

**LIFE LONG LEARNING PROGRAMME**

**TRANSVERSAL PROGRAMME**

KA3 – ICT-Multilateral projects

**Project title :** Telepathological ASsessment of histopathological and cytological TEchniques

**Project Acronym:** TASTE

**Project number:** 519108 - LLP-2011-IT-KA3-KA3MP

**Grant Agreement:** 2011-4018/001-001



# **Pathology of the Breast : Possible artefacts Benefits of Standardization**

## **2nd TASTE Workshop Proceedings**

**Brussels, Belgium, April 20 th, 2013**  
Jules Bordet Institute (IJB-ULB), Brussels, Belgium

## Preface

The following presentation contains the contributions presented at the Second TASTE workshop, “TASTE Project: "Pathology of the Breast : Possible artefacts - Benefits of standardization”, held on the 20th april 2013 in Brussels, Belgium. The workshop has been realized in the context of the activities of the TASTE project “Telepathological ASsessment of histopathological and cytological TEchniques“, funded with the support from the European Commission.

This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

It is our hope that these pages will lead to a constructive discussion on the issue of the definition of a new “process and workplace ergo-designer” profile and the establishment of a training model based on this profile.

These proceedings are published on the TASTE project website [www.tasteproject.eu](http://www.tasteproject.eu) .



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## Workshop Introduction

The Institute **Jules Bordet** (IJB-ULB) is a leading European institution for breast cancer diagnosis and treatment.

The **TASTE project** is part of the European Commission's Lifelong learning project. It is devoted to set technical standards in pathology.

Standardized optimal technical quality is certainly important on every day practice of every pathology department.

Digital pathology allows open circulation of virtual slides throughout many countries of the world. It will be used in the TASTE project. The goals of discussion, training and technical standardization will be achieved by collecting top quality, average quality and artefact-damaged preparations and archiving them in a digital web based archive. The images will thereafter be standardized through user assessment sessions and made available for the interested professionals all over the world.

**This second TASTE workshop** will focus on the possible artefacts in breast pathology and will illustrate the benefits of standardization.

## **The TASTE workshop and system**

**Gianni Bussolati**

COREP, University of Turin, Italy

### **Background:**

**Histological and cytological preparations are not standardized and their quality level is variable.  
This can affect diagnoses.**

**Optimal, standardized procedures are crucial if a high standard of test results is to be achieved, which is what each patient deserves.**

Groenen et al., Histopathol. 59, 1-7, 2011

In designing a clinical trial, it is no longer acceptable to state that

“tissue will be collected by standard protocol”

when in fact protocols are not standard between hospitals...

Hewitt et al., Arch. Pathol. Lab. Med. 132, 1929, 2008

**Goal:**

**To exploit Telepathology to enhance (at European level) knowledge and recognition of artifacts and ultimately to improve the quality of histological and cytological preparations.**

- The ultimate goal of this approach (unprecedented at world level) is to fuel a comprehensive Web-based community of students and staff personnel, aimed to a harmonisation and improvement of histopathological and cytopathological preparations, thus leading to an innovative training and more reproducible diagnoses, a basic requisite for disease treatment.

## ARTIFACTS.

the goal of the TASTE project, must recognize definite landmarks, since one should acknowledge that no preparation can properly be defined as "perfect". The Project is accordingly pursuing the goal of an "acceptable level", i.e. of preparations permitting sound and reproducible diagnoses.

**Presentation of examples of artifacts “preventing correct diagnoses”.**



- A Project focused on the use of Telepathology for the Assessment of Histopathological Techniques at European level (TASTE) was approved and financed by a European grant from – ICT (Information and communications technology) - Multilateral projects, project number : 519108 - LLP-2011-IT-KA3-KA3MP, Grant Agreement number: 2011-4018/001-001 KA3.

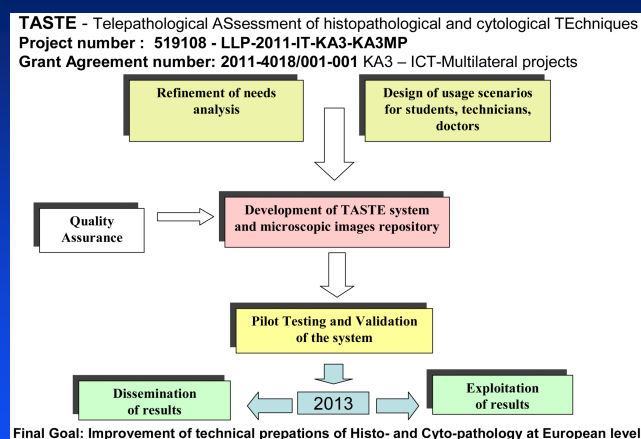
- What is a top quality histological section?
- What is an **internationally accepted standard level** of section or staining quality?
- Which technical artifacts are not acceptable in a slide of standard quality?
- What is the acceptable technical quality having no impact on diagnostic assessment?
- How to define digital slide of standard quality?
- *These questions are certainly important in everyday practice of every pathology departments and there is an increasing demand for the answers in the era of digital pathology characterized with open circulation of virtual slides throughout many countries of the world*

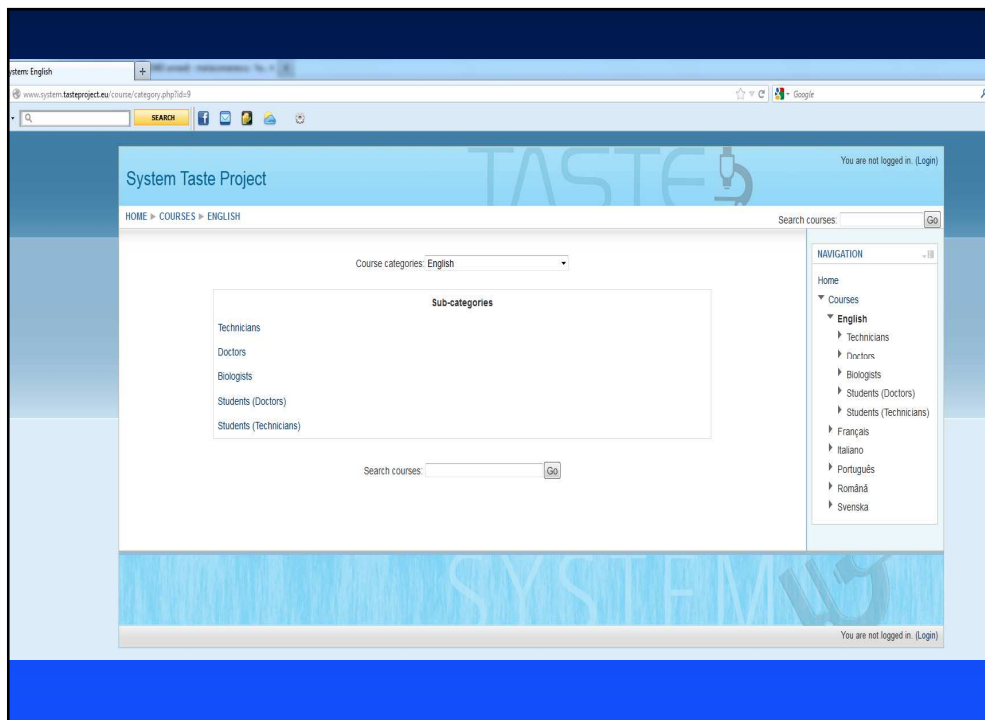
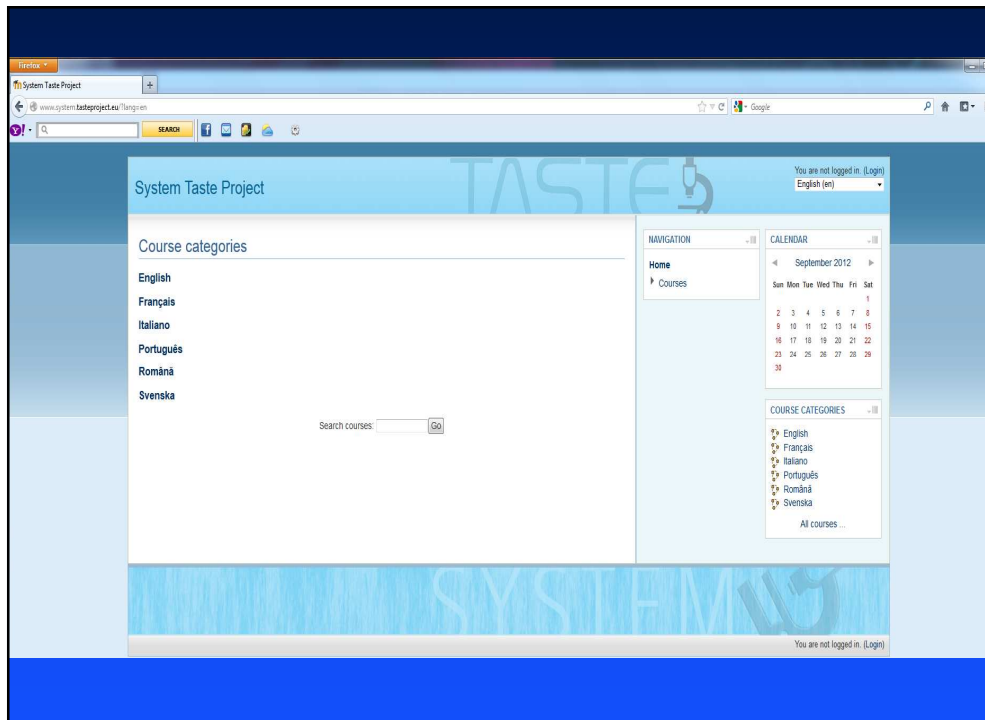
- **The Project tackles these problems =**
  - building-up an ICT environment TASTE System
  - Technicians and students connected via the WorldWideWeb,
  - (Italy, Romania, Portugal, Belgium and Sweden -
  - other countries WELLCOMED )
- 
- microscopic images of their own preparations to a panel of internationally-recognized experts who will give them comments and suggestions.

- Practicing pathologists as well as Residents in Anatomic Pathology will be involved. Assessment with real users will be organized in order to smooth-down the major problems encountered.
- A step-wise approach for the assessment (and hopefully the improvement) of the various histopathological and cytopathological preparations will be conducted, so that the exchange of images will start with basic routine stains (Haematoxylin-Eosin stained slides, Papanicolaou-stained smears) in order to check quality and reproducibility of fixation, processing and staining procedures.



- The 3 years program will then proceed with more sophisticated techniques such as special stains immunohistochemical and FISH Preparations.
- It will be achieved by collecting top quality, average quality and artifact-damaged preparations and archiving them in a digital web-based archive. The images will thereafter be standardized through user assessment sessions and made available for the interested professionals all over the world.





**Breast Biopsy**

HOME » MY COURSES » BREAST BIOPSY

**Breast Biopsy**

**Topic 1**

- ? Exercise 1-3
- ? Exercise 4
- ? Exercise 5
- ? Exercise 6
- ? Exercise 7
- ? Exercise 8
- ? Exercise 9
- ? Exercise 10

**NAVIGATION**

- Home
- My home
- Site pages
- My profile
- My courses
  - Breast Biopsy**
    - Participants
    - General
    - Topic 1

**SEARCH FORUMS**

Go

Advanced search

**UPCOMING EVENTS**

There are no upcoming events

Go to calendar...

New event...

**RECENT ACTIVITY**

Activity since Wednesday, 5 September 2012, 8:54 AM

Full report of recent activity...

Nothing new since your last login

**SETTINGS**

- Course administration
  - Grades
- My profile settings

You are logged in as taste1 taste1 (Logout)

**Exercise 1-3**

Continue

(\*)Answers are required to starred questions.

**Virtual microscope**

**Slide A**

**Virtual microscope**

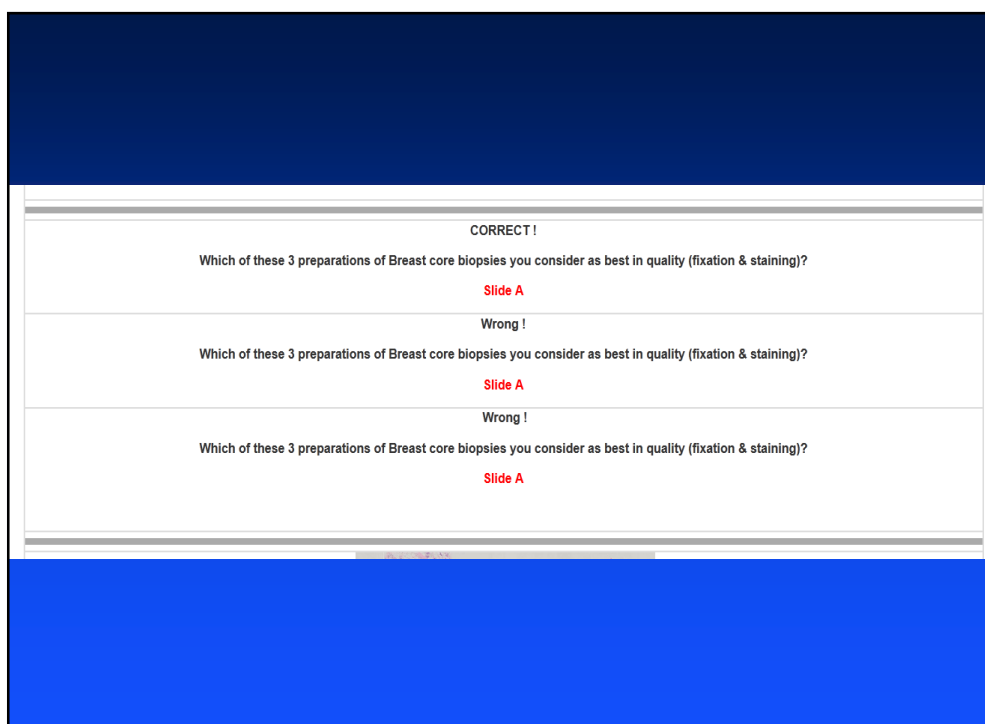
**Slide B**

**Virtual microscope**

**Slide C**

Which of these 3 preparations of Breast core biopsies you consider as best in quality (fixation & staining)?\*

☐ Slide A ☐ Slide B ☐ Slide C





**Virtual microscope**

This slide of a breast core biopsy\*

☐ is ☐ is not

of acceptable quality. In areas of comedo necrosis sectioning artifacts\*

☐ are ☐ are not

present. Staining of nuclei\*

☐ is ☐ is not

of good quality.



**The correct answer is:**

"Biopsy is of acceptable quality. In areas of comedo necrosis sectioning artifacts are present. Staining of nuclei is of good quality"



# The Belgian guidelines for HER2/neu testing in breast cancer. Personal experience and comments.

Cecile Colpaert MD, PhD  
Department of Pathology  
GZA ziekenhuizen/UZA,  
Antwerp, Belgium



## JCO January 2007 Arch Pathol Lab Med 2007

### American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

*Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, and Daniel F. Hayes*

#### A B S T R A C T

##### **Purpose**

To develop a guideline to improve the accuracy of human epidermal growth factor receptor 2 (HER2) testing in invasive breast cancer and its utility as a predictive marker.

##### **Methods**

The American Society of Clinical Oncology and the College of American Pathologists convened an expert panel, which conducted a systematic review of the literature and developed recommendations for optimal HER2 testing performance. The guideline was reviewed by selected experts and approved by the board of directors for both organizations.

##### **Results**

Approximately 20% of current HER2 testing may be inaccurate. When carefully validated testing is performed, available data do not clearly demonstrate the superiority of either immunohistochemistry (IHC) or in situ hybridization (ISH) as a predictor of benefit from anti-HER2 therapy.



## MONITEUR BELGE - BELGISCH STAATSBLAD 21.05.2007 : reimbursement of Herceptin

De specialiteit komt eveneens in aanmerking voor vergoeding indien zij wordt toegediend in het raam van een adjuvante behandeling van een borstkanker met een tumorale overexpressie van de humane epidermale groeifactor receptor-2 (HER2 of Human Epidermal growth factor Receptor-2), bewezen door gen-amplificatie via ten minste één positieve In Situ Fluorescentie Hybridisatie test (FISH-test of Fluorescence In Situ Hybridisation test) uitgevoerd door een erkend Centrum voor Moleculaire Diagnostiek. De test wordt als positief beoordeeld indien er meer dan 6 copieën van het gen per nucleus aanwezig zijn of een ratio HER2 signalen/chromosoom 17 signalen > 2,0\*. In geval van een intermediair resultaat (aanwezigheid van 4 à 6 copieën of een ratio tussen 1,8 en 2,2) moet er een 2de FISH test uitgevoerd worden evenals een test in immunohistochemie waarvan het resultaat 3+ moet zijn om de overexpressie van het eiwit te bevestigen.

Ref. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. 2007. [www.jco.org/cgi/doi/10.1200/JCO.2006.09.2775](http://www.jco.org/cgi/doi/10.1200/JCO.2006.09.2775)

ISH centres need ISO15189 accreditation by July 2009

## Belgian guidelines for HER2/neu testing in breast cancer

**Authors** C. Colpaert and R. Salgado (on behalf of the Belgian Working Party for Molecular Pathology)

**Key words** Human epidermal growth factor receptor 2 (HER2), trastuzumab, breast cancer, immunohistochemistry (IHC), Fluorescence-In-Situ-Hybridisation (FISH).

### Summary

The Belgian guidelines for HER2/neu testing in breast cancer are based on the recommendations by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), recently published in the

Archives of Pathology and Laboratory Medicine<sup>1</sup> and in the Journal of Clinical Oncology.<sup>2</sup> A Review of National Testing Guidelines published in Modern Pathology in 2003 was also taken into account.<sup>3</sup>  
(*BJMO* 2007;1;22-9)

## KEY MESSAGES OF THE BELGIAN GUIDELINES



1. Immunohistochemistry (IHC) is used for primary HER2 testing, detecting overexpression of the HER2 protein. Fluorescence In Situ Hybridisation (FISH) testing is performed in all tumours with IHC scores 2+ or 3+.
2. Tumour tissues should be fixed in 10% buffered formalin for 6-48 hours. The interval between tissue acquisition and fixation should be as short as possible, preferably less than 1 hour.
3. The use of controls of known HER2 levels is mandatory. A positive and negative control, determined by IHC and FISH, is a minimum. An additional control close to cut-off values is also recommended.

## KEY MESSAGES OF THE BELGIAN GUIDELINES



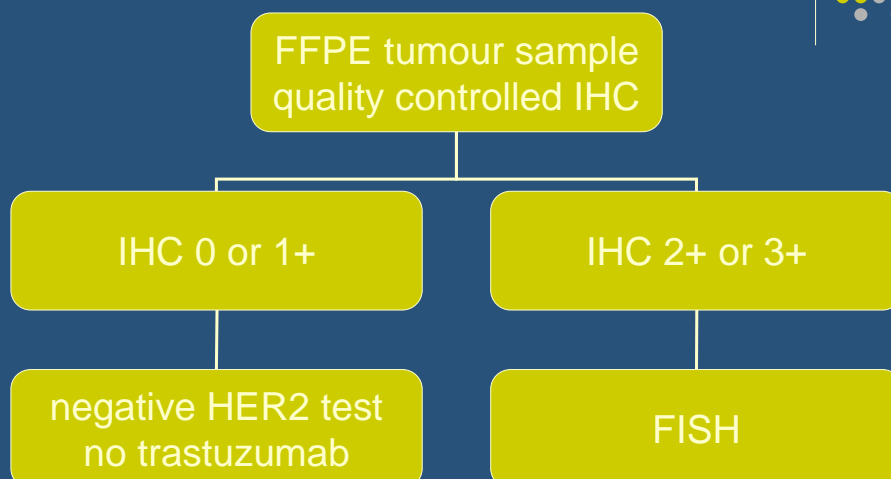
4. Laboratories need to document their concordance of IHC versus FISH annually. Concordance in IHC 0 and IHC 3+ categories should be at least 95%. Importantly, concordance in IHC 1+ category should be documented to be > 95% before limiting FISH testing only to IHC 2+ and 3+ results.
5. A yearly audit of HER2-positive results in an unselected breast cancer population should show that these are within the reported limits of 10-25%.
6. Participation in EQA external quality assessment rounds (CAP, UKNEQAS, NordiQC,...) is mandatory for labs performing ISH (ISO 15189) and encouraged for labs performing only IHC.



## Key message 1

Immunohistochemistry (IHC) is used for primary HER2 testing, detecting overexpression of the HER2 protein. Fluorescence In Situ Hybridisation (FISH) testing is performed in all tumours with IHC scores 2+ or 3+.

## HER2 testing algorithm in Belgium



## Results of a Belgian multicenter retrospective study to determine the incidence of HER2 gene amplification in patients scored as immunohistochemistry 0 or 1+.



*D. Larsimont, C. Colpaert, R. Salgado, N. Vermeesen, V. D'hondt, T. De Celle.*

*Jules Bordet Institute, Brussels, Belgium; St Augustinus, Antwerp, Wilrijk, Belgium; Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; Roche Diagnostics, Brussels, Belgium; Roche Pharmaceuticals, Brussels, Belgium*

J Clin Oncol 29: 2011 (suppl; abstr 549)

- Objective: to determine the proportion of patients who are not currently retested (i.e. IHC 0/1+) but who are HER2-positive by SISH.
- IHC 0/1+ samples from 34 participating laboratories
- **HER2-positive status (HER2/CEP 17 ratio > 2.0) was detected in 3.1% (n=14 of 456) of samples in total:**
  - 2.5% (n=4 of 163) of samples classified as IHC 0
  - 3.4% (n=10 of 293) of samples classified as IHC 1+.

## Key message 2



The interval between tissue acquisition and fixation should be as short as possible, preferably less than 1 hour.

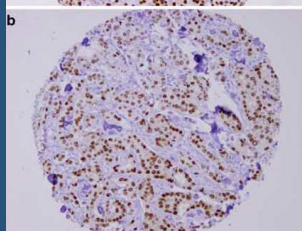
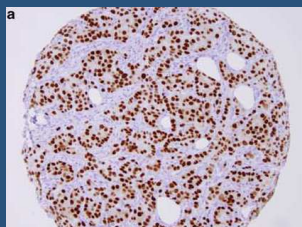
- Time between tissue removal and initiation of fixation must be recorded (cold ischaemia time): TTF
- Recent studies challenge the < 1 h guideline (e.g. Portier BP et al. *Mod Pathol* 2013) ↔ frozen tissue for tumour tissue banking

**Tumour tissues should be fixed in 10% buffered formalin for 6-48 hours.**

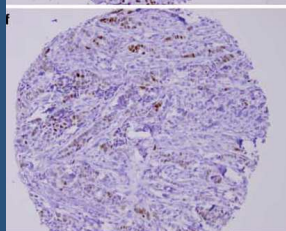
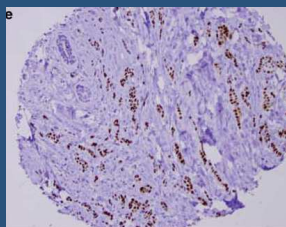
- Underfixation is more critical than overfixation.
- Duration of fixation (FT) should be routinely recorded

## interval between tissue acquisition and fixation should less than 1 hour

*Khoury T et al. Delay to formalin fixation effect on breast biomarkers. Modern Pathology 2009;22:1457-1467.*



ER: delay 0 versus 4 h



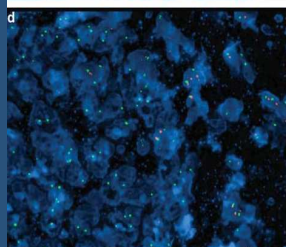
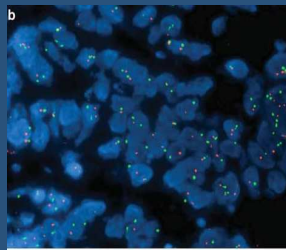
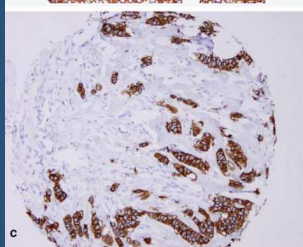
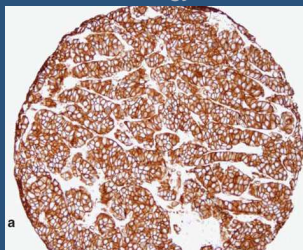
PR: delay 0 versus 1 h

For ER (DAKO 1D5), Allred score started to decline when delay to formalin fixation was > 2 h

For PR (DAKO PgR636), Allred score started to decline when delay to formalin fixation was > 1 h

## interval between tissue acquisition and fixation should less than 1 hour

*Khoury T et al. Delay to formalin fixation effect on breast biomarkers. Modern Pathology 2009;22:1457-1467.*

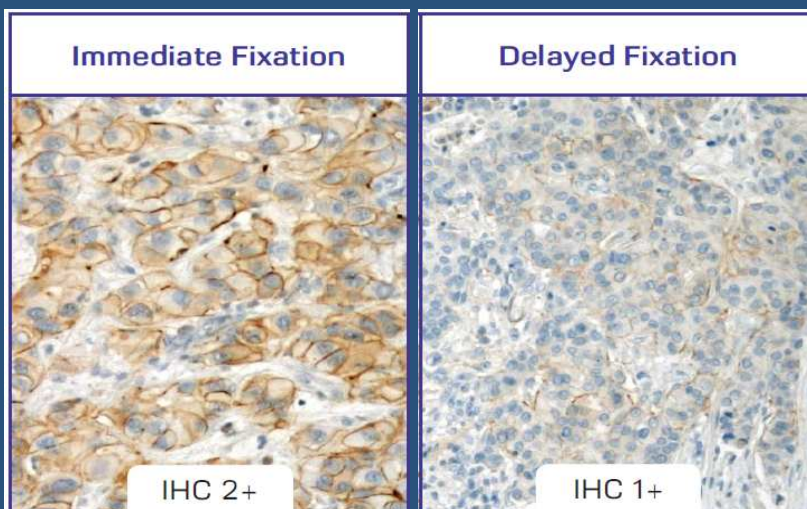


Delay to formalin fixation had no effect on HER2 IHC (DAKO rabbit polyclonal).

