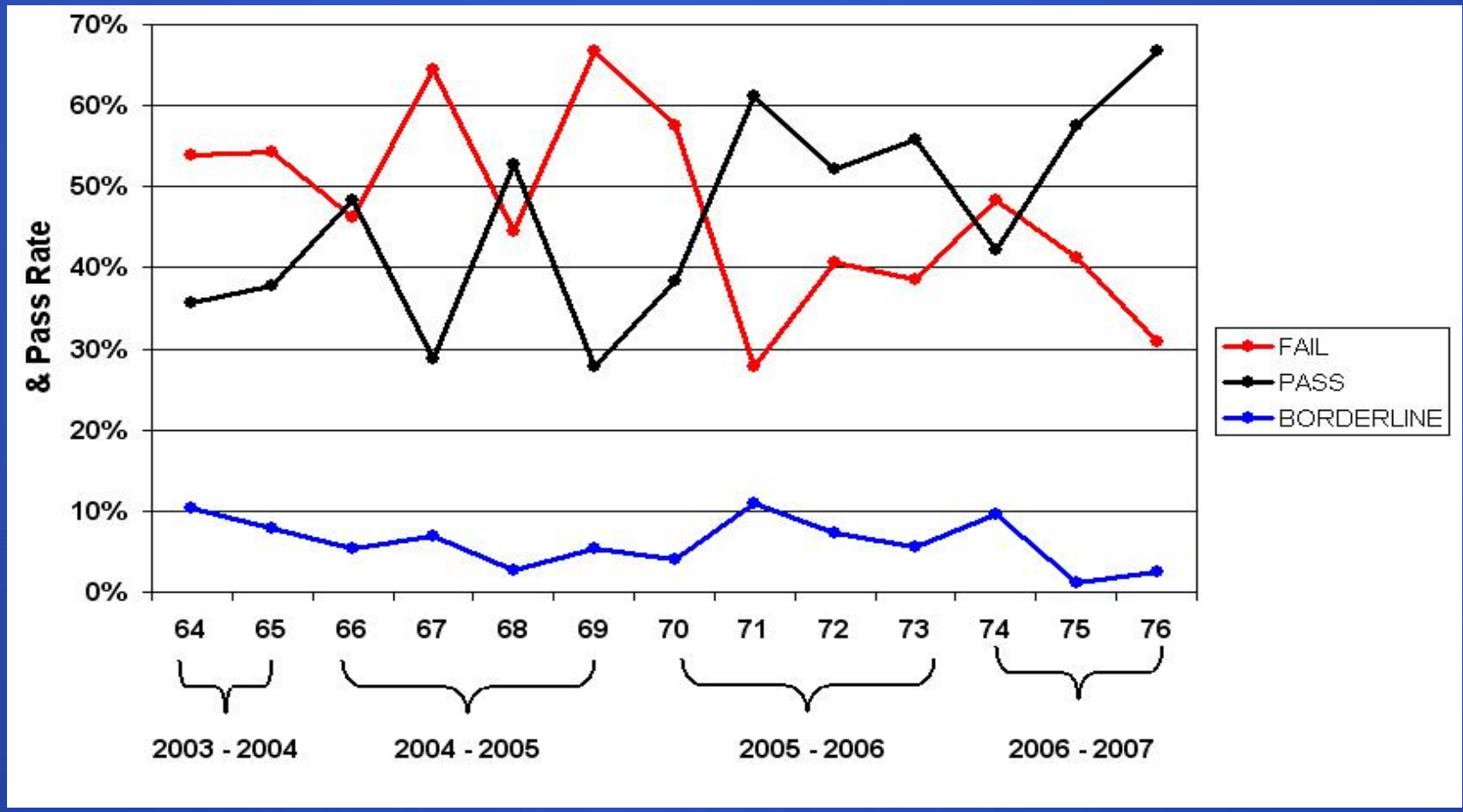


UK NEQAS Updated HER-2 Pass Rates: Data From UK only (2003- present)