HER2 FISH interpretation started to be compromised with delays to formalin fixation of > 1 h:

- vague cellular outline
- poor nuclear resolution
- faded non-uniform signals (red>green)

## Delayed fixation has an impact on HER2 IHC score



Images : Courtesy of UCL Brussels (C. Galant/Y. Guiot)

## Formalin fixation time between 6 and 48 hours for all specimens

- ASCO/CAP 2007 recommendation based on only 1 abstract describing a small experiment with 3 carcinomas:

*Hofmann M, Gloeckner-Hofmann K, Maass G et al. Influence of different fixatives and fixation times on immunohistochemical detection of HER2/neu in breast cancer using Herceptest. Pathology Research and Practice 2003: 199(4). Abstract*

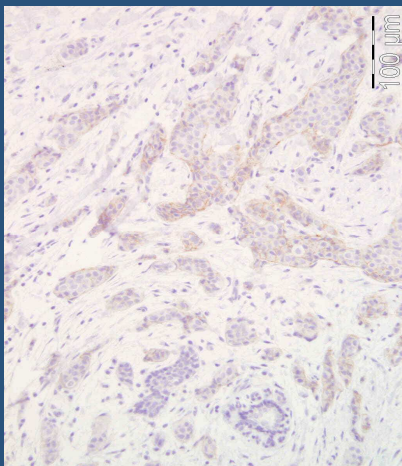
- ASCO/CAP 2010 ER / PgR recommendation: FT 6-72h
- Most laboratories try to implement the FT of 6-48 h, but prolonged fixation is sometimes inevitable during long WE.
- Underfixation is more critical than overfixation and leads to false-negative ER results, false-positive HER2 IHC results and “empty nuclei” in FISH.

## Underfixation is more critical than overfixation: overfixation experiment

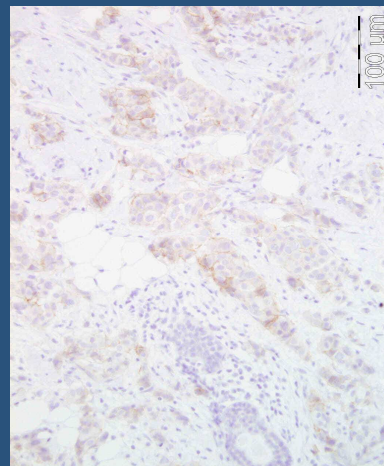


- 32 resection specimens of invasive breast cancers:  
one tissue section adjacent to the tissue section used for diagnosis and immunohistochemistry was fixed in formalin 10% for a prolonged time between 75 h and 300 h, mean 118 h.
- HER2 IHC score (DAKO Herceptest) was similar in overfixed and standard fixed tissue sections.

## Underfixation is more critical than overfixation: overfixation experiment



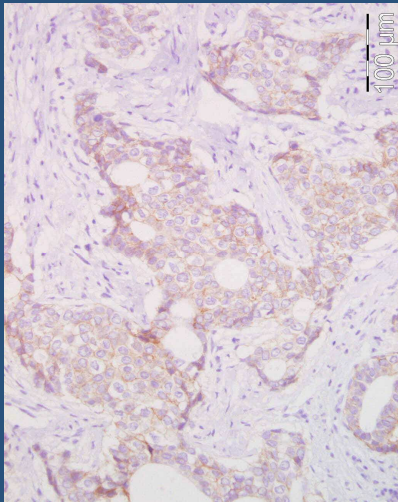
IHC score 1+: fixation time 6-48 h



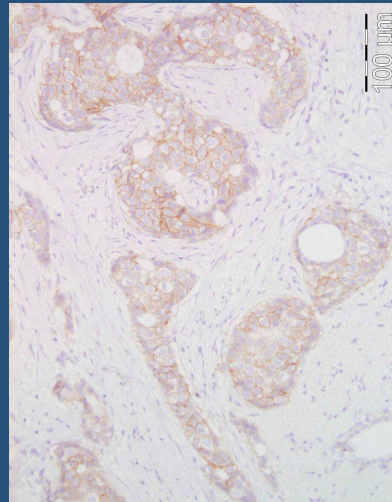
IHC score 1+: fixation time 80 h



## Underfixation is more critical than overfixation: overfixation experiment

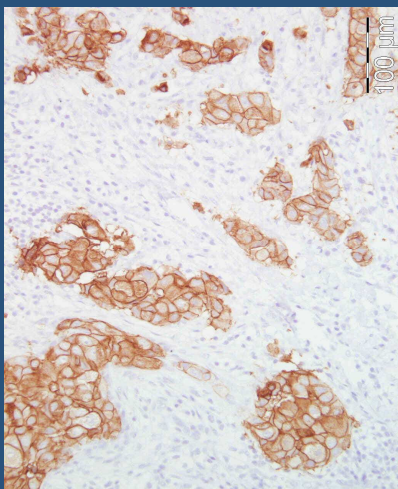


IHC score 2+: fixation 6-48 h

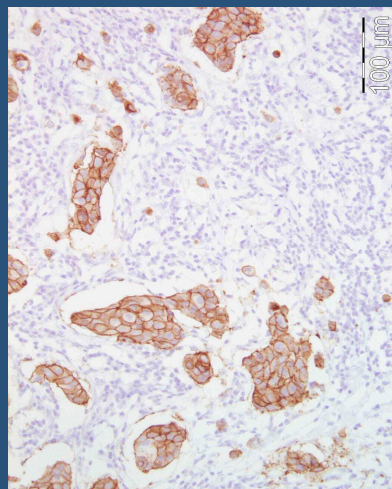


IHC score 2+: fixation 150 h

## Underfixation is more critical than overfixation: overfixation experiment



IHC score 3+: fixation 6-48 h

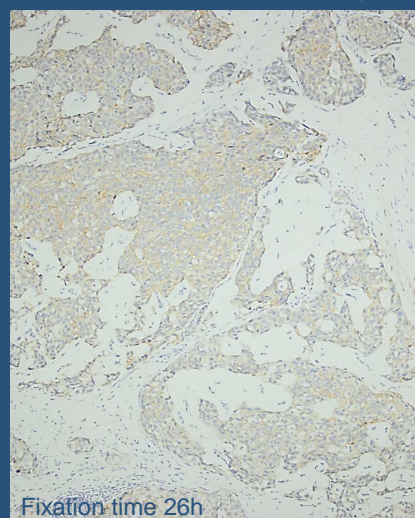
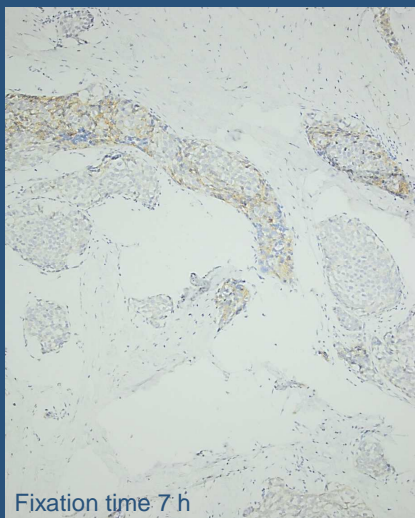


IHC score 3+: fixation 300 h

## Underfixation is more critical than overfixation: overfixation experiment

- FISH (DAKO pharmDx) performed on 9 IHC score 1+ cases, 7 IHC score 2+ cases and 3 IHC score 3+ cases.
- Quality of FISH assay was good (CAP/ASCO criteria) in all but one case: 216 h formalin fixation time.  
In 4 other cases: focal loss of hybridisation signals (red>green); all 4 cases fixation times  $\geq 100$  h.
- fixation time 6 – 48 h is recommended, but can be prolonged to a maximum of 100 h

## Minimum fixation time of 6h may be insufficient for resection specimens



## Key message 3

The use of controls of known HER2 levels is mandatory.

- A positive (IHC3+/amplified) and negative (0-1+/non-amplified) control, determined by IHC and FISH, is a minimum.
- An additional control close to cut-off values is also recommended, e.g. a tumour with IHC score 2+ and HER2/CEP17 between 2 and 2,5.

## Key message 3: IHC control materials



- Adequate control materials include small **tumour blocks** with well defined HER2 protein expression level and HER2 gene amplification status.
- Composite control tumour block can be made by every laboratory.
- Cell lines available in some kits.
- HER2 IHC **score 2+ block is more informative** about sensitivity of the technique than 3+ block.
- **Ideally place control tumour blocks on the same slide as the patient's tumour** to detect technical errors e.g. failure to apply reagents.



## Belgian Working Group for Breast Pathology

Survey on the implementation of CAP/ASCO HER2 guidelines

In 2012 survey sent to ISH labs → correspond with referring labs

Response: 12 labs with ISO 15189 accreditation and 12 labs without ISO 15189 accreditation

### →Accredited labs:

- test more HER2 IHC score 1+ cases with ISH
- more standard reporting of TTF and FT, but almost all labs aim to have TTF<1h en FT 6-48h

- use 1st line control with 0-1+ and 3+

5/12 also use 2+

### ↔ non accredited labs: no control or only 3+ control

- all accredited labs and only some non accredited labs have a database for prospective recording of IHC/ISH concordance



## Key message 4

Laboratories need to document their concordance of IHC versus ISH annually.

○Concordance in IHC 0 and IHC 3+ categories should be at least 95%.

○Importantly, concordance in IHC 1+ category should be documented to be >95% before limiting ISH testing only to IHC 2+ and 3+ results.



## Key message 4

### Concordance of IHC versus ISH



Concordance in IHC 0 and IHC 3+ categories should be at least 95%.

- 100% concordance in IHC3+ group → IHC test is not sensitive enough
- Concordance <<< 95% in IHC3+ group → IHC test is too sensitive

Importantly, concordance in IHC 0-1+ category should be documented to be > 95% before limiting ISH testing only to IHC 2+ and 3+ results.

- ISH testing of a random sample of IHC 0-1+ cases?
- ISH testing of all IHC 0-1+ cases?

## Concordance of IHC versus ISH

Excel file for prospective collection of data on HER2 IHC/ISH concordance: yearly audit



**Suggestion: designate 1 person in the lab responsible for keeping the file**

Patient identity	lab number of specimen excision/trucut	Time to formalin fixation	Fixation time	HER2 IHC score 0, 1+, 2+, 3+	FISH ratio HER2/CEP17	Absolute number of HER2 signals

- Time to formalin fixation: < 1h, >1h\*, unknown\*
  - Fixation time: <6h\*, 6-48h, 48-72h\*, >72h\*, >100h\*, unknown\*
- for all fixation conditions marked with an \*, a disclaimer is added in the report

## Key message 5

A yearly audit of HER2-positive results in an unselected breast cancer population should show that these are within the reported limits of 10-25%.

MR 2013 GZA: 381 resection specimens in 2012

- HER2 IHC 3+: 7,1%, 2+: 32,5%, 1+: 54,9%, 0: 5,5%
- HER2 gene amplification: 96,2% of IHC3+, 17,7% of IHC2+, 1% of IHC1+, 0% of IHC0

→HER2 gene amplification in 13% of resection specimens  
(17,9% in 2011, 14,3% in 2010, 16,3% in 2009)

## Key message 6

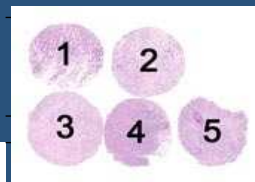
Participation in EQA external quality assessment rounds (CAP, UKNEQAS, NordiQC,...) is mandatory for labs performing ISH (ISO 15189) and encouraged for labs performing only IHC.

## NordiQC

### Assessment Run B14 2012

#### HER-2 IHC

The slide to be stained for HER-2 comprised the following 5 tissues:



	IHC HER-2 Score* (0, 1+, 2+, 3+)	FISH HER-2/chr17 ratio**
1. Breast ductal carcinoma	0-1+	1.1 – 1.4
2. Breast ductal carcinoma	0-1+	1.2 – 1.5
3. Breast lobular carcinoma	1-2+	1.3 – 1.7
4. Breast ductal carcinoma	2-3+	2.5 – 2.8
5. Breast ductal carcinoma	3+	> 6.0* HER-2

*immunohistochemical score (guidelines below) as achieved by using  
two FDA approved kits and antibodies  
(HercepTest™, Dako & PATHWAY®, Ventana)*

## Key message 6: EQA

### Anonymous results from NordiQC run B12 (HER2 IHC) sent to BWGBP:

- Belgian labs *with* ISO15189 accreditation:  
9/11 optimal, 1/11 good, 1/11 poor result
- Belgian labs *without* ISO15189 accreditation:  
21/36 optimal, 1/36 good, 14/36 (39%) poor

*NordiQC: “staining is assessed as poor in case of a false negative staining (e.g. the 3+ tumour and the 2+ tumour with gene amplification showing a 0 or 1+ reaction) or a false positive staining (e.g. the 0, 1+ and 2+ tumours without gene amplification showing a 3+ reaction).”*

## Adhere strictly to CAP/ASCO scoring criteria – tune with colleagues!

**Table 1. Scoring of HER2 immunohistochemistry.**

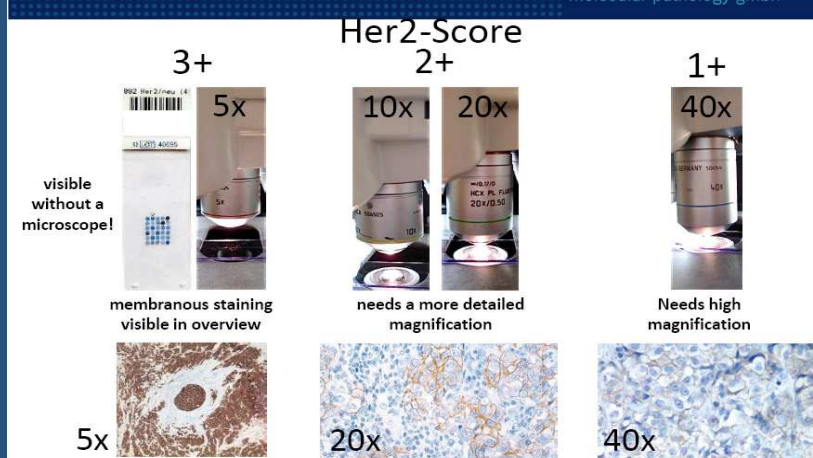
- **Score 0:** no staining is observed in invasive tumour cells ("negative").
- **Score 1+:** weak, incomplete membrane staining in any proportion of invasive tumour cells, or weak, complete membrane staining in less than 10% of cells. Incomplete means that the cells are only stained in part of their membrane ("negative").
- **Score 2+:** complete membrane staining that is non-uniform or weak but with obvious circumferential distribution in at least 10% of invasive tumour cells, or intense complete membrane staining in 30% or less of invasive tumour cells ("equivocal: weakly or focally positive").
- **Score 3+:** intense complete membrane staining is observed in more than 30% of invasive tumour cells ("positive").

*Intense staining: easily visualised with 4x or 10x objective*

*Weak staining: visualisation requires 40x objective*

## Adhere strictly to CAP/ASCO criteria

targos  
molecular pathology gmbh



Rüschhoff J et al. Histopathology 2010

## Concordance of IHC versus FISH yearly audit of HER2-positive results



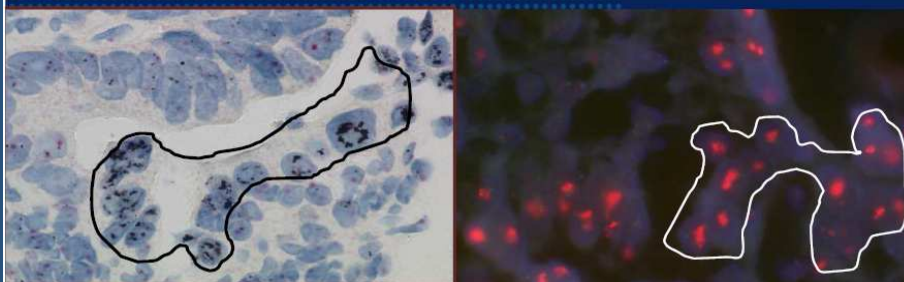
In 2009: 295 resection specimens of breast cancer:

- 16,7 % of specimens with IHC score 2+ (DAKO Herceptest): FISH confirmed amplification
- 94,9 % of specimens with IHC score 3+ (DAKO Herceptest): FISH confirmed amplification

→ In 16,3% of the 295 resection specimens FISH confirmed amplification of HER2 (reported limits are 10-25%)

How to deal with tumor heterogeneity  
In ISH analysis (brightfield, fluorescent)

targos  
molecular pathology gmbh



Scan all of the tumor tissue on the slide for focal amplification or other gene count alteration (e.g. polysomy, interspersed amplified cells).

Count signals in 20 *adjacent* tumor cells, skip cells that don't meet the quality criteria.

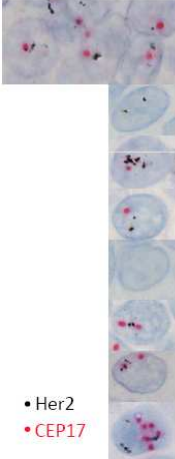
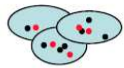







BDISH allows a more thorough scanning of the tissue (20-40x magnification), and the counting can be done at 40x or 60x magnification as opposed to 100x in FISH.

02.06.09

## ISH signal enumeration

targos  
molecular pathology gmbh

Her2 BDISH – proposed analysis guidelines based on criteria for FISH

 <p>• Her2 • CEP17</p>		Don't count within overlapping nuclei
		2 black signals – don't count cells that show one type of signal only
		1 red and 15 black signals
		1 red and 2 black signals (distance between black signals < 1 signal diameter)
		no signals
		2 red and 2 black signals?
		2 red and 5 black signals
		6 red and 7 black signals

## Her2 ISH :

selection of the area makes the difference!

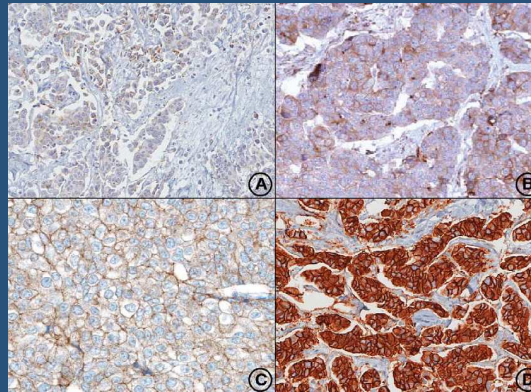
- H&E: tumor morphology? necrosis? inflammation? In-situ component?
- Scan all of the tumor tissue, look for areas with any kind of gene alteration, e.g. focal amplification, interspersed amplified cells, polysomy...).
- Count 20 adjacent tumor cells within the chosen area, only leaving out cells that don't meet the quality criteria (esp. overlapping nuclei).
- In case of a ratio between 1.8 and 2.2 count additional 40 cells in a different area. The final cut off is then 2.0



## Immunohistochemistry IHC

Utilizing cell lines, it was possible to establish a standardized immunohistochemical procedure and scoring system

- A: cells containing less than 20,000 receptors show no staining (**score 0**)
- B: cells containing 100,000 receptors show partial membrane staining with less than 10% of the cells showing complete membrane staining (**score 1+**)
- C: cells containing 500,000 receptors show light to moderate complete membrane staining in more than 10% of the cells (**score 2+**)
- D: cells containing 2,300,000 receptors show strong, complete membrane staining (**score 3+**)



### Pathology of the Breast: Possible artefacts

BENEFITS OF STANDARDIZATION

## The Formalin-free Hospital

Brussels, Belgium, 20<sup>th</sup> April 2013

**Gianni Bussolati**  
*University of Turin*



**Pathology of the Breast:  
Possible artefacts**

BENEFITS OF STANDARDIZATION

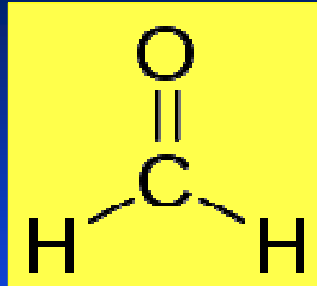
## **A Formalin-free Hospital ?**

**Brussels, Belgium, 20<sup>th</sup> April 2013**

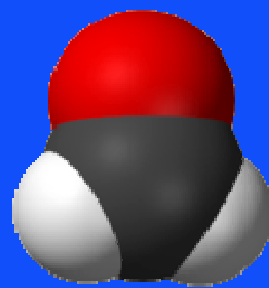
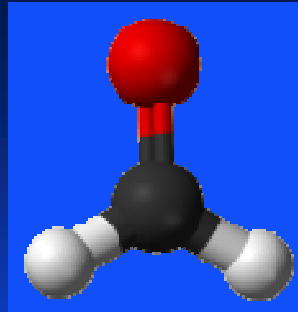
***Gianni Bussolati***  
***University of Turin***

### **Questions:**

- **Is a Formalin-free Hospital feasible?**
- **Is a Formalin-free Pathology feasible?**



Formaldehyde



## FORMALDEHYDE

- Discovered by Butlerow in 1859
- Synthesis procedure: von Hoffman in 1868
- Patent by Trillar in 1889
- Anti-septic properties : F. Blum in 1893
- Formaldehyde as a FIXATIVE: F Blum (1893, 1894)

Ferdinand Blum  
(1940)



## Formalin Fixation:

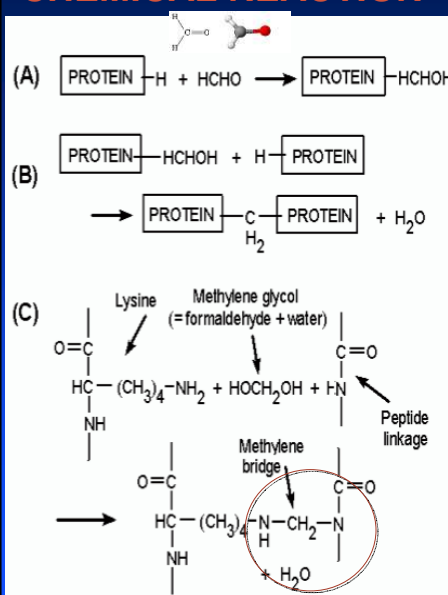
### Advantages

- Cost
- Safe for tissue
- Fast penetration
- Excellent morphology
- ICC

### Disadvantages

- Unsafe for operators
- Effect on Nucleic Acid

## CHEMICAL REACTION



The main effect of formaldehyde in tissues is linked to the **formation of methylol groups on amino groups** first, followed by the **establishment of cross-linking methylene groups** that lead to proper fixation

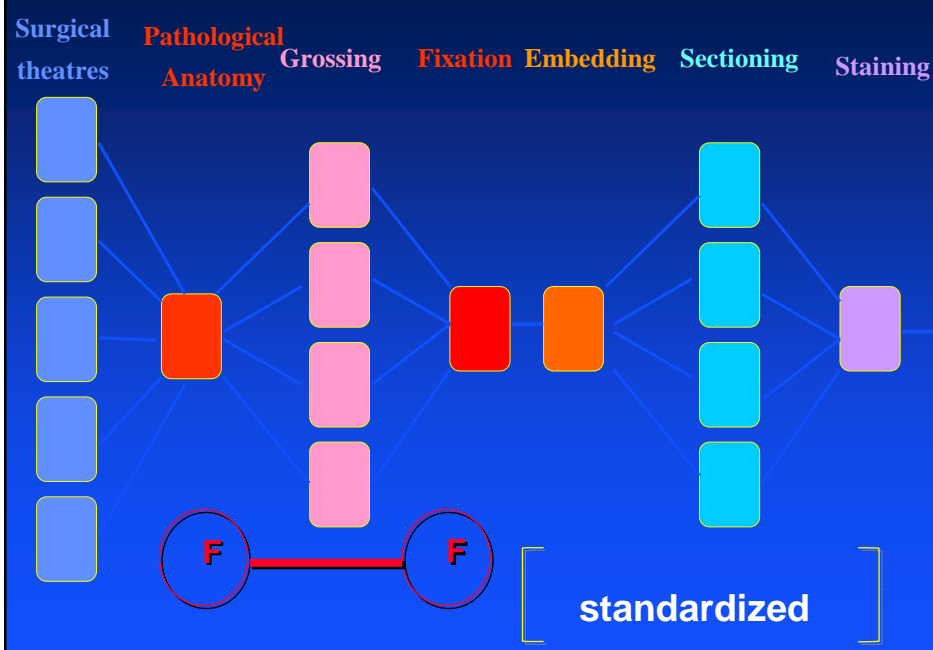
Initial binding of formaldehyde to protein is  
 • largely completed in 24 hours but the formation of methylene bridges proceeds much more slowly.

• still relatively weak and reversible; stronger cross-linking continues to occur over time.  
 Complete fixation is thought to take at least 7 days. Even after this time cross-links continue to form slowly.

Depending upon the time of interruption, the periphery may show adequate cross-linking, whereas the remainder of the tissue is fixed by coagulant alcohol during processing.

This may have disastrous effects upon IHC staining. This will occur whether the tissue is a small biopsy or a 4 mm slice.

## Pre-analytical procedures in histopathology



## **When (where) is Formalin used?**

- 1) TRANSFER of surgical specimens to Pathology labs.**
- 2) FIXATION of biopsies**
- 2) PRESERVATION of residual tissues.**

## **Interval 2: From the surgical table to the pathology laboratory**

**“cold ischemia time”**

### **Alternatives :**

- a) Tissues left fresh**

## “Cold ischemia time interval”

Alternative: a) Tissues left fresh

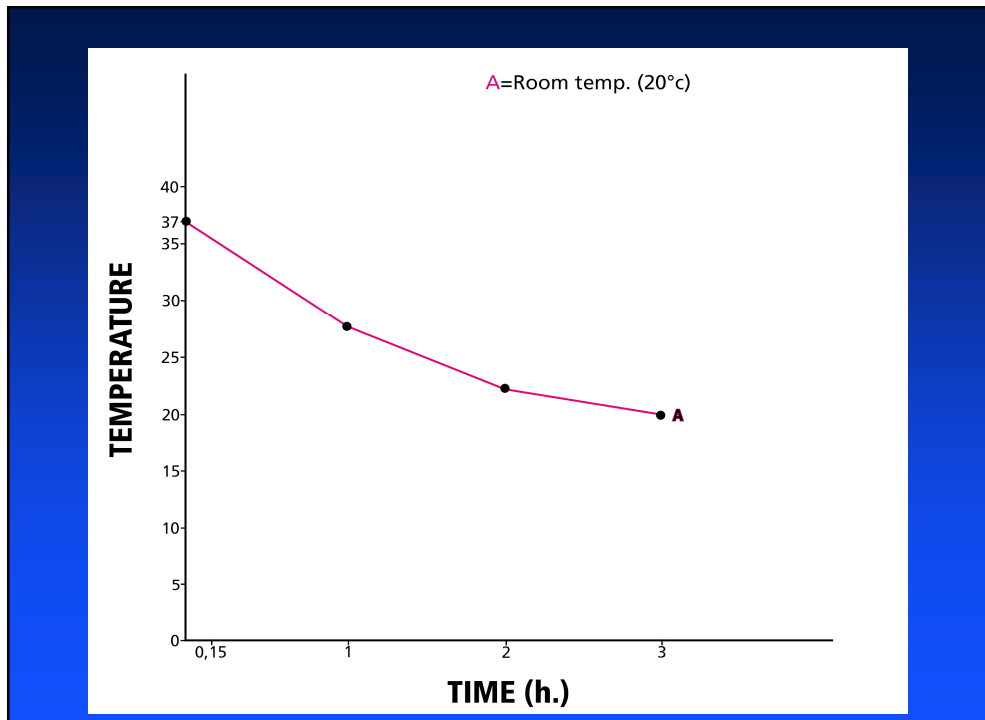
Temperature: Room Temperature (about 20°C)

In some realities, tissues (free in a vessel or in a bag) are transferred to the grossing room.

- Time interval : relatively short (it depends)
- Time before grossing: variable from a few minutes up to several hours. Optimal: 30 minutes
- Up to 4 hours according to Grizzle et al. 2001.<sup>[3]</sup>



How cold is cold ischemia?



## “Cold ischemia time interval”

Alternative: a) Tissues left fresh

### Merits:

- No Fixation

(material available for banking)



## **“Cold ischemia time interval”**

**Alternative: a) Tissues left fresh**

### **Drawbacks:**

- Drying of tissues  
(Even of tissues left in refrigerator)**
- Loss of antigens and RNA related to the time spent at room temperature before grossing**

## **ASCO / CAP Guidelines for Breast Cancer Fixation**

- 1) Reduce time of “tissue ischemia” before grossing - fixation to < 1 h**
- 2) Fixation time 12 – 48 h**



### **“Cold ischemia time interval”**

**From the surgical table to the pathology lab**

#### **Alternatives :**

- a) Tissues left fresh
- b) Tissues immersed in formalin



Molinette  
Hospital  
year 2005

### **“Cold ischemia time interval”**

**From the surgical table to the pathology**

**Alternative: b) Tissues immersed in formalin**

**Temperature: Room Temperature (generally)**

- **Time interval : from a few minutes up to days.**

**Formalin: penetration is fast initially (1mm/h), then much slower (1cm/24h).**

**This is followed by fixation (slow);  
subtotal binding plateau at 24 h.**

**Fixation time at least 6-8h in 3mm thick specimens.**

**“Cold ischemia time interval”****From the surgical table to the pathology lab****Alternative: b) Tissues immersed in formalin****Merits:**

- In small blocks it rapidly affects:  
structure, antigens and nucleic acids  
(preservation /de-naturation)

**Tissues immersed in****Phosphate – buffered Formalin****Small Biopsies <1****Uniform****cm. = fixation****Large specimen ( > 2 cm)**

- Outside = Fixed
- Inside = Autolysis

## From the surgical table to the pathology lab

### Alternative: b) Tissues immersed in formalin

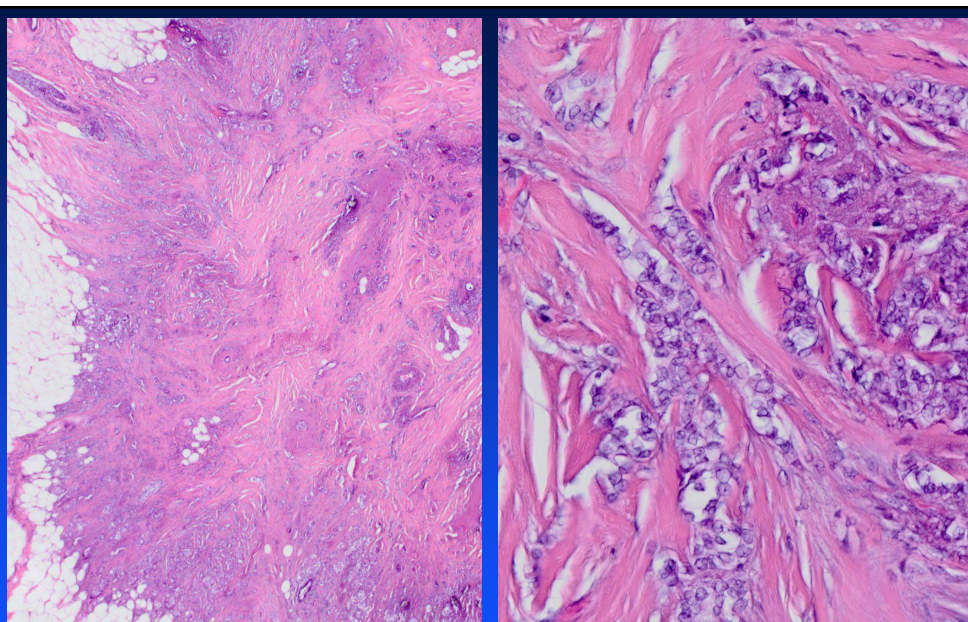
#### Drawbacks (in large specimens):

- Degradation continues in deep areas
- Tissue banking is hampered
- Formalin containing vessels heavy to carry
- Spilling of formalin may occur
- Fumes dispersed while grossing
- Nurses refuse to handle this “carcinogen” in surgical theatre (and without hoods)
- Tissue forgotten by the surgeon because “already safe in formalin”

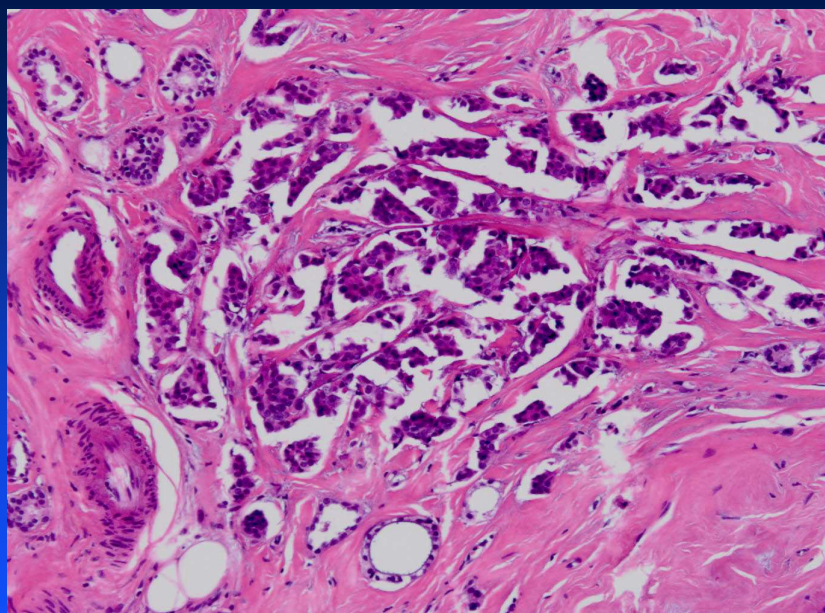
**Jan. 2006:**

**Surgeons start to operate  
on  
Friday afternoon.**

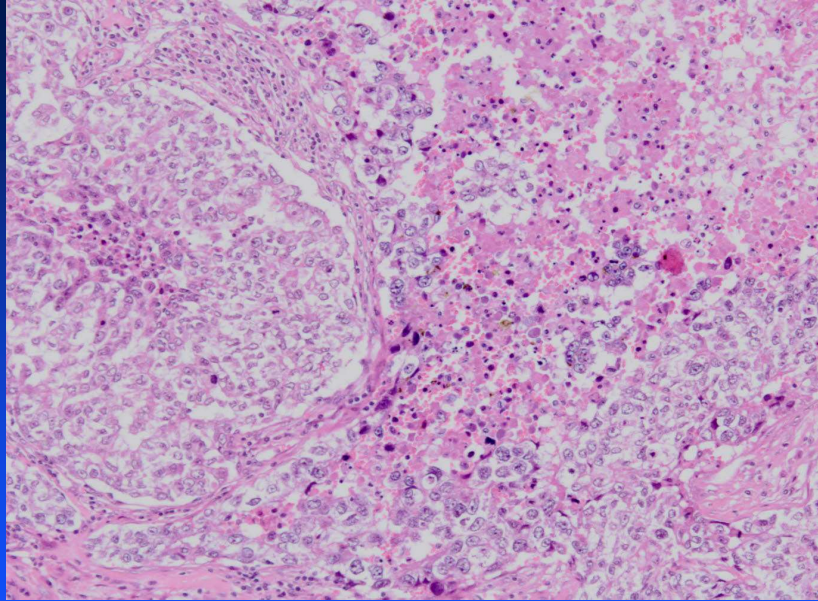




**Breast Cancer.** Operated on Friday; Immersed in Formalin; Arrived on Monday.  
Jan. 2006



**Breast Cancer.** Operated on Friday; Immersed in Formalin; Arrived on Monday.  
Jan. 2006



**Breast Cancer.** Operated on Friday; Immersed in Formalin; Arrived on Monday.  
Jan. 2006

**“Cold ischemia time interval”**  
**From the surgical table to the pathology lab**

**Alternatives :**

- a) Tissues left fresh
- b) Tissues immersed in formalin
- c) Under Vacuum Sealing and Cooling (UVSC)**



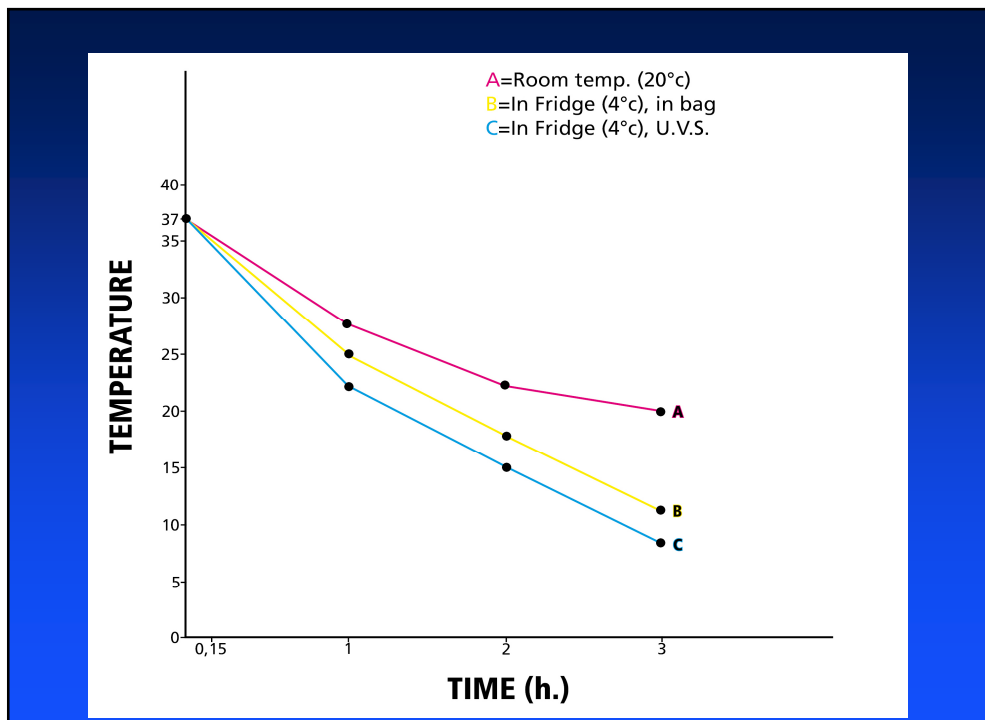
Virchows Arch  
DOI 10.1007/s00428-007-0529-x

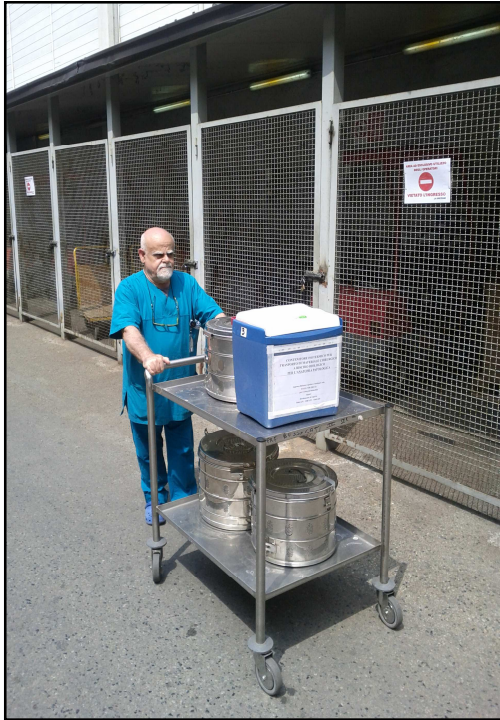
LETTER TO THE EDITOR

## **Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin**

**Gianni Bussolati • Luigi Chiusa • Antonio Cimino •  
Giuseppe D'Armento**



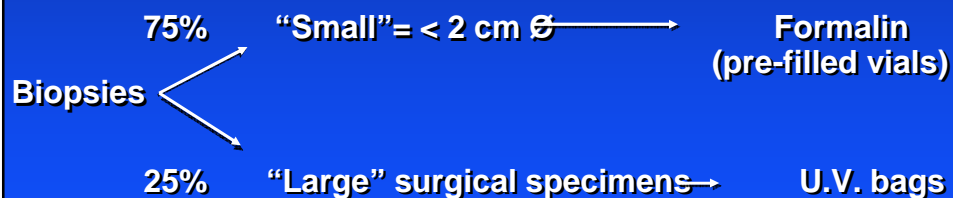




**Molinette Hospital  
year 2010**

**Project: “...Towards a Formalin-free Hospital”  
“Molinette” Hospital, Turin**

- 1162 Beds; >54.000 yearly admissions; > 40.000 histopathological exams. (2008)



Science of the Total Environment 408 (2010) 3092–3095

Contents lists available at [ScienceDirect](#)

**Science of the Total Environment**

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)




Vacuum-based preservation of surgical specimens: An environmentally-safe step towards a formalin-free hospital

Cinzia Di Novi <sup>a</sup>, Davide Minniti <sup>b</sup>, Silvana Barbaro <sup>b</sup>, Maria Gabriella Zampirolo <sup>b</sup>, Antonio Cimino <sup>c</sup>, Gianni Bussolati <sup>c,\*</sup>

## Staff satisfaction

<i>Level of Satisfaction</i>	<i>Freq.</i>		<i>Percent</i>	
	<i>formalin</i>	<i>under-vacuum</i>	<i>formalin</i>	<i>under-vacuum</i>
Low	42	6	39.25	8.57
average	44	17	41.12	24.29
high	21	47	19.63	67.14
Total	107	70	100.00	100.00

C. Di Novi et al. / *Science of the Total Environment* 408 (2010) 3092–3095

## Gross anatomic preservation

	FORMALIN		UNDER-VACUUM	
	Mean	Std. Dev.	Mean	Std. Dev.
# 1= esofagus and stomach				
STRUCTURE	1.108696	0.3146964	2.977778	0.1490712
COLOUR	1.086957	0.2848849	2.956522	0.2061846
CONSISTENCY	1.913043	0.2848849	2.782609	0.4170288
# 2= colon				
STRUCTURE	1.021739	0.147442	2.913043	0.2848849
COLOUR	1.130435	0.3405026	2.955556	0.2084091
CONSISTENCY	1.911111	0.287799	2.652174	0.4815434
# 3= kidney and prostate				
STRUCTURE	1.934783	0.2496374	2.326087	0.4739596
COLOUR	1.173913	0.383223	2.913043	0.2848849
CONSISTENCY	2.021739	0.147442	2.23913	0.431266
# 6= endocrine / thyroid				
STRUCTURE	1.934783	0.2496374	2.326087	0.4739596
COLOUR	1.23913	0.431266	2.934783	0.2496374
CONSISTENCY	2	0.2108185	2.152174	0.3631584
# 8= liver / spleen				
STRUCTURE	1.904762	0.2971018	2.690476	0.4679011
COLOUR	1.190476	0.3974366	3	0
CONSISTENCY	1.97619	0.1543033	2.404762	0.4967958

1= weak  
2= satisfactory  
3= good

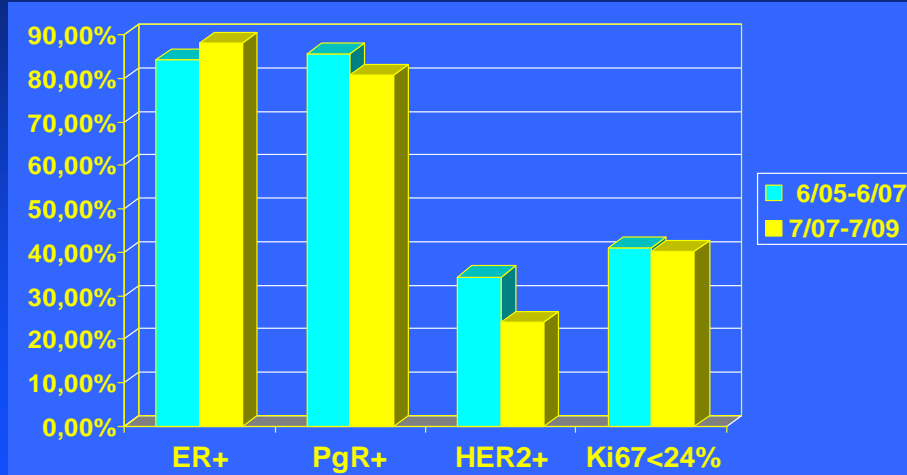
C. Di Novi et al. / Science of the Total Environment 408 (2010) 3092–3095

## Result of Survey among Staff Operators (October 2008 – April 2009)

- Satisfaction:
  - Low for Formalin
  - High for U.V.
- Handling & Gross Anatomy
  - Histopathol. + ICC - U.V. = no drawbacks

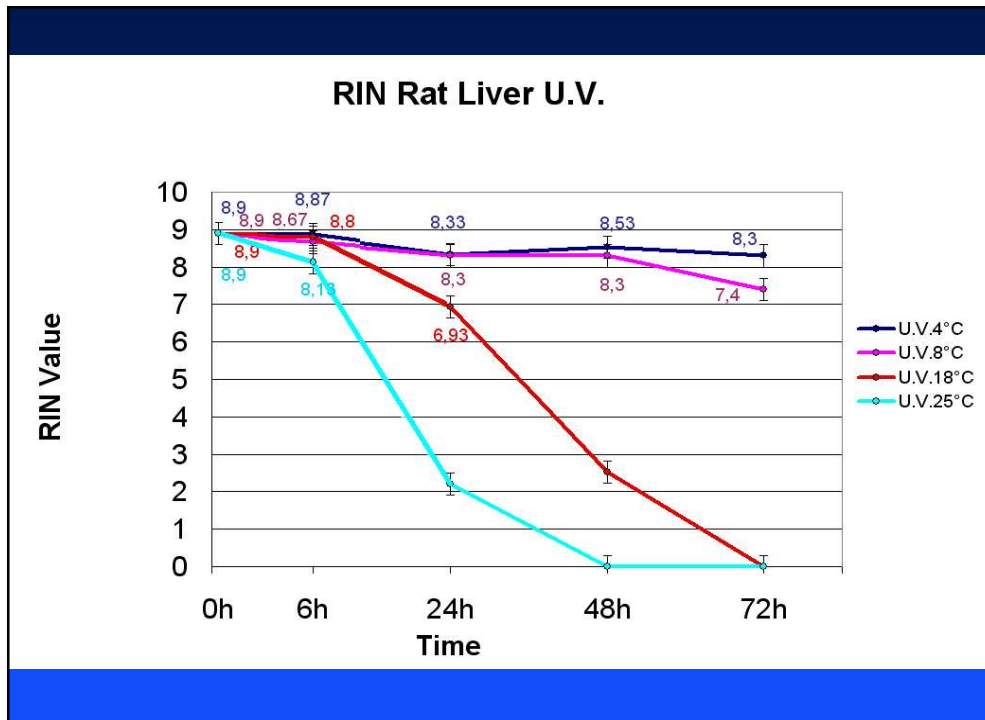
Project: ————— F-f

### ICC evaluation of therapeutic/prognostic parameters in a continuous series of breast cancers Years 6/05-6/07 vs. 7/07-7/09. N=375