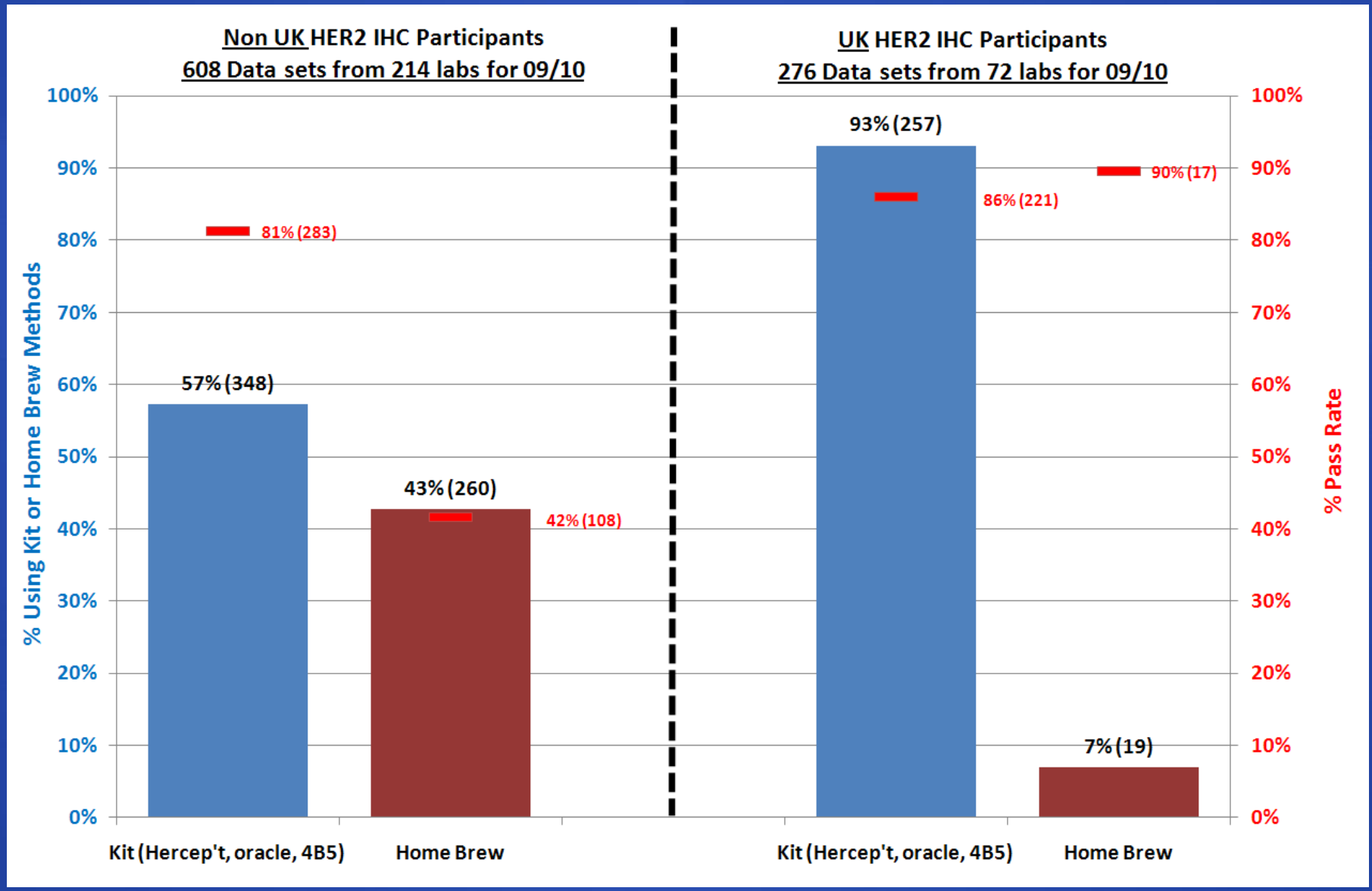


- UK labs have to pass the NEQAS assessments to be accredited (CPA)

HER-2 Assessment Pass Rates:
Data From 36 countries - UK & Overseas



Standardised Kit or Home Brew Method?