#### Breast cancers processed by USV and cooling (4°C)

histo ID	gap	RIN	histo ID	gap	RIN	3400/09/3	24h	8,7	8687/09/3	7h	8,6
1285/09/3	1h	9	6054/09/3	6h	8,6	3673/09/3	1h	9,6	9000/09/3	24h	7,4
1385/09/3	60h	7,9	6056/09/3	24h	7,3	3657/09/3	72h	7,5	9112/09/3	24h	7,8
1394/09/3	60h	7,9	6058/09/3	24h	7,9	3764/09/3	48h	6,6	9183/09/3	7h	6,9
1599/09/3/B	60h	7,4	6226/09/3	2h	6,9	3889/09/3	72h	7,8	9248/09/3	48h	8
1271/09/3	1h	7,8	6263/09/3	5h	7,8	3888/09/3	72h	8,2	9324/09/3	24h	8,7
1498/09/3	5h	7,5	6368/09/3	72h	7,6	3850/09/3	70h	7,4	9452/09/3	5h	7,9
1469/09/3	5h	7,9	6369/09/3	72h	8	3993/09/3	72h	7,6	9461/09/3	7h	7,5
1422/09/3	2h	8,7	6422/09/3	24h	7,1	4014/09/3	2h	7,6	9585/09/3	24h	8,6
1267/09/3	5h	8,2	6520/09/3	24h	7,7	4073/09/3	6h	9,1	9687/09/3	4h	7,1
1698/09/3/m	24h	8,7	6552/09/3	24h	7,1	4075/09/3	5h	7,9	108/10/3	5h	7,7
1783/09/3	60h	7,9	6753/09/3	24h	7,8	4122/09/3	20h	7,3	143/10/3	5h	8
1793/09/3	60h	7,6	6849/09/3	72h	7,8	4221/09/3	70h	7,4	305/10/3	2h	7,2
1844/09/3	1h	8,7	6907/09/3m	24h	8,7	4211/09/3	70h	6,2	435/10/3	5h	8,2
1886/09/3	1h	8,5	7004/09/3	6h	8,3	4275/09/3	7h	9,4	506/10/3	6h	8,6
1904/09/3	24h	7,3	723/09/Domen	2h	8,6	4298/09/3	24h	6,6	651/10/3	5h	8,5
1992/09/3	5h	7,3	7129/09/3	6h	8,1	4471/09/3	48h	8,6	650/10/3	3h	6,8
1995/09/3	72h	8,1	7249/09/3	2h	9,1	4553/09/3	30h	8	649/10/3	23h	6,8
2032/09/3	7h	7,5	7250/09/3	4h	9,6	4594/09/3	72h	6,2	1022/10/3	72h	8,6
2163/09/3	60h	8,1	7362/09/3	6h	9,4	4617/09/3	2h	8,9	1267/10/3	7h	7,8
2241/09/3	1h	8,9	7405/09/3	6h	7,9	4668/09/3	24h	8,6	1316/10/3	5h	7,7
2238/09/3	6h	8,4	7489/09/3	5h	9,1	4831/09/3	5h	8,3	1453/10/3	24h	6,5
2237/09/3	5h	8	7514/09/3	24h	8	4937/09/3	72h	8	1677/10/3	6h	9
2574/09/3	6h	7,9	7613/09/3	6h	7,3	5038/09/3	24h	8,2	1775/10/3	7h	9,2
2621/09/3	24h	7,6	7781/09/3	8h	7,9	5118/09/3	72h	6,2	1944/10/3	7h	8
2622/09/3	24h	6,8	7782/09/3	6h	7,7	5357/09/3	24h	7,3	1945/10/3	4h	7,4
2712/09/3	72h	7,4	7805/09/3	5h	7	5525/09/3	5h	7,8	2033/10/3	5h	9,1
2665/09/3	20h	7,8	7886/09/3	5h	7,9	5598/09/3	1h	7,9	2036/10/3	24h	7,4
2743/09/3	72h	6,4	7904/09/3	24h	8,6	5661/09/3	7h	7,1	2096/10/3	24h	9,5
2820/09/3	24h	7,2	8153/09/3	7h	7	5764/09/3	5h	6,2	2082/10/3	5h	6,6
2817/09/3m	24h	7,9	8215/09/3	72h	9	5822/09/3	5h	7,4			
3216/09/03	1h	7	8413/09/3	72h	6,5						
3365/09/3	7h	8,1	8542/09/3	5h	7,2						
3368/09/03	7h	7,7	8623/09/3	5h	7,6						

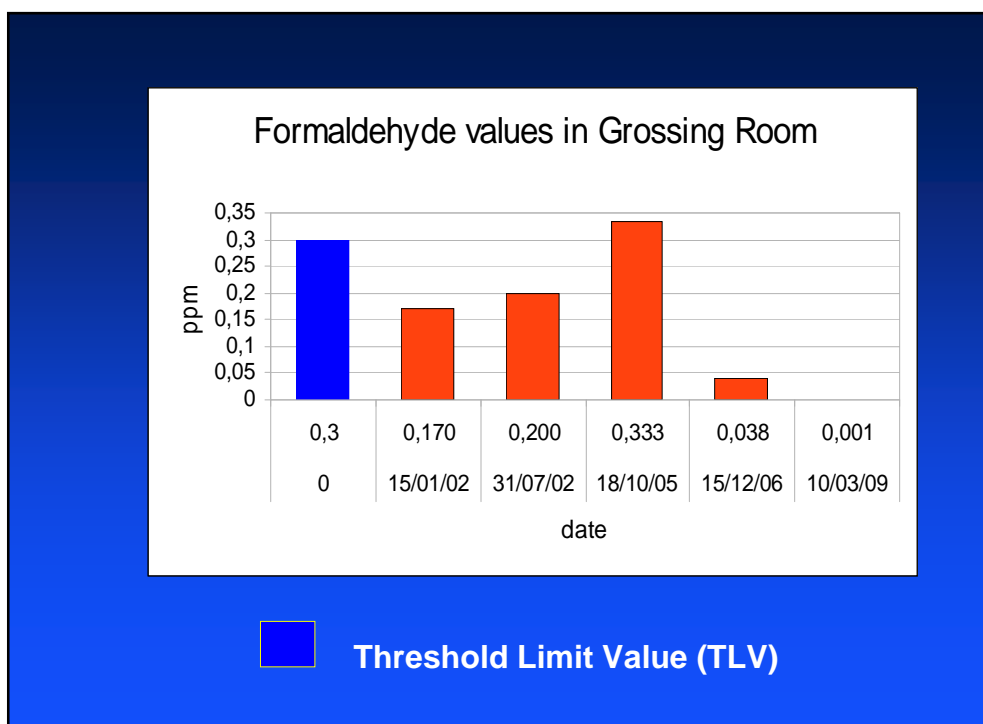
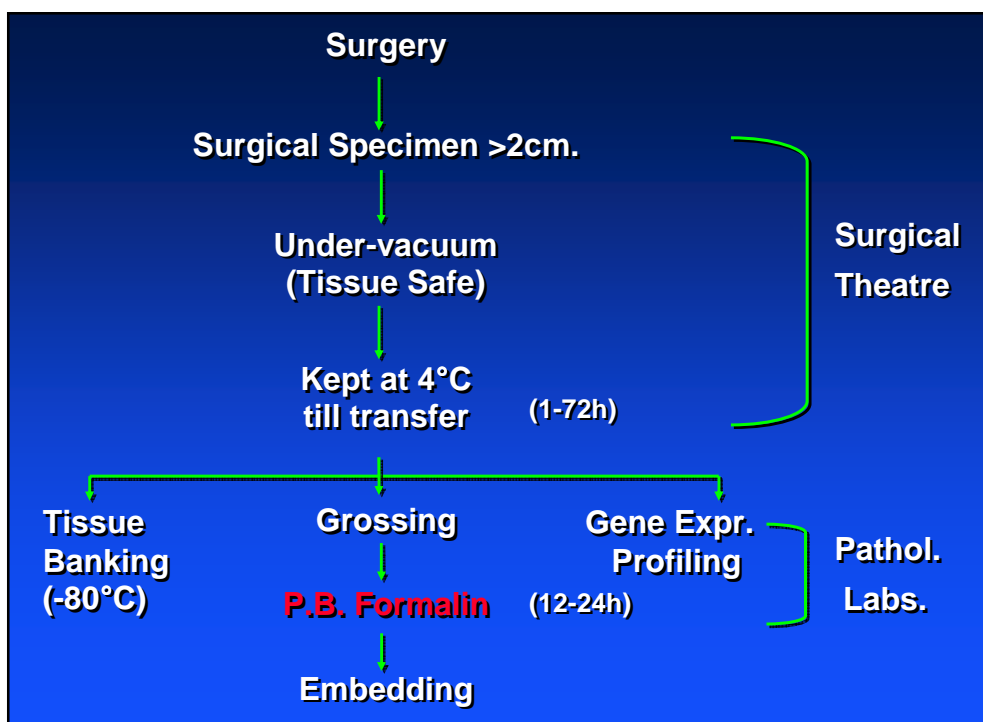


### Alternative c) Tissues preserved under vacuum

#### Merits:

- No more formalin in surgical theatre (except for small specimens, where pre-filled tubes are employed)
- No spilling
- No fumes
- No drying of tissues
- Colours preserved
- Lack of insulating air around tissues allows fast cooling
- Tissues (bags) light and easy to carry
- Structure (RNA, Antigens) preserved up to days
- Banking (selective) allowed
- Demonstrating of operated tissues is convincing for students and surgeons





### When (where) is Formalin used?

- 1) **TRANSFER** of surgical specimens to Pathology labs.
- 2) **FIXATION** of biopsies  
**Ratio Formalin:tissue = 20:1**
- 2) **PRESERVATION** of residual tissues.

.... PAXPE fixation offers some advantages concerning molecular analysis. However, these advantages would **not justify substituting formalin** fixation in any routine pathology laboratory.

Will PAXgene substitute formalin? A morphological and molecular comparative study using a new fixative system  
Belloni B. et al, [jcp.bmj.com](http://jcp.bmj.com) on April 1, 2013

## When (where) is Formalin used?

1) TRANSFER of surgical specimens  
to Pathology labs.

2) FIXATION of biopsies

2) PRESERVATION of residual tissues.  
Ratio Formalin:Tissue = 2:1



The preset amount of formalin has been added, the bag is vacuum-sealed



The specimen is ready for storage

**Answers:**

- Is a Formalin-free Hospital feasible?
  - YES (almost)
- Is a Formalin-free Pathology feasible?

**NO**

(but use can/must be reduced)

## AJSP-D-07-00718 “Tissue transfer to Pathology labs: under-vacuum is the safe alternative to Formalin”

*Reviewer N. 1.* No question that formalin is a serious health hazard and that we as Pathologists need to pay attention to potential economics of its use. This article propose one possible solution. The concept of vacuum storage supplanting formalin is intriguing. However .....

## The CEP 17 amplification

A. Sapino, Turin, Italy

### The Second TASTE Workshop Pathology of the Breast: Possible artefacts BENEFITS OF STANDARDIZATION

Accreditation has been requested in the category 'Ethics and Economics'

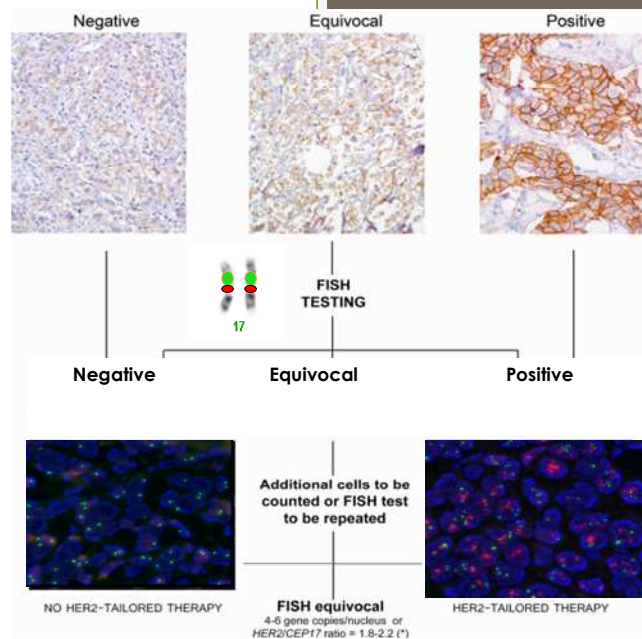


## HER2 AND CEP17

## HER2 oncogene and target for therapy

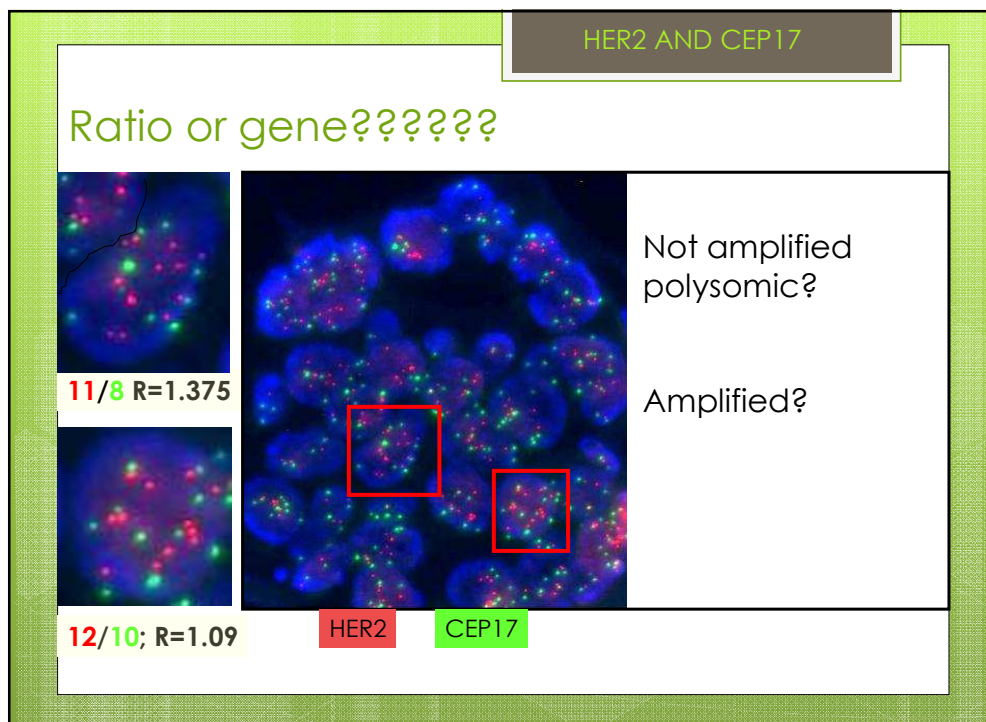
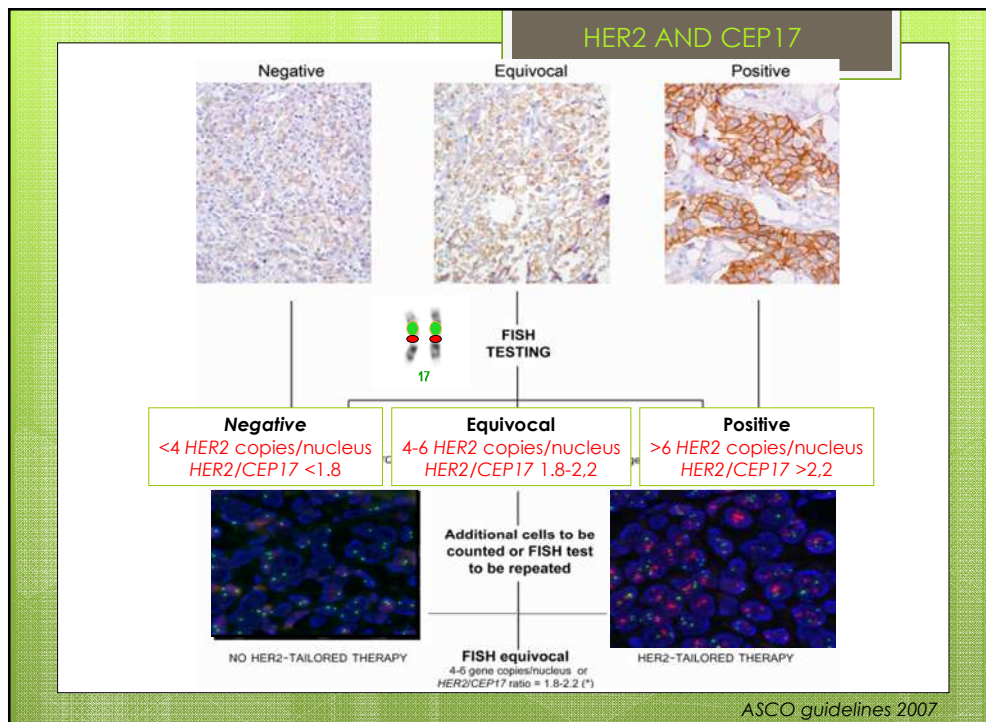
- HER2 overexpression: 15-18% of breast cancer
- HER2 overexpression correlates with gene amplification
- Both correlate with response to Herceptin therapy
- HER2 gene activating mutations independent from HER2 amplification have been recently described

## HER2 AND CEP17



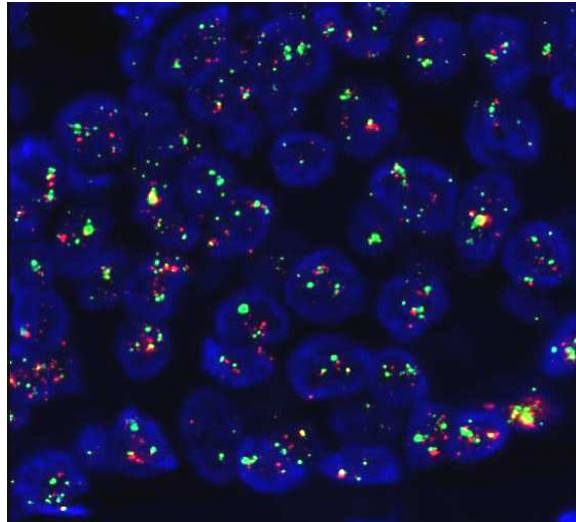
ASCO guidelines 2007







## HER2 AND CEP17



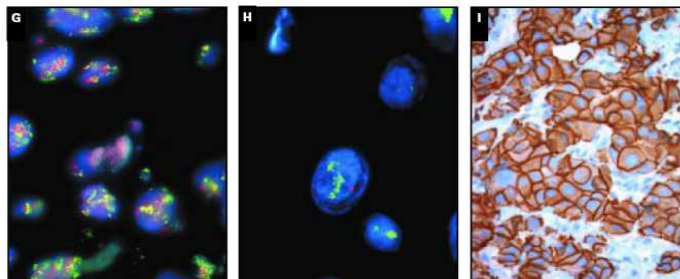
Something else?

## HER2 AND CEP17

*Am J Clin Pathol* 2006;126:709-716

**Evaluation of Her-2/neu Status in Carcinomas  
With Amplified Chromosome 17 Centromere Locus**

Megan L. Troxell, MD, PhD,<sup>1\*</sup> Charles D. Bangs,<sup>2</sup> Helen J. Lawce,<sup>3</sup> Ilana B. Galperin, MS,<sup>2</sup>  
Daniel Baiyee, MD, PhD,<sup>2</sup> Robert B. West, MD, PhD,<sup>2</sup> Susan B. Olson, PhD,<sup>3,4</sup>  
and Athena M. Cherry, PhD<sup>2</sup>



## HER2 AND CEP17



*Am J Clin Pathol* 2006;126:709-716

Coamplification of chromosome 17 centromere approximately 4 (0.47%) of 858 cases.

## HER2 AND CEP17

Is the ratio always precise in defining  
HER2 status?

Polysomy?

## HER2 AND CEP17

**POLYSOMY: a matter of debate**

*“There is no accepted definition of what constitutes polysomy and authors have used different criteria to define it.*

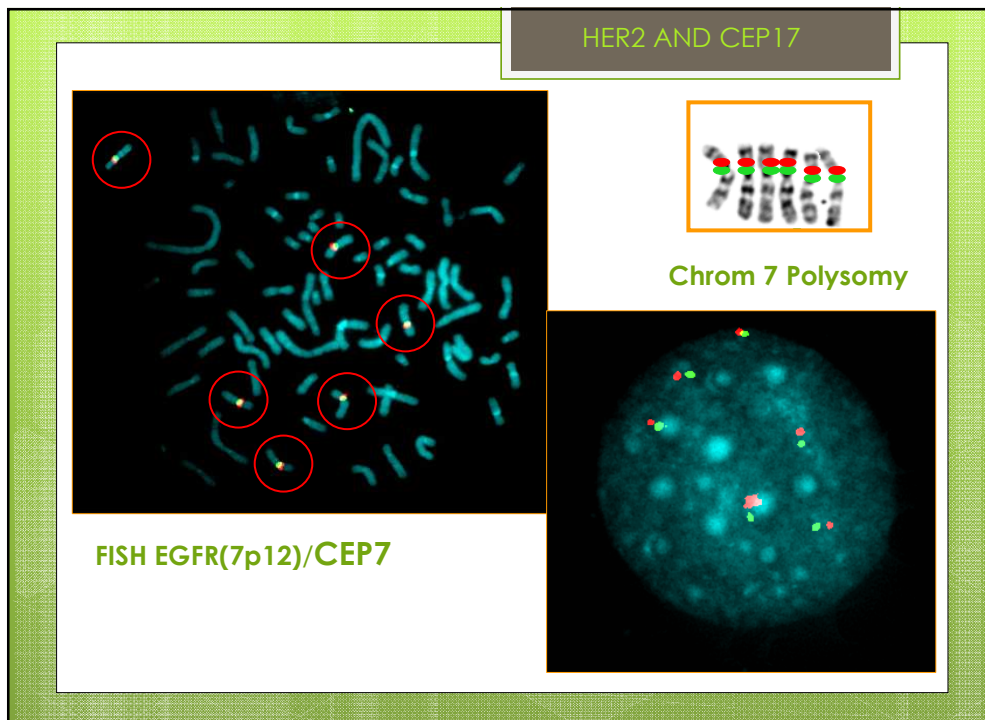
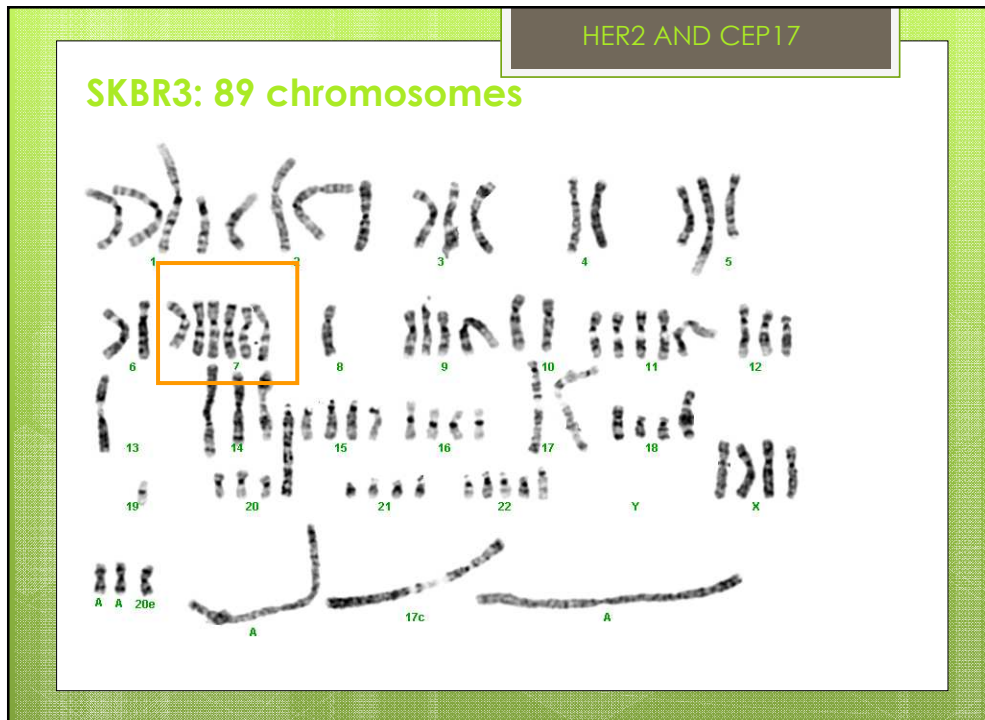
ASCO guidelines 2007  
Wolff AC et al, J Clin Oncol. 2007;25:118-45

## HER2 AND CEP17

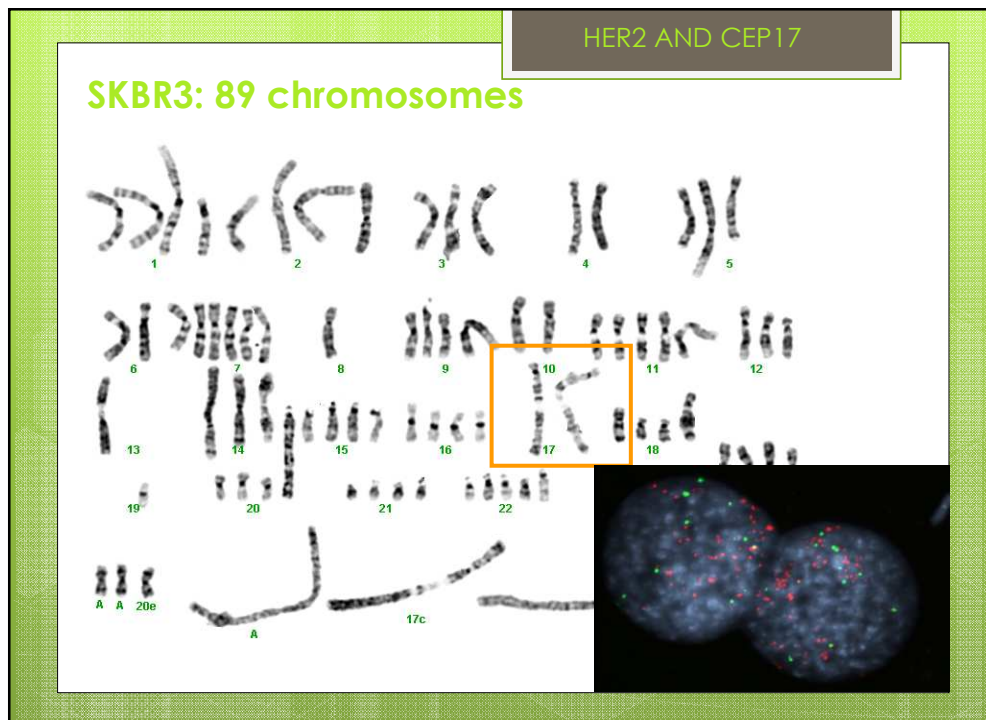
Polysomy

Πολυσ + σωμα

Several      The whole body  
                 of something







HER2 AND CEP17

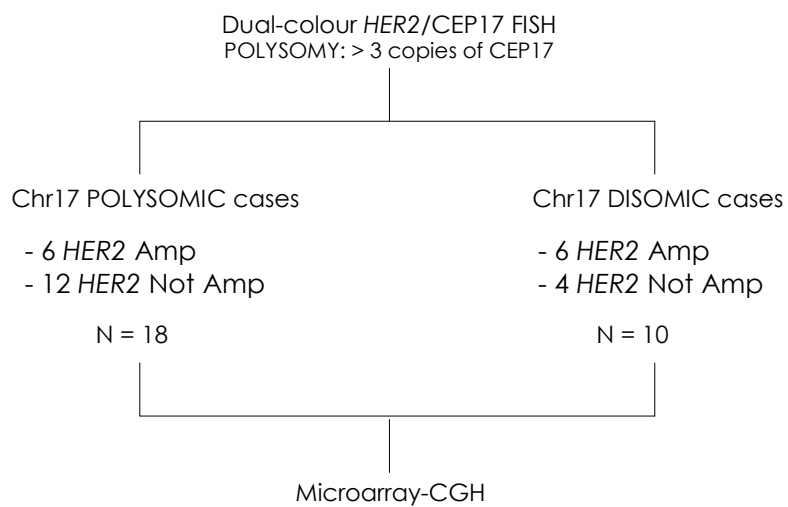
**Chromosome 17 in breast cancer**

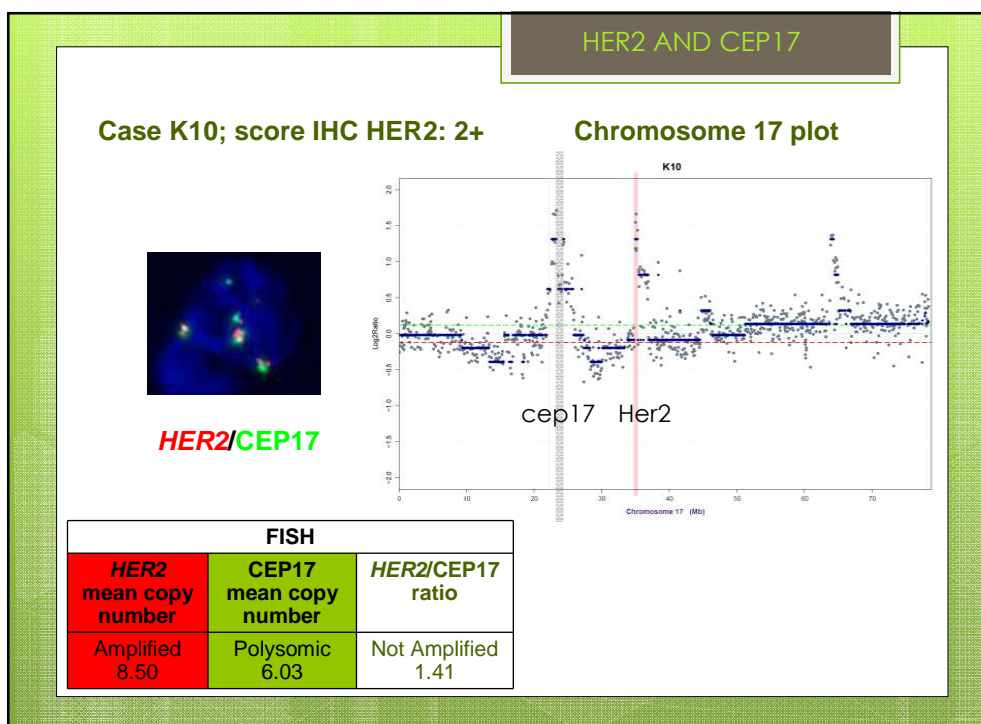
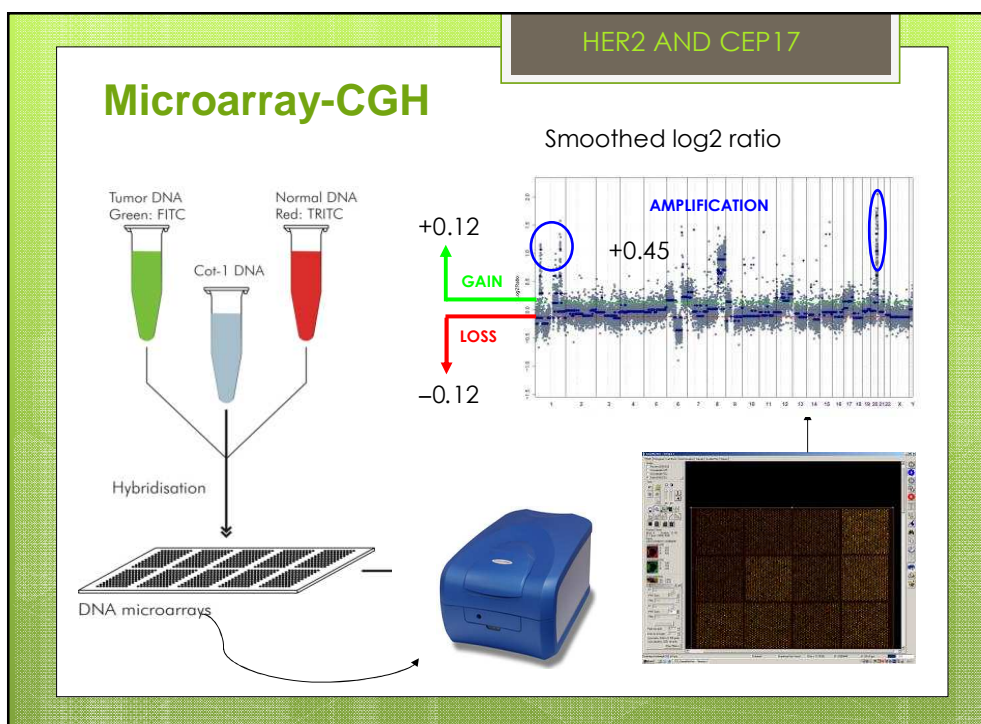
Chromosome 17 is one of the smallest human chromosomes and the second most dense in genes. It is rearranged in at least 30% of breast cancers. Chromosome 17p is mainly involved in genetic losses, some of them possibly focal and targeting potential tumour suppressor genes (eg, *TP53*), whereas 17q is targeted by complex combinations of gains, amplifications, and losses affecting multiple loci. Array-based comparative genomic

*Moelans CB et al, Lancet Oncol. 2011 Nov;12(12):1087-9. Epub 2011 Sep 6.*

**HER2 AND CEP17**

Alternative methods to study HER2 amplification

**The CEP 17 amplification**

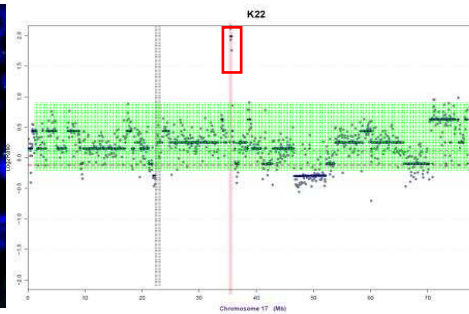
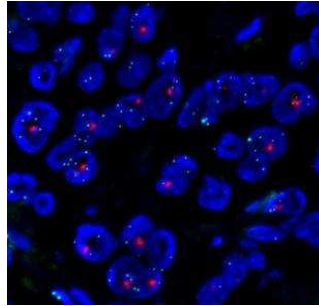




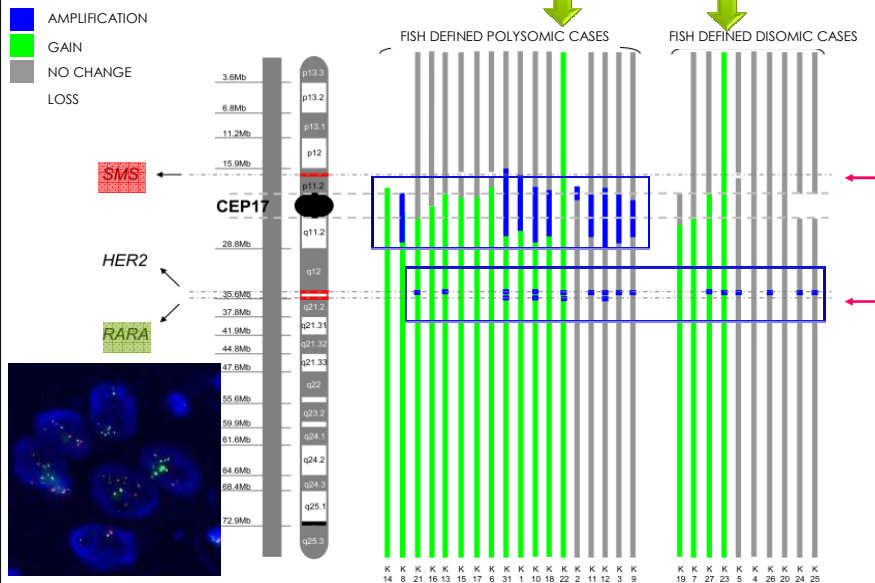
## HER2 AND CEP17

Case K22: amplified polysomic

Chromosome 17 plot



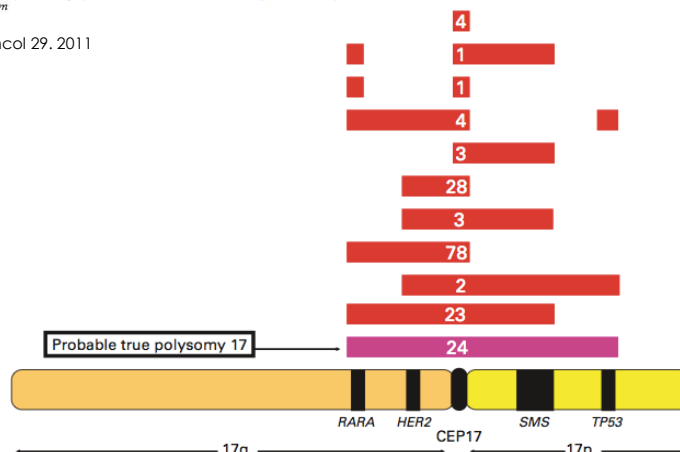
## Polysomy



### Determining True *HER2* Gene Status in Breast Cancers With Polysomy by Using Alternative Chromosome 17 Reference Genes: Implications for Anti-*HER2* Targeted Therapy

Chun Hing Tse, Harry C. Hwang, Lynn C. Goldstein, Patricia L. Kandalafi, Jesse C. Wiley, Steven J. Kussick, and Allen M. Gown

J Clin Oncol 29. 2011



### HER2 AND CEP17

#### Original Paper

#### Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence *in situ* hybridization and microarray-based CGH analysis

Caterina Marchio,<sup>1,2</sup> Maryou B Lambros,<sup>1</sup> Patrizia Gugliotta,<sup>2</sup> Ludovica Verdun Di Cantogno,<sup>2</sup> Cristina Botta,<sup>2</sup> Barbara Pasini,<sup>3</sup> David SP Tan,<sup>1</sup> Alan Mackay,<sup>1</sup> Kerry Fenwick,<sup>1</sup> Naninder Tamber,<sup>1</sup> Gianni Bussolati,<sup>2</sup> Alan Ashworth,<sup>1</sup> Jorge S Reis-Filho<sup>1\*</sup> and Anna Sapino<sup>2\*</sup>

*J Pathol* 2009

- ✓ Chr17 polysomy cannot be accurately defined with CEP17 probes
- ✓ True Chr17 polysomy is not the common denominator of increased numbers of CEP17
- ✓ Abnormal CEP17 copy numbers may stem from high-level gains/amplification of CEP17
- ✓ Correction with CEP17 probes may provide misleading *HER2* gene status assessment results in some cases (CEP17 high-level gains/amplification )

## HER2 AND CEP17

**Journal of Pathology**  
*J Pathol* 2009; **219**: 1–2  
 Published online in Wiley InterScience  
 (www.interscience.wiley.com) DOI: 10.1002/path.2593

## Invited Commentary

**Be precise! The need to consider the mechanisms for CEP17 copy number changes in breast cancer**

Giuseppe Viale\*  
 Division of Pathology, European Institute of Oncology, Università degli Studi di Milano, Milan, Italy

## Comment

Moelans CB *et al*, *Lancet Oncol*. 2011 Nov;12(12):1087-9. Epub 2011 Sep 6.

**Implications of rarity of chromosome 17 polysomy in breast cancer**

Amplification of the *HER2/neu* gene on chromosome 17q21, present in about 15% of breast carcinomas, correlates with a poor outcome and is a predictive marker of benefit from the anti-HER2 antibodies trastuzumab and lapatinib. Standard HER2 testing methods include protein immunohistochemistry and analysis of HER2 gene copy number by fluorescence in-situ hybridisation (FISH), chromogenic in-situ hybridisation (CISH), or silver in-situ hybridisation (SISH). In FISH (and to a lesser extent CISH and SISH) scoring, correction of the HER2 gene copy number with the copy number of chromosome 17 centromere (CEP17) has long been believed to be crucial

17 but lacking HER2 gene amplification.<sup>2</sup> Despite the substantial interest in chromosome 17 polysomy, its definition and clinical significance are far from being completely understood.

Chromosome 17 is one of the smallest human chromosomes and the second most dense in genes. It is rearranged in at least 30% of breast cancers. Chromosome 17p is mainly involved in genetic losses, some of them possibly focal and targeting potential tumour suppressor genes (eg. TP53), whereas 17q is targeted by complex combinations of gains, amplifications, and losses affecting multiple loci. Array-based comparative genomic



Published Online  
 September 7, 2011  
 DOI:10.1002/path.2593  
 204511270234-9

**Implications of rarity of chromosome 17 polysomy in breast cancer**

Amplification of the *HER2/neu* gene on chromosome 17q21, present in about 15% of breast carcinomas, correlates with a poor outcome and is a predictive marker of benefit from the anti-HER2 antibodies trastuzumab and lapatinib. Standard HER2 testing methods include protein immunohistochemistry and analysis of HER2 gene copy number by fluorescence in-situ hybridisation (FISH), chromogenic in-situ hybridisation (CISH), or silver in-situ hybridisation (SISH). In FISH (and to a lesser extent CISH and SISH) scoring, correction of the HER2 gene copy number with the copy number of chromosome 17 centromere (CEP17) has long been believed to be crucial



Despite the great interest in the development of robust biomarker assays, the ideal method for HER2 status determination remains controversial. Definitive studies of the clinical and biological importance of CEP17 are needed. Until then, we fully support the recommendations previously made by Viale:<sup>9</sup> tumours should be classified as *HER2* amplified on the basis of the mean copy number of *HER2* gene signals (greater than six *HER2* signals per cell) irrespective of the number of CEP17 signals. This approach will not deny any patient with breast cancer a potentially useful HER2-targeted treatment and would be consistent with the present ASCO/CAP guidelines for *HER2* gene status assessment.

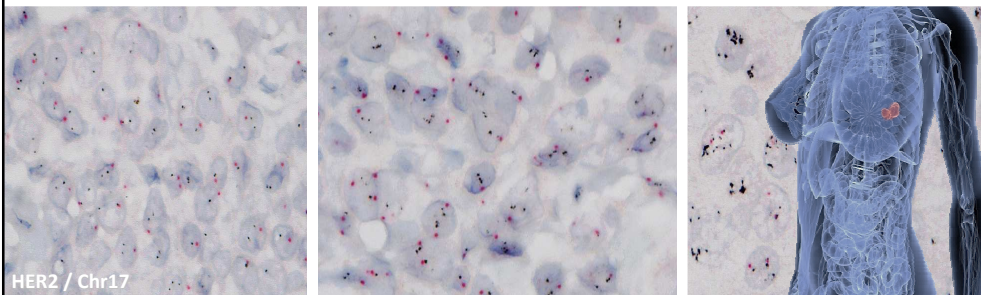
We therefore strongly encourage pathology reports to mention the absolute *HER2* gene copy number in addition to the *HER2*:CEP17 ratio.

## HER2 AND CEP17

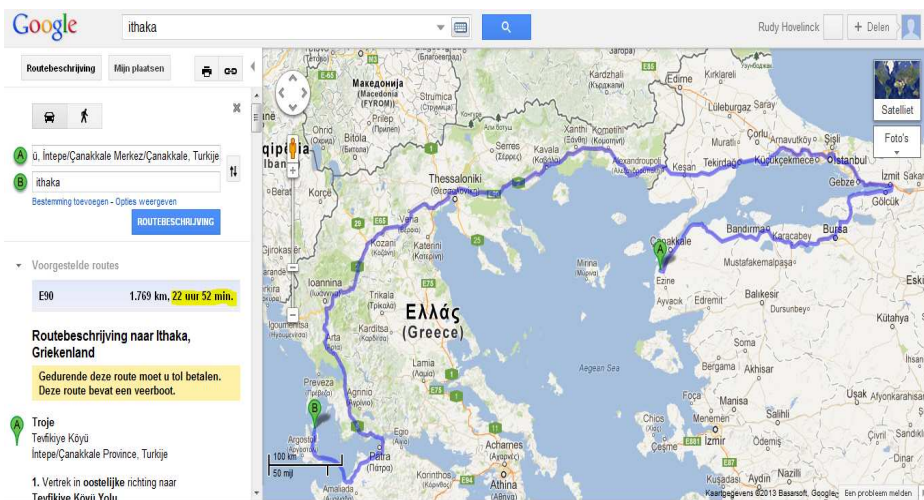


# HER2 diagnosis, a modern

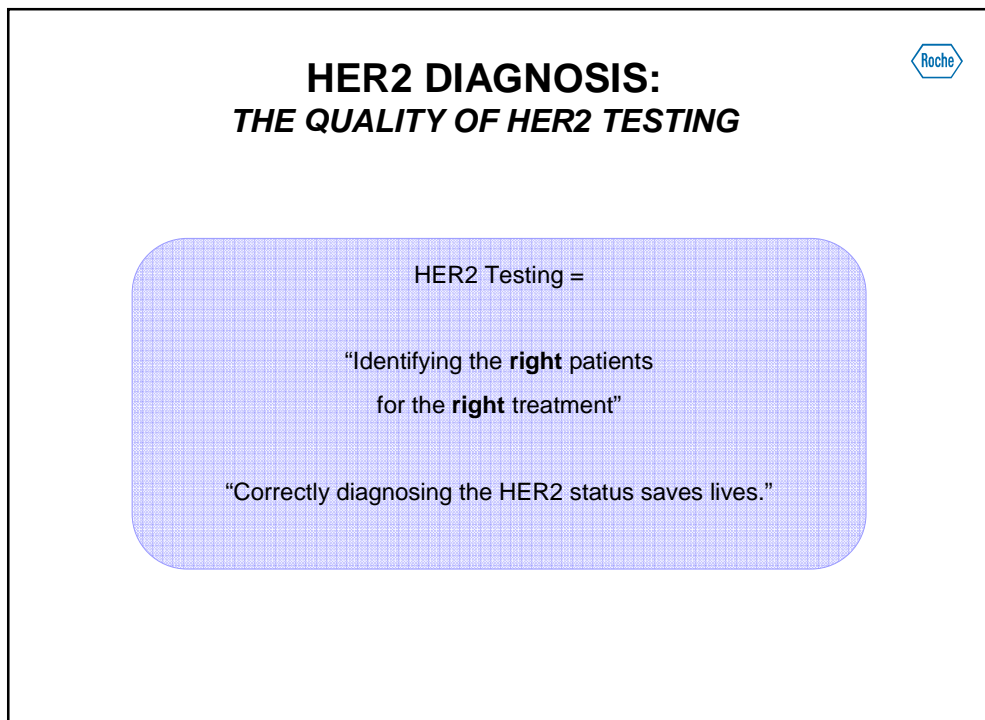
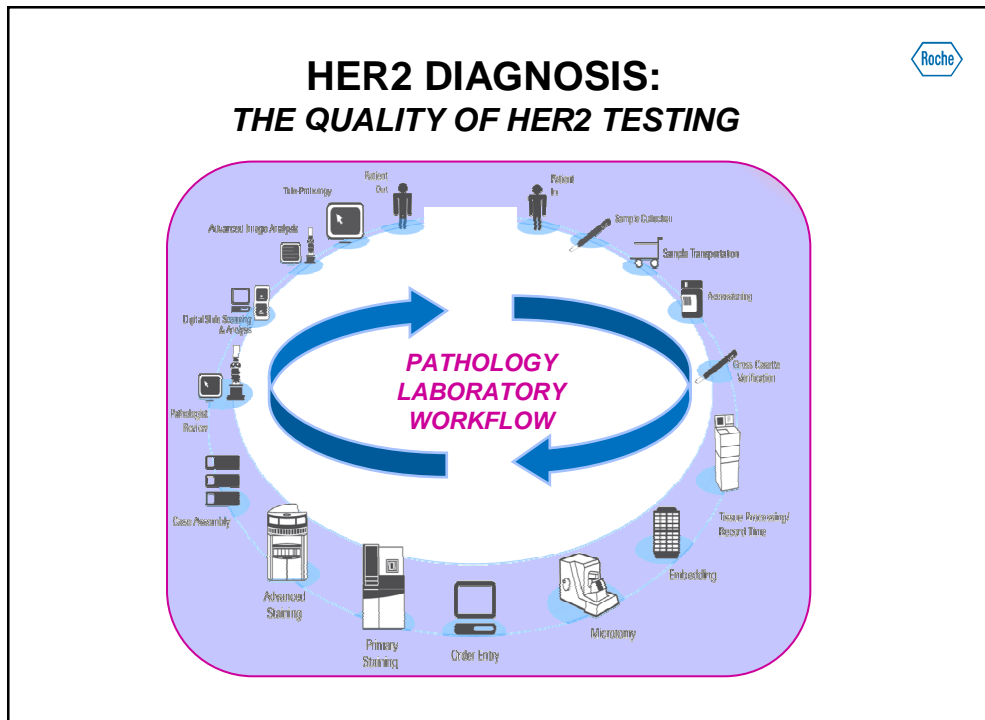
**Odyssey?**  
Orly Sasse & Rudy Hovelinck