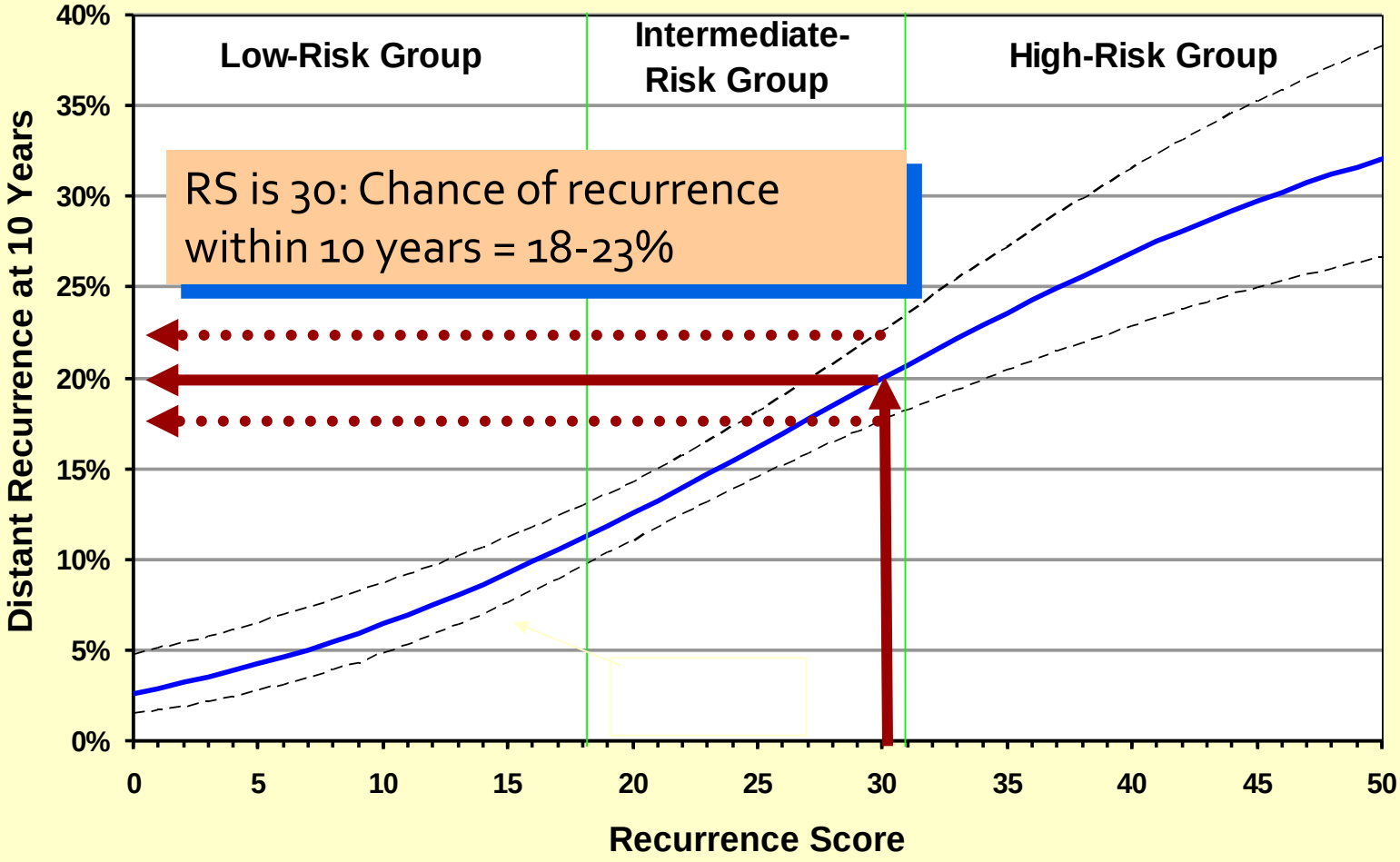
Conclusion

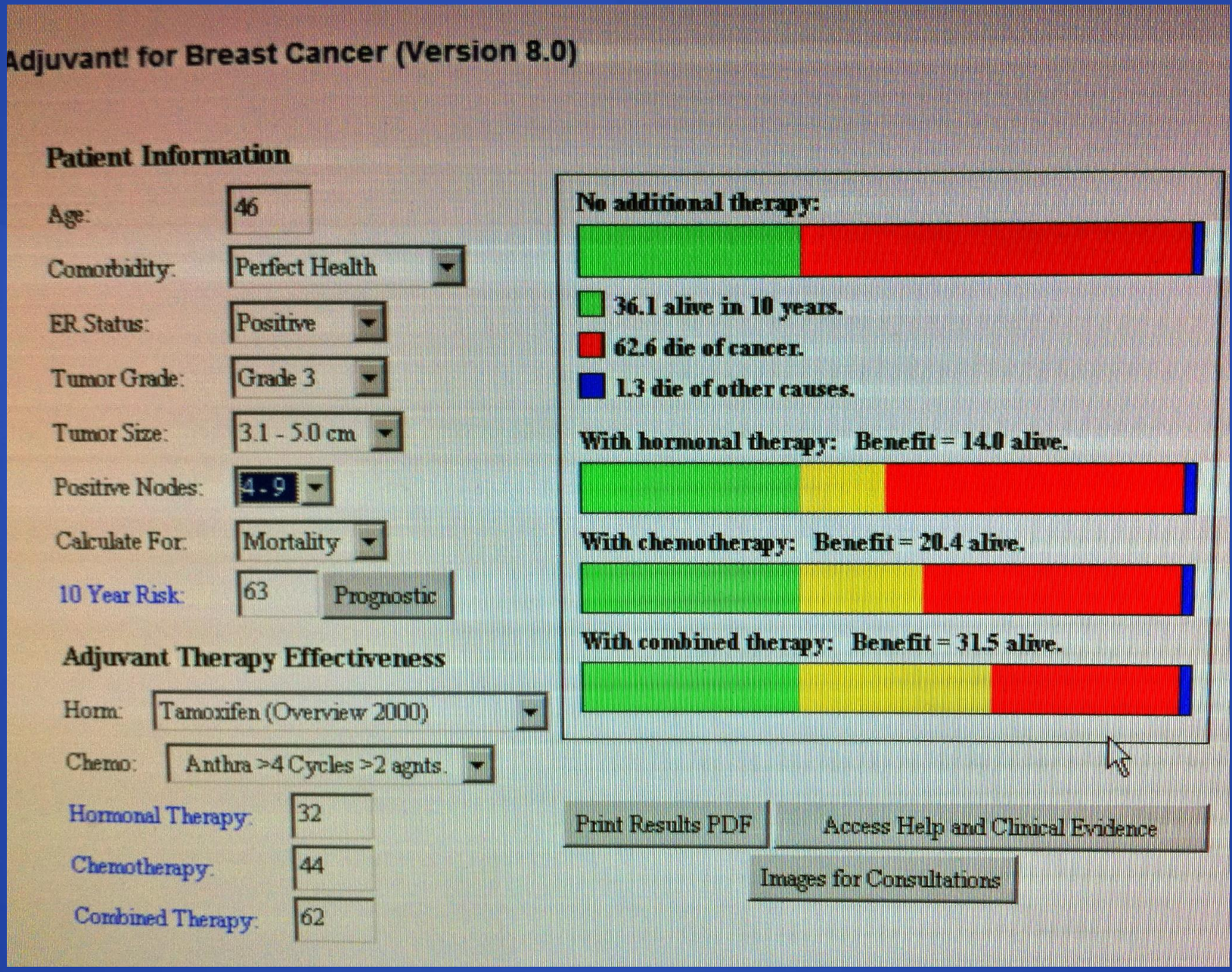
- Quality of analyticall performance can be improved through:
  - Use of recommended optimised kit based reagents and methods
  - Regular participation in External Quality Assurance Schemes