## HER2 diagnosis, a modern Odyssey?





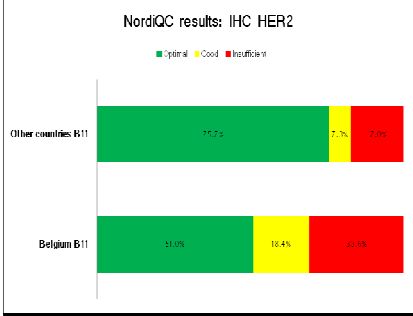




## HER2 DIAGNOSIS: THE QUALITY OF HER2 TESTING

NordQC results: IHC HER2


■ Optimal ■ Good ■ Insufficient



Region	Optimal (%)	Good (%)	Insufficient (%)
Other countries N=11	75.2%	7.7%	17.1%
Belgium B=11	51.0%	18.4%	30.6%


**ROCHE HAS A LONG SUPPORT TRADITION**  
**IN HER2 TESTING QUALITY**  
Ensuring the highest Quality Assurance

- \* Targos Training (theoretical and hands-on training in regional centers)
- \* Educational Sessions & Satellite Symposia
- \* Retrospective study : Retesting of IHC 0/1+ by ISH<sup>1</sup>



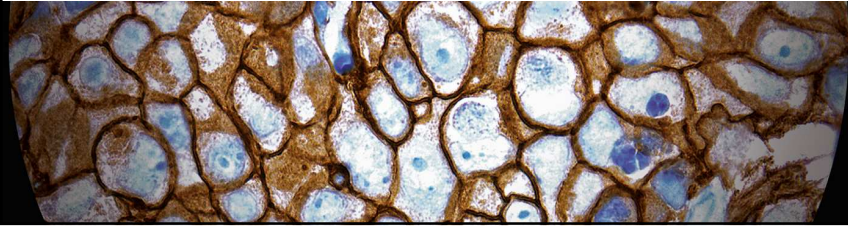
The expert service provides in-lab support  
in line with your specific needs

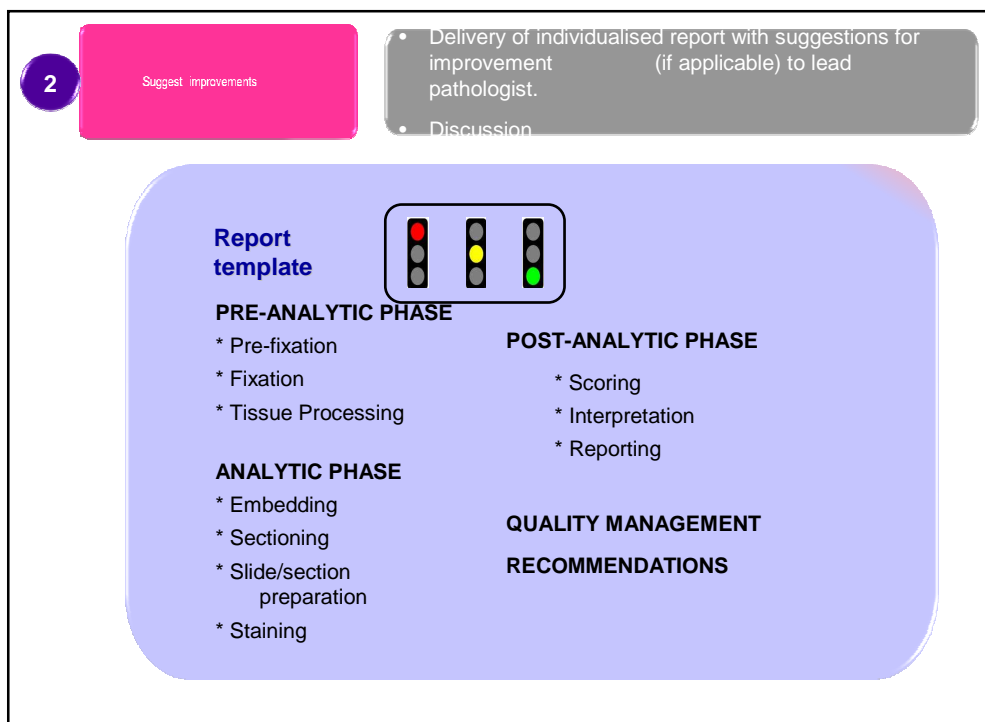
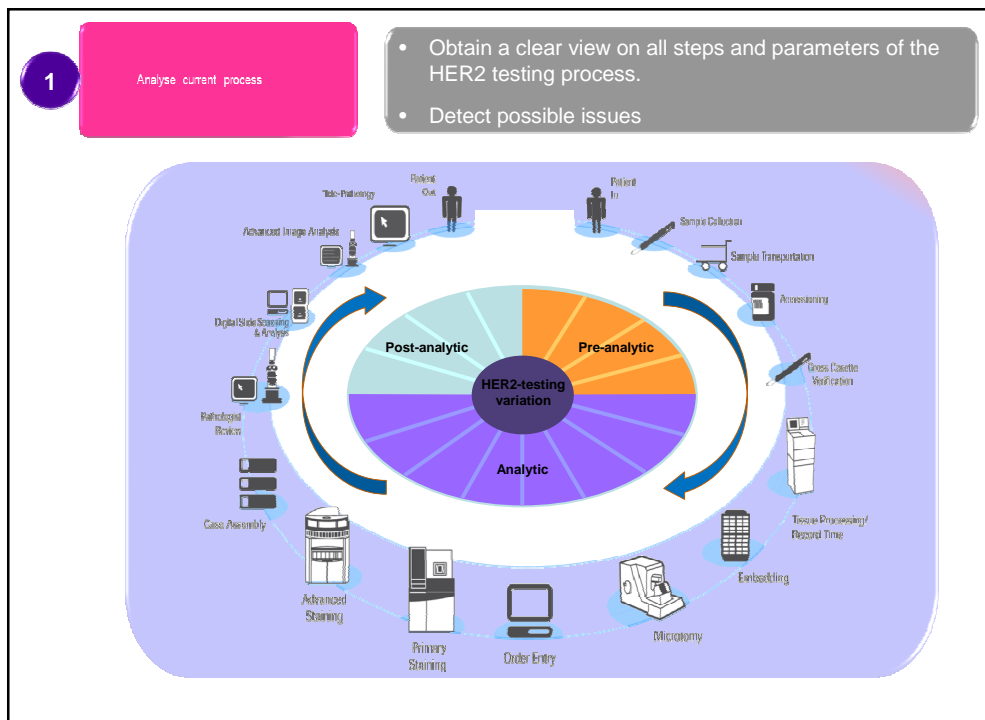
1: **Larsimont et al.** : Results of a Belgian multicenter retrospective study to determine the incidence of HER2 gene amplification in patients scored as immunohistochemistry 0 or 1+; ASCO Annual Meeting, Chicago, USA; June 3–7, 2011, poster 549



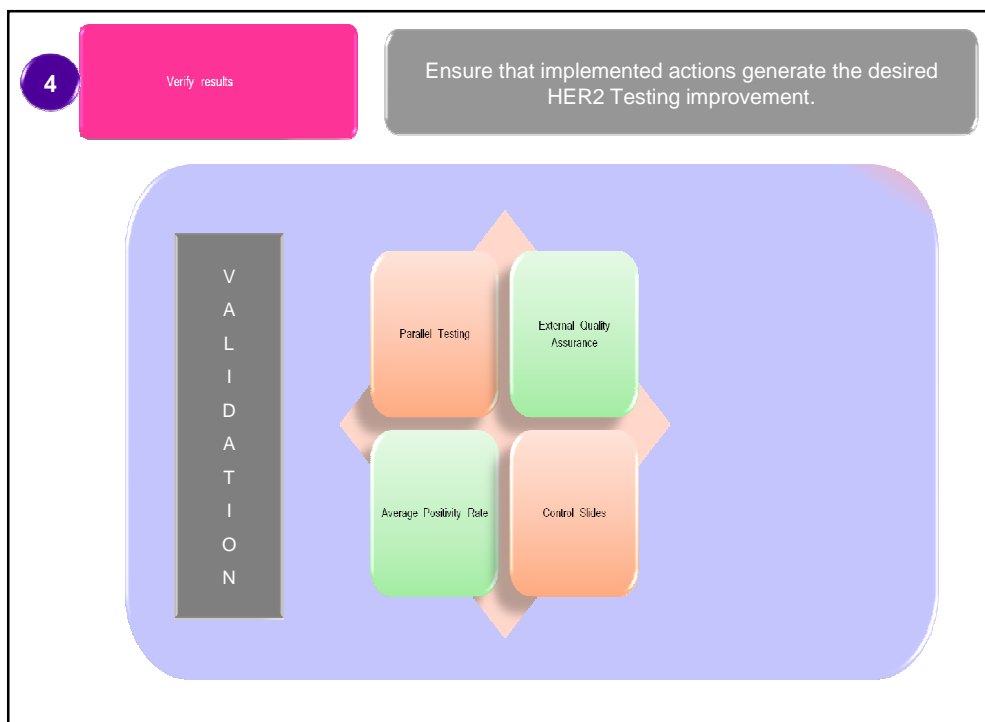
## HER2 Testing Service:

*a 4-step approach*







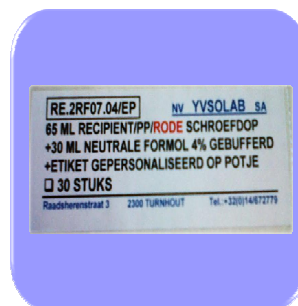
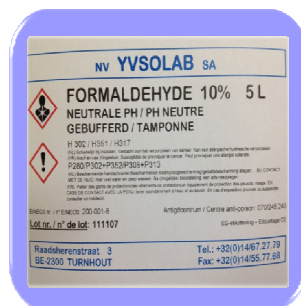


## Example 1

Pre-analytic Phase  
Fixation:  
Type of fixative



10% Neutral Buffered Formalin = 4% Neutral Buffered Formaldehyde



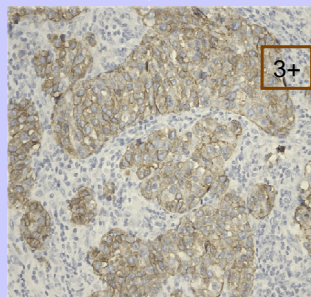
## Example 2

Analytic Phase  
Staining



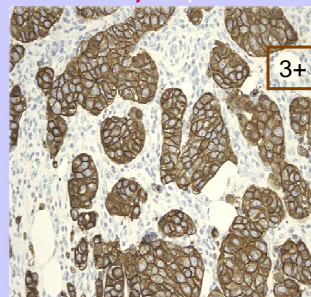
## Baking temperature

Lab protocol

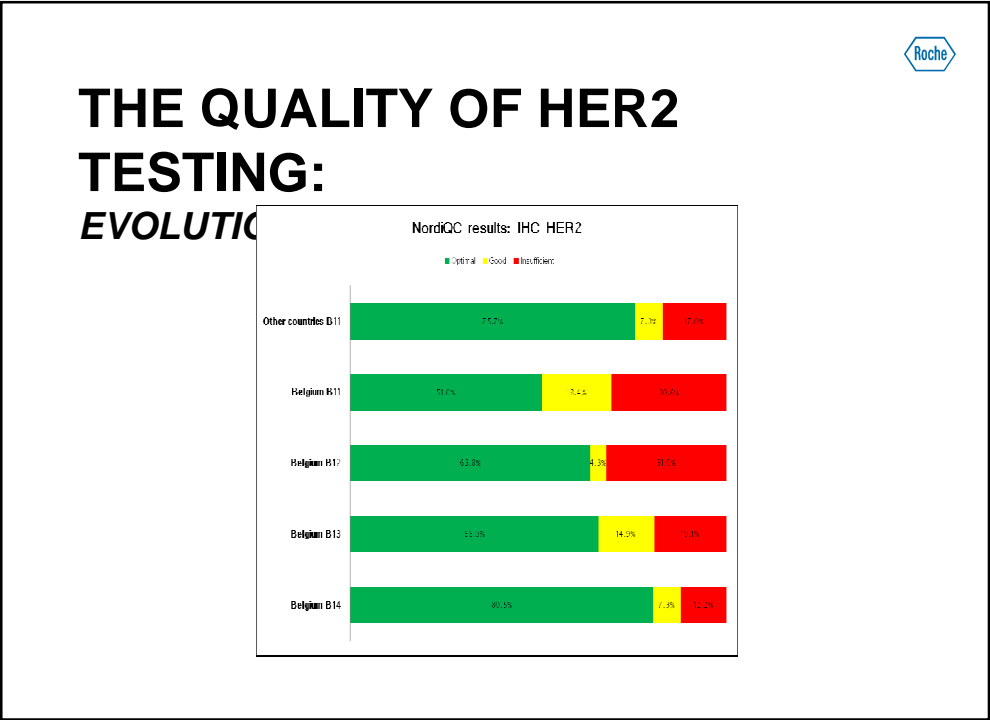
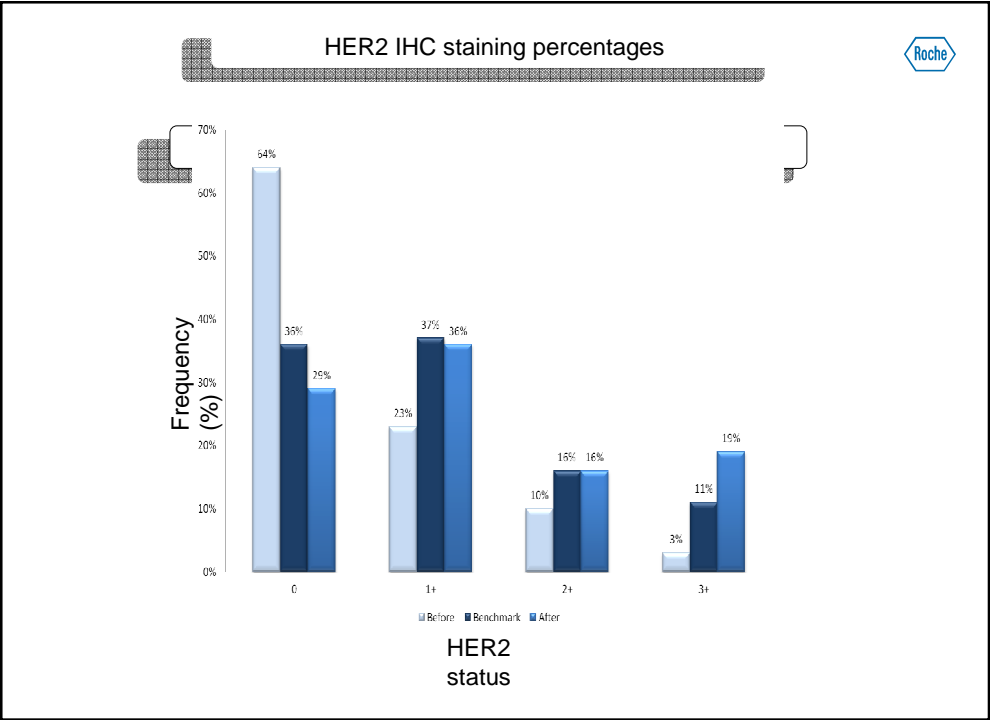


60°C - Overnight

Adjusted protocol



RT - Overnight





## Save the date!



- Satellite Symposium: IMPAKT 2nd May 2013
- Targos HER2 training: 1st week of July 2013
- Nordiqc Run B16: 12th August 2013
- New website: [www.HER2testing.be](http://www.HER2testing.be)

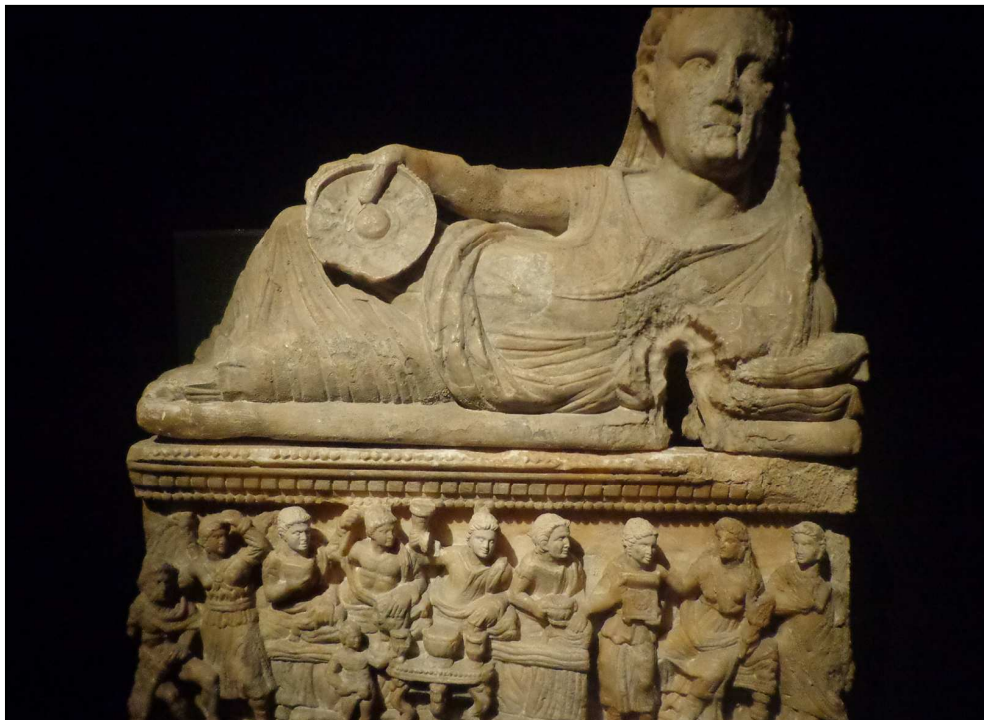
→ HER2 testing service:
[elisabeth.coene@roche.com](mailto:elisabeth.coene@roche.com)





HER2 / Chr17

port Team



## Analogies to Radiology



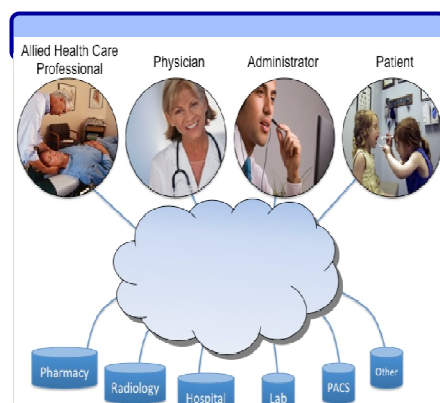
- Both disciplines undergoing **transition from analog to digital** based information systems, including digital image management
- Pathology and radiology both provide crucial **phenotypic evidence required for patient management** that is often **complementary**
- **Narrative reports** based on analysis and **interpretation of image data**
- Opportunity to learn from and



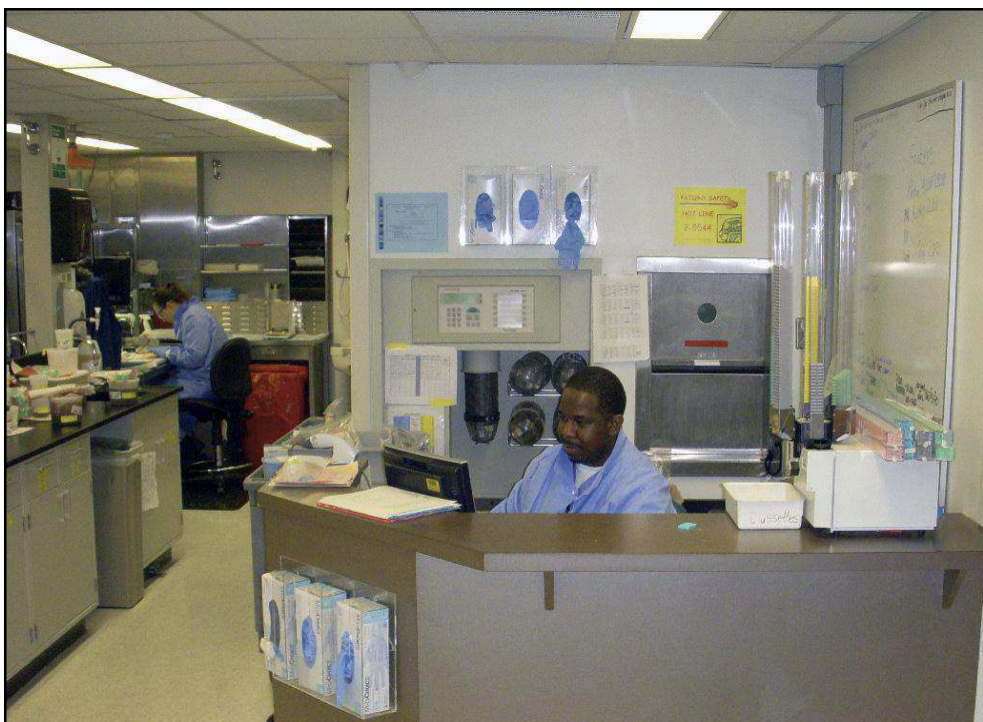
## Convergence is essential



- **Fully digital** process/workflow steering – Paperless
- **Link to lab data results** (biomarkers, gene sequences,...)
- **Integration** of image data in **patient report**
- Integrated documentation of **quality** deviations
- **Single point of entry** to access data via LIS
- Critical success factor is **IT integration**

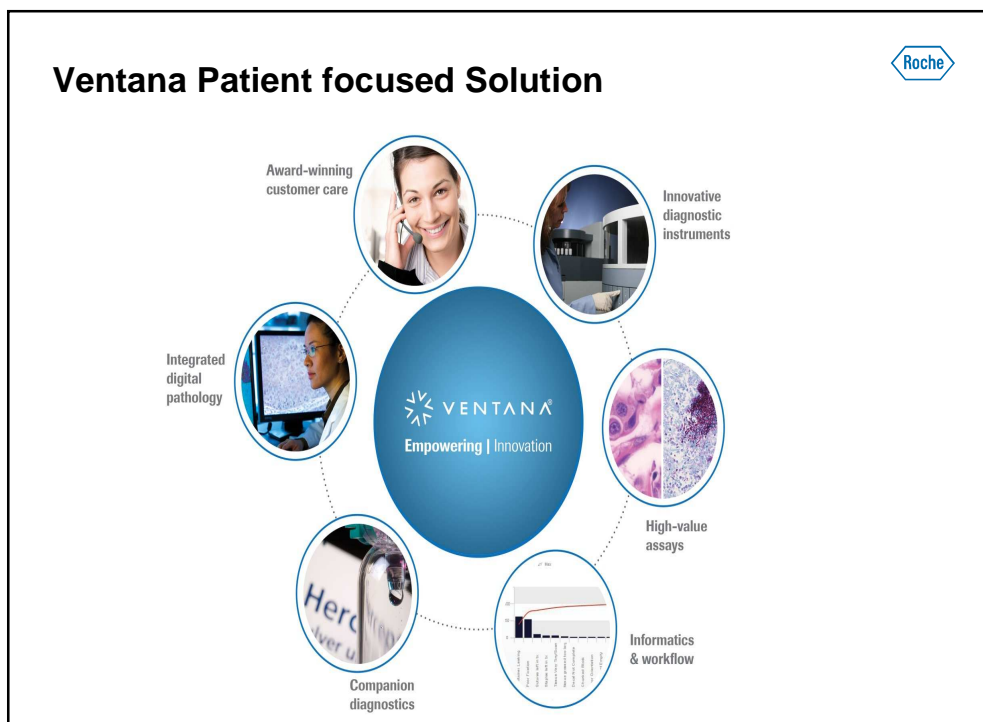






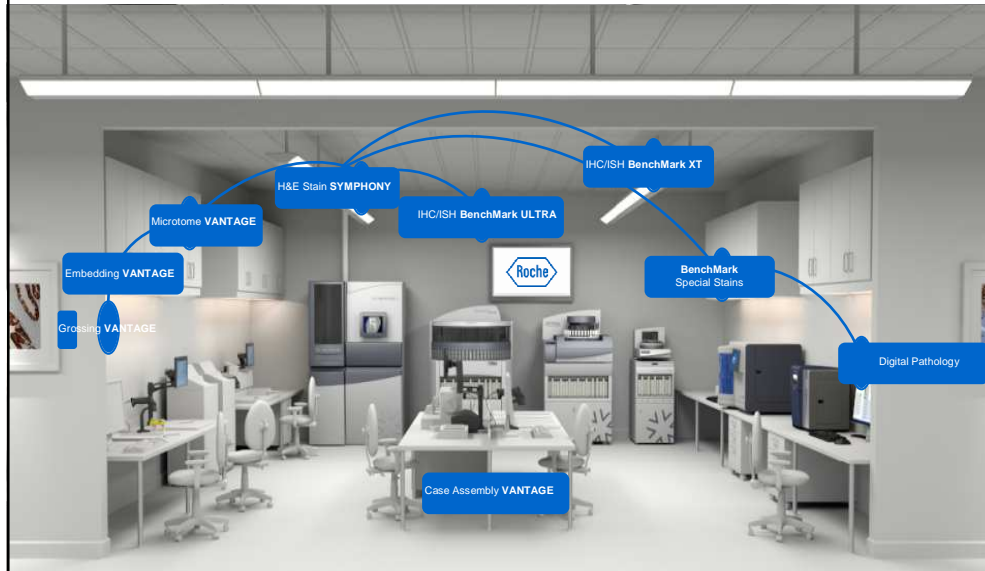






## AP Lab Workflow, Quality Management, Automation

*Full AP-lab automation available today*



## Roche Digital Pathology

*Fits with our Patient Focused Solution*

## Scanners for digitizing slides

- High speed image acquisition

## Image Management

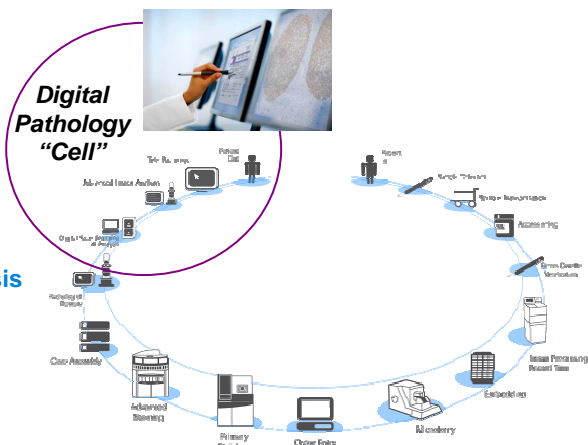
- Case assembly, sorting and routing
- Telepathology
- QC, archival & retrieval


## Case Review and Image Analysis

- Image viewing and annotation
- Image analysis for Ventana assays
- Patient report with lab information


## Workflow Management

- Integration with Vantage and LIS
- Positive specimen control, data mining for rework cases, uploading data into LIS






## Ventana Scanner family tailored to Customer needs




**iScan Coreo**  
*Low to Medium-throughput brightfield labs*




**iScan HT**  
*High-throughput brightfield labs*

Optics	4x, 10x, 20x, 40x 2 mm	20x, 40x
Time to View 20x (15x15 mm)		40 sec
Time to View 40x (15x15 mm)	5 min	50 sec
Loader size	160 slides	360 slides
Workflow	Batch	Continuous access



## Virtuoso - Web based software application that allows users to view, manage, manipulate, analyze, report and collaborate

- Case Management
- Image Viewing
- Image Analysis
- Telepathology (peer review, remote consultations)
- Real-time Collaboration
- Customised Reporting
- Vantage middleware connectivity
- LIS Connectivity

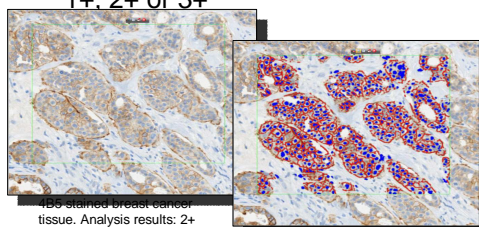


## HER2 Digital Algorithms



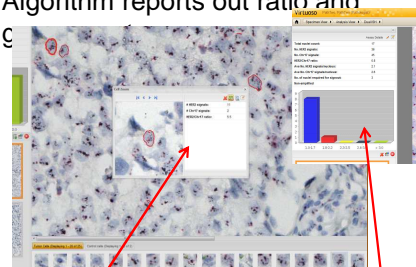
### HER2 IHC algorithm

- FDA Approved with clone 4B5
- Within user selected area, algorithm distinguishes between epithelial and non-epithelial areas
- Algorithm determines complete, partial or non stained membrane
- Algorithm reports out score of 0, 1+, 2+ or 3+



### HER2 SISH algorithm

- Under development
- Within user selected area, algorithm identifies nucleus
- Algorithm determines chr17 and HER2 signal
- Algorithm reports out ratio and



Ability to

Slide

## Convergence is essential



- **Fully digital** process/workflow steering – Paperless
- **Link to lab data results** (biomarkers, gene sequences,...)
- **Integration** of image data in **patient report**
- Integrated documentation of **quality** deviations
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- Critical success factor is **IT integration**





***Doing now what patients need  
next***



The Second TASTE Workshop

Pathology of the Breast:  
Possible Artefacts  
Benefits of Standardization

Francesco Feoli MD

*Institut Bordet, Brussels.*

Breast Cytopathology using LBC samples



Breast FNA Cytology.

Studies Before Modern  
Population Screening Programs  
Reported Satisfactory Accuracy


Authors	Area	Year	Total cases reported	Histological diagnosis malignant	Cytological Diagnosis				
					Pos. No.	Sus. No.	Neg. No.	Unsatisf. No.	Sensitivity (POS.+SUS.) No. %
Adair	England	1949	1579	1579	1343 (85)	—	236	—	1343 85
Smith	Canada	1959	294	100	92 (92)	—	—	—	92 92
Franzen	Sweden	1968	3479	873	662 (76)	117	(— 94 — — —)	—	779 89
Laumonier	France	1968	1000	456	335 (73)	21	51	49	356 78
Zajicek	Sweden	1970	4700	1068	823 (77)	139	(— 106 — — —)	—	962 90
Cornillot	France	1971	2267	1335	1173 (88)	—	62	100	1173 88
Zajdela	France	1975	2772	1745	1539 (88)	54	63	89	1593 91
Geier	Germany	1975	974	72	57 (79)	10	5	—	67 93
Takeda	Japan	1976	860	71	56 (79)	—	15	—	56 79
Schondorf	Germany	1978	2778	307	283 (92)	18	6	—	301 98
Kline	USA	1979	3545	341	213 (62)	89	35	4	301 88
Garadicki	U.K.	1980	233	129	96 (74)	16	6	11	112 87
Kudoh	Japan	1983	167	40	35 (88)	—	5	—	35 88
Feldman	USA	1983	300	100	80 (80)	15	—	5	95 95
Eisenberg	USA	1986	1874	1480	984 (66)	152	173	171	1136 77
Spierer*	Switz	1986	1768	80	65 (81)	7	8	—	72 90
Kan	China	1986	8129	1012	772 (76)	60	173	7	832 82

Authors	Area	Year	Total cases reported	Histolog. or follow up Diag. benign	Cytological Diagnosis				Spe* %
					Unsatisf.	Neg.	Sus.	Pos.	
Franzen	Sweden	1968	3479	807	(— 783 — )	—	23	1	> 99.0
Laumonier	France	1968	1000	544	(84 424)	—	23	13	97.6
Zajicek	Sweden	1970	4700	1009	— 980 —	—	28	1	> 99.0
Cornillot	France	1971	2267	932	86 831)	—	—	15	98.4
Zajdela	France	1975	2772	1027	66 916	42	3	—	> 99.0
Kreuzer	German	1976	602	355	—	305	46	4	98.9
Kline	USA	1979	3545	3177	—	3188	59	—	100.0
Feldman	USA	1985	300	200	35	160	5	—	100.0
Spierer*	Switz	1986	1768	146	—	142	4	—	100.0
Kan	China	1986	8129	635	9	595	30	1	> 99.0

\*Specificity

\*Gantenbein and Spierer.

1949 - 1986



The Royal College of Surgeons of England

Ann R Coll Surg Engl 2001; 83: 110-112

Original article

**Replace fine needle aspiration cytology with automated core biopsy in the triple assessment of breast cancer**

D Clarke, N Sudhakaran, CA Gateley

Department of Surgery, Royal Gwent Hospital, Newport, Gwent, UK

All patients presenting with a symptomatic breast lump are assessed by means of triple assessment (clinical examination, radiology in the form of mammography and cytology by means of a fine needle aspiration) performed by the clinician in the rapid access breast clinic at the Royal Gwent Hospital, Newport, UK. In our initial experience, it was found that a significant number of patients were returning to clinic for the results of the triple assessment to find that the cytology was not conclusive and hence needed a core biopsy, thus delaying diagnosis and definitive treatment.

Compared to Biopsy  
FNA of The Breast,  
Generated Too Many  
Unsatisfactory Samples and  
Uncertain (C3 & C4 Diagnoses)



### Core Biopsy Is More Accurate.

Complete Sensitivity **98.5** vs 95.1  
 Absolute Sensitivity **96.7** vs 83.1  
 Specificity **98.7** vs 84.0  
 Inadequate Rate **0.05** vs 12.8

(Britton PD Review 1999)

**More Expensive** (1 Needle: 25 Euros at Least).  
 More Traumatic. **Local Anesthesia.**  
**Delayed Results**

**Areas Of Gray** (Inconclusive Dx)  
 False Positives up to 0.4-1.  
 False Negatives up to 4.9-13.7.

### FNA

*Rapid Diagnosis.*

*Multiple Lesions, Very Small Lesions.*

Axilla

Periclavicular, Very Close To The Chest Wall, Under The Skin.

74

CANCER

CYTOPATHOLOGY

Is There Still a Role for Fine-Needle Aspiration Cytology in Breast Cancer Screening?

Experience of the Verona Mammographic Breast Cancer Screening Program With Real-time Integrated Radiopathologic Activity (1999–2004)

54472 Women

2008

Screening: Benign Abnormalities More Frequent.

1286 Abnormalities.

1263 (98,2%) FNA

510 (39,6%) Open Biopsies.

Benign vs. Malignant Biopsy Ratio 0.2

Recently the role of breast cytopathology  
 has been strongly supported  
 by the results of multidisciplinary groups  
 working in a real time integrated manner.

*Fine Needle Aspiration Cytology of the Breast:*  
*Feoli F. et al. Acta Cytol 2008 ; 52:145-151.*

ACTA  
CYTOLOGICA

Robust Diagnostic Criteria.

Experience.

Systematic Internal Review.

Patient Selection.

Alcohol Fixed Material.

292 Conventional FNA With Paired Biopsies  
 Were Blindly Reviewed 4 Years Later

(2.183 FNA)

Variety of Reporting Styles,

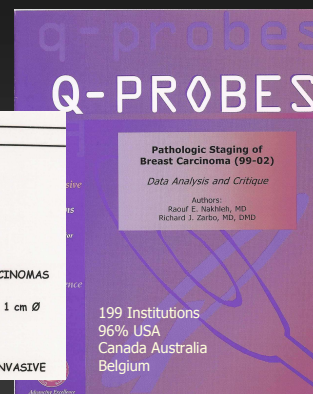
Diversity Of Classification Schemes

Differences In Follow-up/Management...

*Ozkara SK Acta Cytol 2002;46:513-518.*



SENOLOGIE CHR MONS 1999			
Résultats des modifications dans la phase pré-analytique			
CHR 99	Q-Probes	CHR 94-95	
16.6 %	{13.8 %}	7.6 %	IN SITU CARCINOMAS
36.6 %	{36.7 %}		IN SITU et < 1 cm Ø
70.8 %	{64 %}		PNO
19.04 mm	{16.5 mm}	2.1 cm	DIAMETER, INVASIVE



**UK NHSBSP Guidelines, 1992:**

C3: Like Benign Aspirates  
  
Possible Hypercellularity.  
Some Loss of Cohesiveness.  
Nuclear Pleomorphism.  
  
ADH, ALH, LG -DCIS, LC,  
TUB. Ca, FA, Radiotherapy,  
Spreading Artifacts, Lactation.

C4: A) Few Malignant Cells.  
  
Suboptimal Smear or  
Overall Benign Pattern.  
  
B) Sample with Atypia -> C3.  
  
*Triple Test For All Cases.*

**Criteria for Reporting Fine Needle Aspiration on Palpable and Nonpalpable Masses of the Breast.**  
  
Editorial. Acta Cytol 1997; 41: 623-627

C3: PBD Without Atypia.  
  
Moderate Hypercellularity & Crowding.  
Slight Nuclear Variability

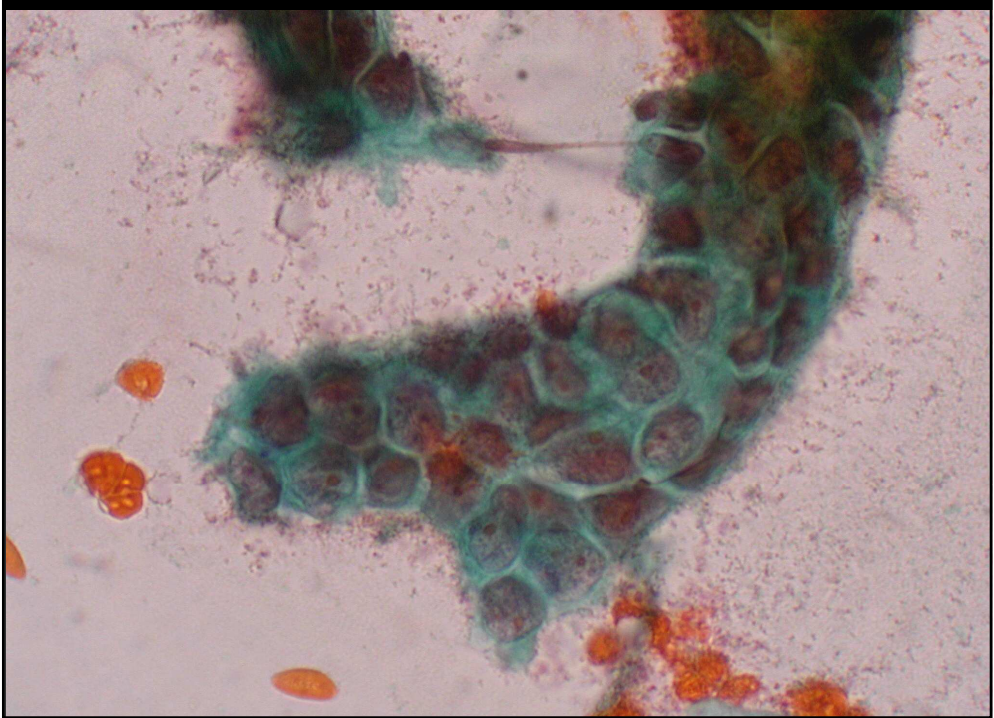
C4: Atypical Duct Hyperplasia.  
  
Cribriform Structures.  
Hyperchromatism, Monotony,  
N/C Ratio  
Discohesion,  
Rare Myoepithelial Cells.

**NCI Bethesda, 1996:**

C2: Abscess, Mastitis, FA  
Fat necrosis, PBD, Etc.

C3: Non Diagnostic.  
  
-PBD/FA vs LG DCIS  
-Papillary Lesions  
-FA vs Phyllodes  
  
*Refer to Triple Test*

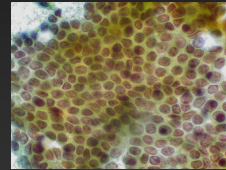
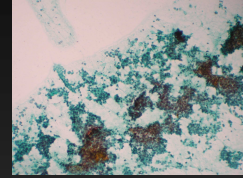
C4: Highly Suggestive of Malignancy  
  
*Tissue Biopsy*



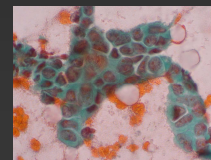
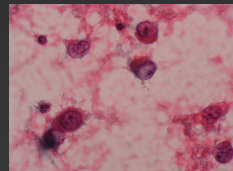
## *Fine Needle Aspiration of the Breast* *A Probabilistic Approach to Diagnosis of Carcinoma* Wang H.H. Acta Cytol 1998; 42:285-289.

### Reporting Categories:

- C1 Inadequate: n.d.
- C2 Benign: PPV 13.8%
- C3 Atypia, Probably Benign: PPV 35%
- C4 Suspicious: PPV 93%
- C5 Malignant: PPV 100%



**Four Features**



2006

### **Fine Needle Aspiration Cytology**

- C1 Inadequate for diagnosis
- C2 Benign epithelial cells
- C3 Atypia probably benign
- C4 Suspicious of malignancy
- C5 Malignant

#### **C1. Unsatisfactory**

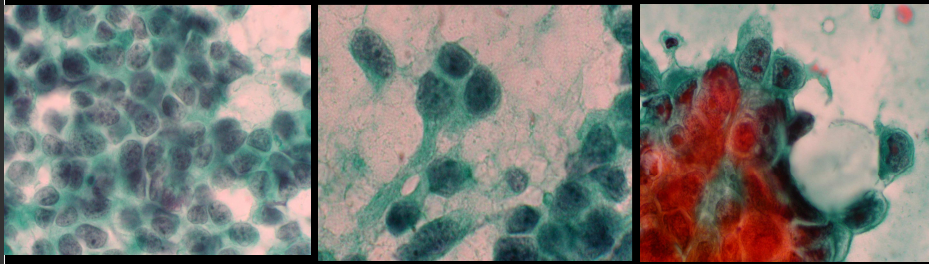
The designation of an aspirate as 'inadequate' is to a certain extent a subjective matter

#### **C4. Suspicious of malignancy**

The sample may show some malignant features without overt malignant cells present. The degree of abnormality should be more severe than in the previous (C3) category

## Alcohol Fixed Material & Papanicolau Staining: Improves Accuracy Reduces Uncertain Diagnoses

### Cytologic Criteria for Black's Nuclear Grading System (Fisher's Modification)



### Fine Needle Aspiration Cytology of the Breast:

F. Feoli, M.D., et al. Acta Cytol 2008 ; 52:145-151.

292 Conventional FNA With Paired Biopsies. 4 Years Later .

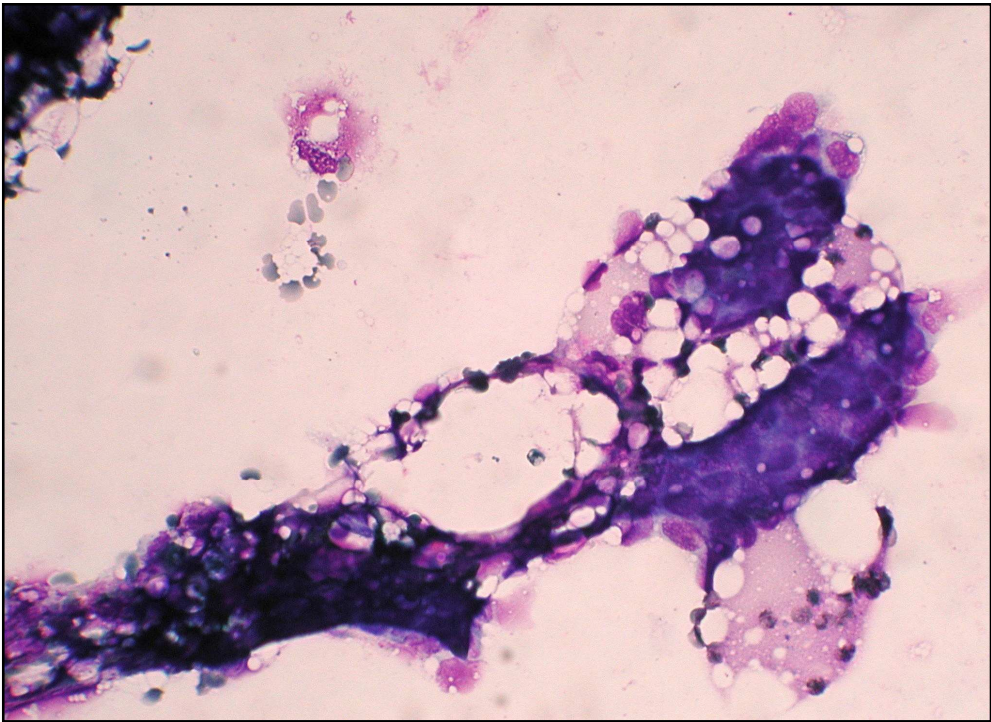
**Table 2.** Minimum Standards proposed for Cytology within the UK Breast Screening Programme

Absolute sensitivity	+13.00%	> 60%
Complete sensitivity	91.70%	> 80%
Specificity	+7.6%	> 60% (including nonbiopsied cases)
Positive predictive value (C5)	100.00%	> 95%
False negative rate	1.84%	< 5%
False positive rate	0.00%	< 1%
Inadequate rate	14.04%	< 25%
Suspicious rate	19.85%	< 20%

They try FNAC for alterations that are not alarming (Chuo CB.2003).

Some of these alterations may be vague by palpation,  
may express uncertain images by ultrasonography  
and be merely composed of fat (Bernier A. 2003)





ACIA  
CYTOLOGICA

Francesco Feoli<sup>a,b</sup> Lieveke Ameye<sup>a</sup> Pascal Van Eeckhout<sup>b</sup>  
Marianne Paesmans<sup>a</sup> Vincenzo Marra<sup>c</sup> Riccardo Arisio<sup>c</sup>

Accepted after revision: February 6, 2013

Retrospective Revue of 190 LBC Cases With Paired Biopsies. No Clinical Information.