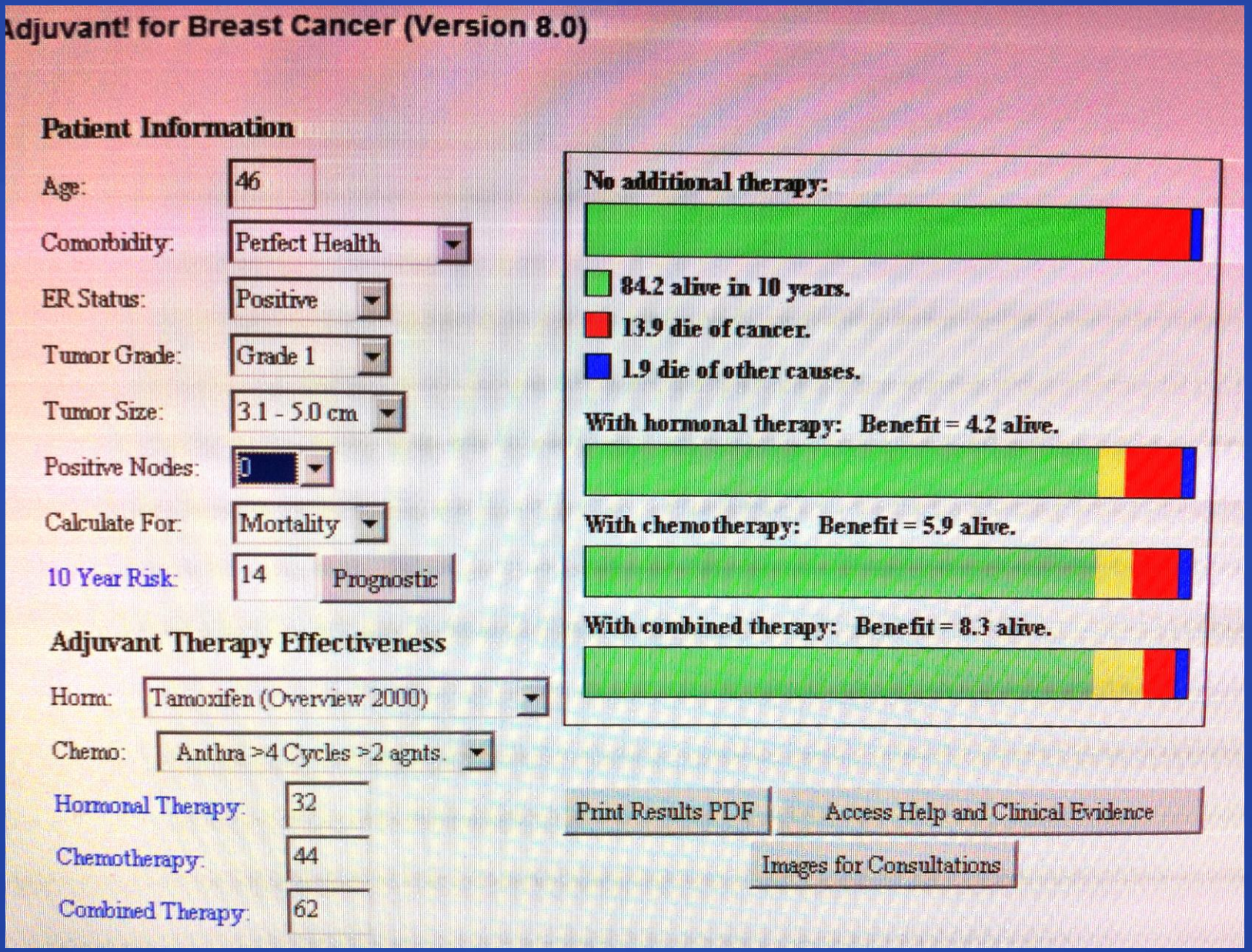
Рахмет / Rakh-met
Спасибо / Spasibo
Thank You

Bharat.jasani@nu.edu.kz

Oncotype DX® Clinical Validation: RS as Continuous Predictor



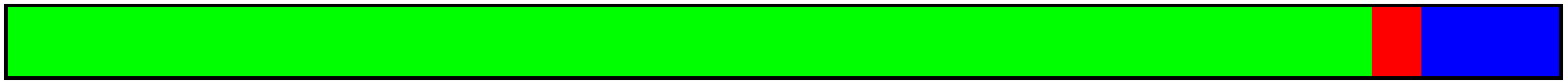

Adjuvant! for Breast Cancer (Version 8.0)
Patient Information
Age: 46
Comorbidity: Perfect Health
ER Status: Positive
Tumor Grade: Grade 3
Tumor Size: 3.1 - 5.0 cm
Positive Nodes: 4 - 9
Calculate For: Mortality
10 Year Risk: 63
Prognostic
Adjuvant Therapy Effectiveness
Horm: Tamoxifen (Overview 2000)
Chemo: Anthra >4 Cycles >2 agnts.
Hormonal Therapy: 32
Chemotherapy: 44
Combined Therapy: 62
No additional therapy:
36.1 alive in 10 years.
62.6 die of cancer.
1.3 die of other causes.
With hormonal therapy: Benefit = 14.0 alive.
With chemotherapy: Benefit = 20.4 alive.
With combined therapy: Benefit = 31.5 alive.
Print Results PDF
Access Help and Clinical Evidence
Images for Consultations


Adjuvant! for Breast Cancer (Version 8.0)
Patient Information
Age: 46
Comorbidity: Perfect Health
ER Status: Positive
Tumor Grade: Grade 1
Tumor Size: 3.1 - 5.0 cm
Positive Nodes: 0
Calculate For: Mortality
10 Year Risk: 14
Prognostic
Adjuvant Therapy Effectiveness
Horm: Tamoxifen (Overview 2000)
Chemo: Anthra >4 Cycles >2 agnts.
Hormonal Therapy: 32
Chemotherapy: 44
Combined Therapy: 62
No additional therapy:
84.2 alive in 10 years.
13.9 die of cancer.
1.9 die of other causes.
With hormonal therapy: Benefit = 4.2 alive.
With chemotherapy: Benefit = 5.9 alive.
With combined therapy: Benefit = 8.3 alive.
Print Results PDF
Access Help and Clinical Evidence
Images for Consultations

Name: _____ (Breast Cancer)
Age: 61 General Health: Good

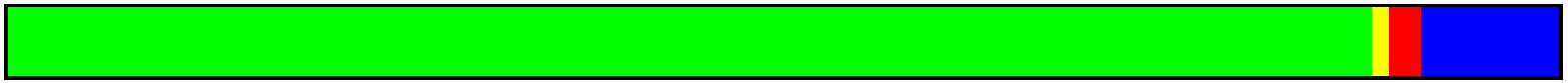
Estrogen Receptor Status: Positive Histologic Grade: 2
Tumor Size: 0.1 - 1.0 cm Nodes Involved: 0
Chemotherapy Regimen: CMF-Like (Overview 2000)

Decision: No Additional Therapy



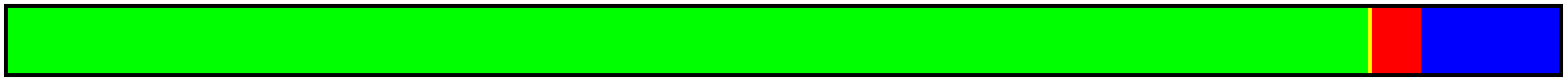
88 out of 100 women are alive in 10 years.
3 out of 100 women die because of cancer.
9 out of 100 women die of other causes.

Decision: Hormonal Therapy



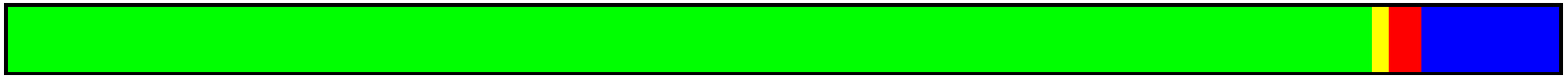
1 out of 100 women are alive because of therapy.

Decision: Chemotherapy



Less than 1 out of 100 women are alive because of therapy.

Decision: Combined Therapy



1 out of 100 women are alive because of therapy.

Nottingham Prognostic Index

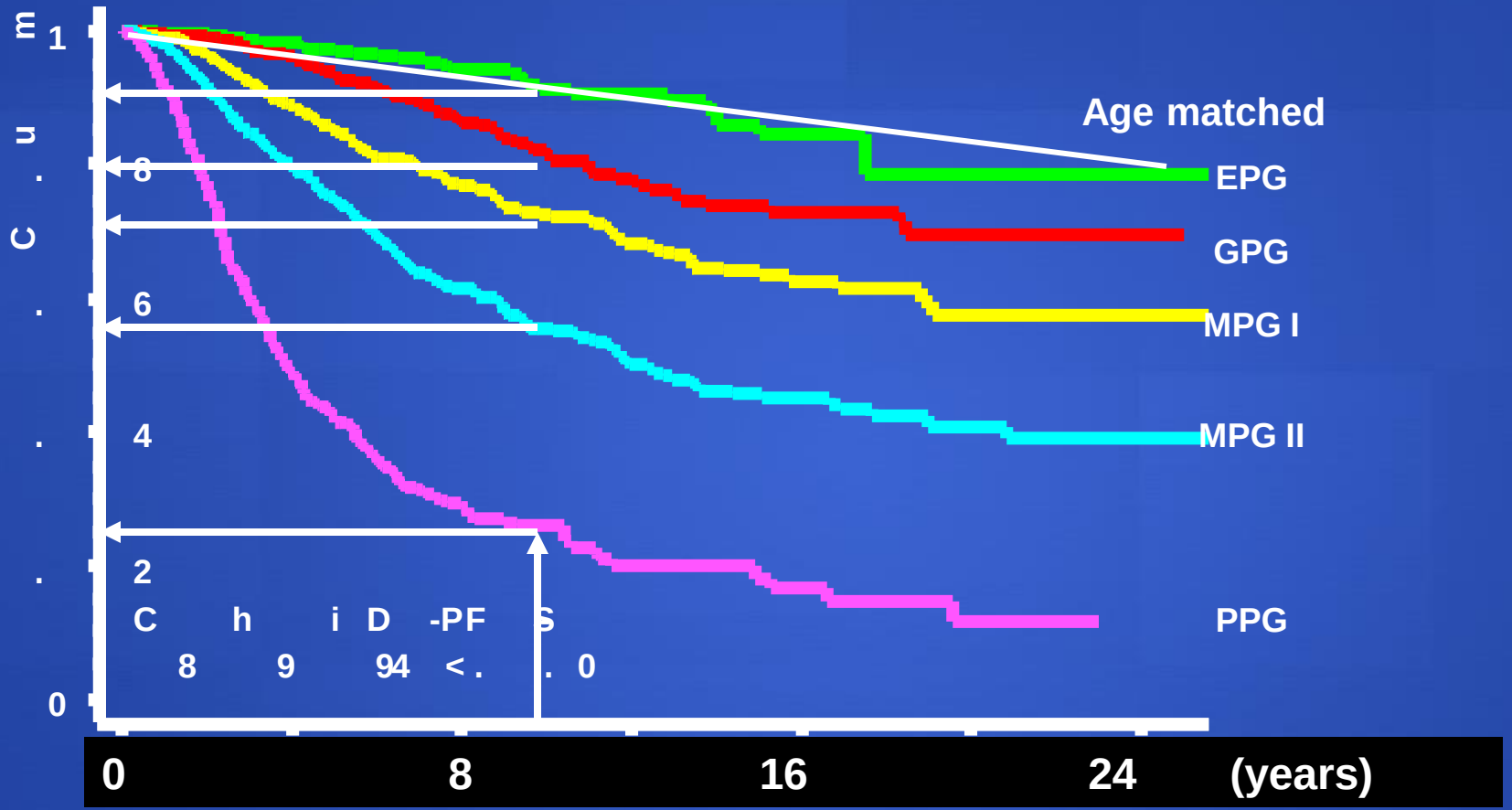
NPI = 0.2 x size (cm)

+ lymph node stage (1, 2, 3)

+ grade (1, 2, 3)

Nottingham Primary Breast Cancer Study

Nottingham Prognostic Index



475	223	46	EPG
744	289	69	GPG
1001	339	68	MPG I
891	241	61	MPG II
590	57	9	PPG