	Suggested Acceptable Values <sup>a</sup>	Readings						P-value		
		FF1 (No Training)		FF+PVE (Collaboration) (No Training)		FF2 (Post Training)		FF1 vs. FF+PVE	FF1 vs. FF2	FF+PVE vs. FF2
Absolute sensitivity:	>60%	55%	(88/159)	57%	(90/159)	57%	(90/159)	0.48	0.72	1
MalignantC5/malignant cases										
Complete sensitivity:	>80%	86%	(137/159)	89%	(142/159)	94%	(149/159)	0.20	0.003	0.008
“Positive” (C3+C4+C5)/malignant cases										
Specificity:	>60%	71%	(22/31)	68%	(21/31)	65%	(20/31)	0.56	0.41	0.56
BenignC2/benign cases										
Suspicious rate:	<20%	27%	(51/190)	29%	(56/190)	35%	(66/190)	0.30	0.03	0.10
(C3+C4)/total number of cases										
Inadequate rate:	<25%	5%	(9/190)	5%	(9/190)	4%	(7/190)	1	0.16	0.16
C1/total										
FP rate:	<1%	0.6%	(1/159)	0.6%	(1/159)	0%	(0/159)			
BenignC5/malignant cases										
FN rate:	<5%	12%	(19/159)	8%	(13/159)	4%	(7/159)	0.11	0.003	0.03
MalignantC2/malignant cases										

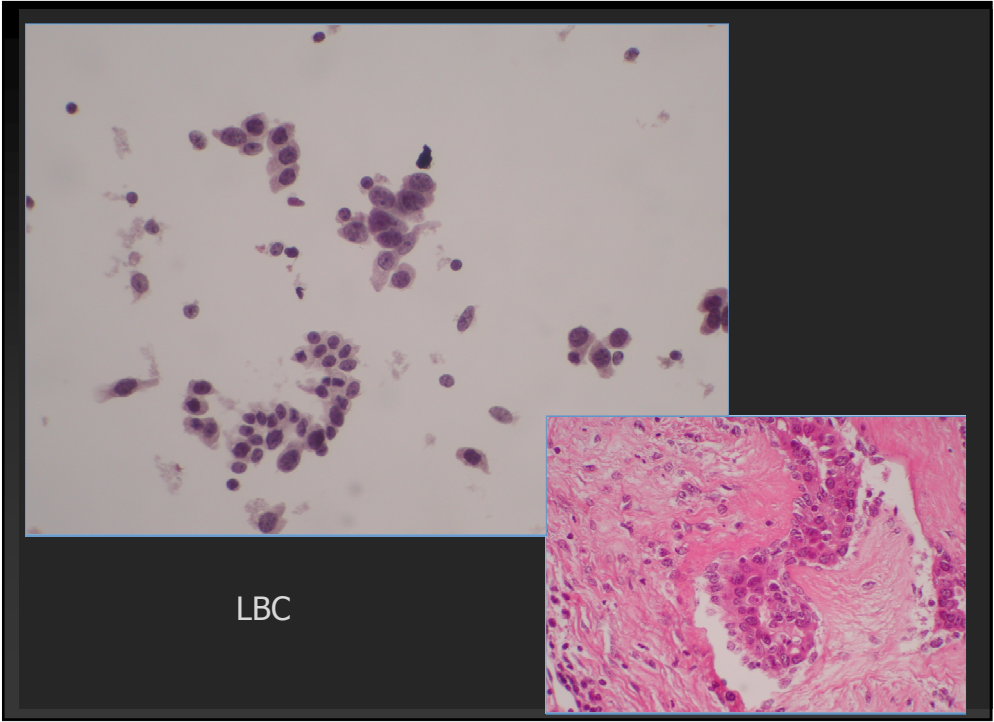


ACTA  
CYTOLOGICA

Francesco Feoli<sup>a</sup> Lieveke Ameye<sup>a</sup> Pascal Van Eeckhout<sup>b</sup>  
Marianne Paesmans<sup>a</sup> Vincenzo Marra<sup>c</sup> Riccardo Arisio<sup>c</sup>

Accepted after revision: February 6, 2013

	Suggested Acceptable Values <sup>d</sup>	Accuracy					
		RA		RA, new criteria 1		RA, new criteria 2	
Absolute sensitivity: #malignantC5/malignant cases	>60%	57%	(90/159)	68%	(108/159)	74%	(118/159)
Complete sensitivity: malignant(#C3+#C4+#C5)/malignant cases	>80%	95%	(151/159)	95%	(151/159)	95%	(151/159)
Specificity: #benignC2/benign cases	>60%	74%	(23/31)	74%	(23/31)	74%	(23/31)
Suspicious rate: (#C3+C4)/total number of cases	<20%	34%	(65/190)	25%	(47/190)	19%	(36/190)
Inadequate rate: #C1/total	<25%	4%	(7/190)	4%	(7/190)	4%	(7/190)
FP rate: #benignC5/malignant cases	<1%	0%	(0/159)	0%	(0/159)	0.6%	(1/159)
FN rate: #malignantC2/malignant cases	<5%	3%	(5/159)	3%	(5/159)	3%	(5/159)



## Challenging Breast Lesions: Pitfalls and Limitations of Fine-Needle Aspiration and the Role of Core Biopsy in Specific Lesions

Aylin Simsir, M.D.\* and Joan Cangiarella, M.D.

Diagn. Cytopathol. 2012;40:262-272.

Fibroepithelial Lesions  
Papillary Lesions  
Mucinous Lesions  
Low grade Cancers

ACTA  
CYTOLOGICA

Francesco Feoli<sup>a,b</sup> Lieveke Ameye<sup>a</sup> Pascal Van Eeckhout<sup>b</sup>  
Marianne Paesmans<sup>a</sup> Vincenzo Marra<sup>c</sup> Riccardo Arisio<sup>c</sup>

Accepted after revision: February 6, 2013

Prospective Validation.  
Modified Criteria. Clinical Information.

Cytology Clarified  
BIRADS 3 and 4  
Uncertain Categorizations.  
75% and 87%

Absolute Sensitivity	91%
Complete Sensitivity	100%
Specificity	69%
FP & FN Results.	0%

C1-C5: K value 0.78 (95% CI=0.67-0.89)  
Combining C3-C4K value 0.94 (95% CI=0.81-1.00)

Future Work **BIRADS 3.**

Radiology	Janet K. Baum, MD Lucy G. Hanna, MS Suddhasatta Acharyya, PhD Mary C. Mahoney, MD Emily F. Conant, MD Lawrence W. Bassett, MD Elta D. Pisano, MD	<b>Use of BI-RADS 3—Probably Benign Category in the American College of Radiology Imaging Network Digital Mammographic Imaging Screening Trial<sup>1</sup></b>
	47,599 Patients Same Day Screen-Film & Digital Mammography	<b>29% Non Compliance with Short-Interval Follow-up</b>
<b>Purpose:</b> To determine (a) bi and Data System American College of Digital Mammography either at the time work-up, (b) how often subjects actually returned for the recommended follow-up examination, and (c) the rate and stages of any malignancies subsequently found in subjects		

**Radiology:** Volume 260: Number 1—July 2011

Mammographic Short-Interval Follow-up can be used safely...  
 ...but thorough Imaging Evaluation is needed.

Future Work **BIRADS 4.**

**A Wide Range of PPV. 2%-95% Cancers at Follow up** (*Univ. of Leiden 2006*).

Inter-observer Variation

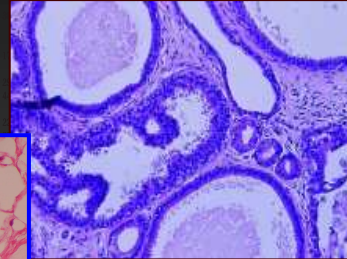
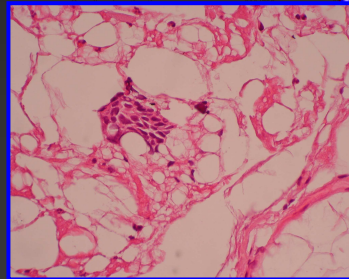
**The Dutch Breast Cancer Screening Programme**  
 Timmers JMH Eur Radiol (2012) 22:1717-1722

Cytology and Biopsy May  
Be Complementary.

Surgical Biopsy Upstaged  
to DCIS or to Invasive Cancer  
36.2% of the B3 Categorizations

Nehmat Houssami, PhD<sup>1,2</sup>  
Stefano Ciatto, PhD<sup>2</sup>  
Ian Ellis, FRCPath<sup>3</sup>  
Daniela Ambrogetti, MD<sup>2</sup>

*Cancer* 2007;109:487–95. © 2006 American Cancer Society.



MISE AU POINT

## Cytopathologie moléculaire. Outils et applications

Molecular biology and cytopathology. Principles and applications

Philippe Vielh<sup>a,\*</sup>, Fernando Carlos Schmitt<sup>b</sup>

*Annales de pathologie* (2012) 32, 444–450

Grazie

# MOLECULAR CYTOPATHOLOGY

*MAIN APPLICATIONS AND TECHNICAL ISSUES*

Prof. Fernando Schmitt  
Medical Faculty of Porto University, Porto, Portugal  
IPATIMUP  
General-Secretary of the International Academy of Cytology



## CYTOLOGY Ancillary Studies

- SPECIAL STAINING.
- IMMUNOHISTOCHEMISTRY.
- FLOW CYTOMETRY.
- **MOLECULAR TECHNIQUES.**

## Ancillary Studies in Cytology Challenges

- To select the correct test for a limited sample quantity.
- Avoid jumping from a histological adapted technique directly to cytological material.
- Use appropriate controls for cytological material.

## ANCILLARY TESTING

**TECHNICAL ASPECTS**

- Cytology specimens
- Fixation
- Controls
- Procedures
  - Techniques
  - Morphology
  - DNA extraction
  - Mutation detection

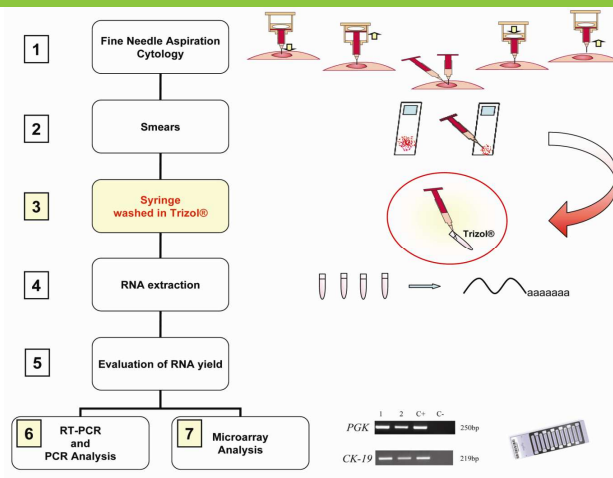


## Ancillary Studies in Cytology

TARGET	PRE-ANALYTICAL ISSUES	FIXATION	TECHNIQUES	APPLICATION
DNA	Very stable	Any kind, including air-dried	PCR, ISH, Sequencing	Detection of mutations, translocations..
RNA	Very unstable	Dedicate sampling for adequate preservative	Microarrays, RT-PCR	Gene expression profiling
PROTEIN	Variable	Alcohol-fixed, formalin-fixed, LBC preservatives	IHC, Flow cytometry	Expression of proteins (markers)

### High-Throughput Molecular Analysis from Leftover of Fine Needle Aspiration Cytology of Mammographically Detected Breast Cancer<sup>1,2</sup>

Laura Annaratone\*, Caterina Marchiò\*, Tommaso Renzulli†, Isabella Castellano\*., Daniela Cantarella†, Claudio Isella†, Luigia Macri\*., Giovanna Mariscotti†, Davide Balmativola\*, Elisabetta Cantanna\*, Cristina Deambrogio\*, Francesca Pietribiasi†, Riccardo Arisio#, Fernando Schmitt\*\*, Enzo Medico† and Anna Sapino\*.,†



Translational Oncology

## Sample types



EDITORIAL

Cytopathology 2011, 22, 355–357

**Molecular cytopathology and flow cytometry: pre-analytical procedures matter**

### Pros and Cons of Cytology Preparation Methods

Pros	Cons
<b>Direct Smear</b>	
<ul style="list-style-type: none"> <li>May do when no extra material</li> <li>No wet material needed</li> <li>Can use what available slides were initially obtained (no expense to extra preparations)</li> <li>Formalin post-fixed in rehydrated air-dried slides bring reliable results in ICC</li> </ul>	<ul style="list-style-type: none"> <li>Background artefact severe</li> <li>Panels unlikely</li> <li>Different antibody levels needed</li> <li>Prior staining or ethanol may affect results</li> <li>Variable results on molecular</li> </ul>
<b>Cytospins</b>	
<ul style="list-style-type: none"> <li>Useful with limited material</li> <li>Panels possible</li> </ul>	<ul style="list-style-type: none"> <li>Background artifact</li> <li>Different antibody levels needed</li> <li>Extra "wet" material needed</li> </ul>
<b>Monolayer Preparations</b>	
<ul style="list-style-type: none"> <li>Possible decreased back-ground</li> <li>Extra material frequently available and easily stored</li> <li>Good DNA and RNA preservation</li> </ul>	<ul style="list-style-type: none"> <li>Different antibody levels needed</li> <li>Extra "wet" material may still be needed</li> <li>Ethanol in fixative may interfere with some antigen</li> </ul>
<b>Cell Block</b>	
<ul style="list-style-type: none"> <li>IHC laboratory can handle like routine material with proper controls</li> <li>Material easily stored</li> <li>Molecular techniques standardized for paraffin-embedded tissues can be easily applied.</li> </ul>	<ul style="list-style-type: none"> <li>Limited cellular specimens cannot be used</li> <li>Methodology of cell block preparation must be tested</li> </ul>

Molecular techniques in cytopathology practice

F C Schmitt,<sup>1,2</sup> A Longatto-Filho,<sup>3,4</sup> A Valent,<sup>5</sup> P Vielh<sup>5</sup> *J Clin Pathol* 2008;**61**:258–267.

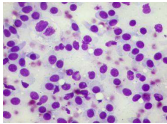
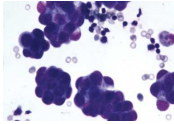
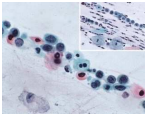
Possible use and role of molecular techniques in fine-needle aspiration cytology (FNAC) practice

Fernando Schmitt  
Helena Barroca  
DIAGNOSTIC HISTOPATHOLOGY 17:7 © 2011 Elsevier Ltd.

Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD<sup>1,2</sup> and Helena Barroca, MD<sup>3</sup>

*Molecular cytopathology (MCP) can be defined as molecular studies applied on all types of cytological specimens, namely gynecology cytology, exfoliative non-gyn cytology and fine needle aspirates.*



MOLECULAR TECHNIQUES				
	live cells	work load	morph control	sensitivity
• Cytogen.	+	+++	+/-	+/-
• PCR/RT-PCR	-	+	+	+++
• Microarrays	-	++	+	+++
• ISH	-	++	+++	++
• Sequencing	-	++	+	++++

## MOLECULAR TESTING MUST TAKE INTO ACCOUNT THE SENSITIVITY OF THE ASSAY

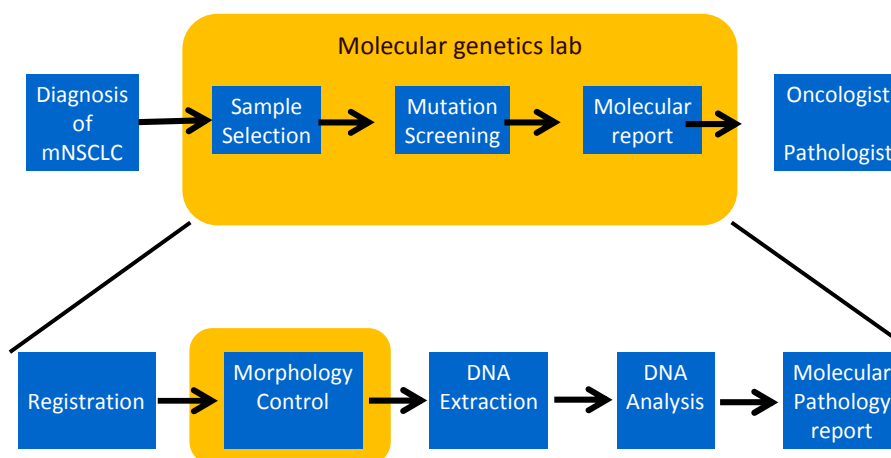
**Table 6** Diagnostic performance (%) of cytology pattern of malignancy and *RET/PTC* testing as a marker of PTC. N= 28 benign nodules, 64 PTC.

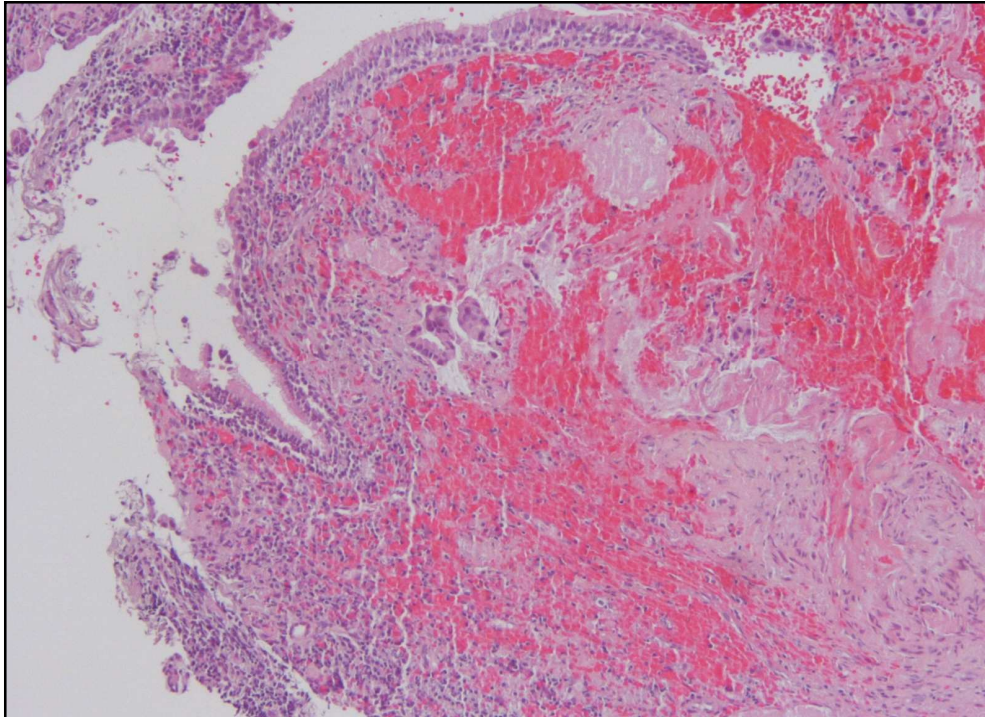
Diagnostic modality	Sensitivity	Specificity	PPV	NPV	Accuracy
Cytology*	94.0	86.7	92.2	89.6	91.2
RT-PCR	18.7	96.7	88.9	40.8	46.2
Southern-blot	35.9	85.7	85.2	44.8	52.5

These results indicate that molecular testing of thyroid nodules for *RET/PTC* induce false positive diagnoses when the highly sensitive assay Southern-blot on RT-PCR is used. Its searching by means of RT-PCR only, has a specificity superior of conventional cytology and can be used to refine inconclusive FNAC.

Guerra A *et al.* Endocrine Journal 2011

## MUTATION STATUS TESTING WORKFLOW





**IPATIMUP**  
Instituto de Patologia e Imunologia Molecular  
da Universidade do Porto  
Diretor: Prof. Doutor Manuel Gesteira-Machado

UNIDADE DE PATOLOGIA E PATOLOGIA MOLECULAR

**RELATÓRIO DE PATOLOGIA MOLECULAR**

Nº do Exame: 20120827-1

Nome: \_\_\_\_\_  
Materia: Lâminas de parafina  
Exame Requisitado: Mutações EGFR  
Termo de responsabilidade: 12021729  
Nº Processo: 14213266 Nº Episódio: 12014343  
Médico/Hospital Requestrante: Dr.ª Ana Barros/Centro Hospitalar de Vila Nova Gaia/Espinho E.P.E.  
Telefone: 227 865100 Fax: 227 666 306 (termos)

**Seleção da amostra**  
Presença de menos de 20% de células tumorais na presente amostra.  
Médico Patologista, Prof. Doutor Fernando Schmitt

**Técnica molecular:**  
A pesquisa de mutações no gene EGFR para os exões 18, 19, 20 e 21 foi efectuada através da técnica de PCR (Polymerase Chain Reaction) com sequenciação directa dos produtos de PCR em DNA obtido a partir de células tumorais.  
A técnica utilizada permite a detecção de 10% de ADN tumoral, o que corresponde a uma amostra com um mínimo de 20% de células tumorais. As mutações do gene EGFR são encontradas em cerca de 11% dos casos de carcinoma de pulmão de células pequenas.

**Resultado:**  
Neste caso não foram encontradas mutações nos exões 18, 19, 20 ou 21 do gene EGFR.

**Nota:** A ausência de mutações no gene EGFR aumenta a probabilidade de resistência ao tratamento com inibidores do EGFR.  
Uma quantidade de células tumorais <20% do total de células da amostra é insuficiente para uma avaliação ótima do estado mutacional do gene EGFR. Nestes casos, e apesar do resultado obtido indicar ausência de mutações, não é possível excluir totalmente a presença de mutações no gene EGFR.

O analista responsável: Dr. Luis Cines  
O responsável pela análise: Prof. Doutor José Carlos Machado

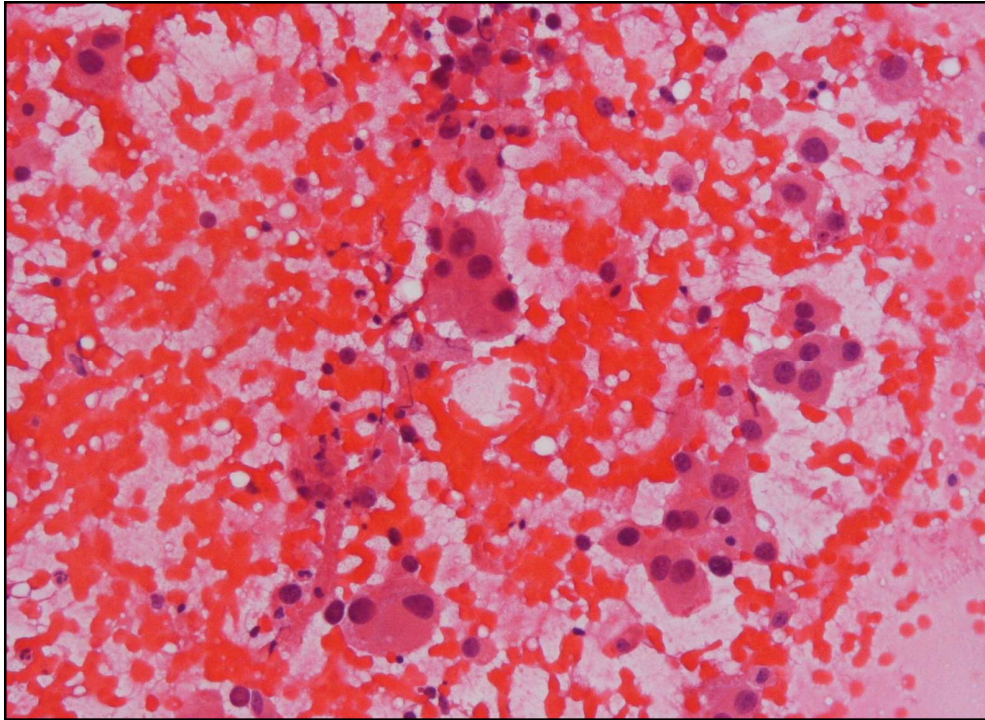
Este laboratório possui Acreditação pelo Código Americano de Patologistas e um Sistema de Gestão que se encontra certificado de acordo com a NP EN ISO 9001:2008

Pág. 1 / 1

Presence of less than 20% of tumor cells in the sample

In this case no mutations were found in exons 18, 19, 20 and 21 of the EGFR





**IPATIMUP**  
Instituto de Patologia e Imunologia Molecular  
da Universidade do Porto  
Director: Prof. Doutor Manuel Sobrinho-Silva

UNIDADE DE PATOLOGIA E PATOLOGIA MOLECULAR

**RELATÓRIO DE PATOLOGIA MOLECULAR**

Nº do Exame: 2012/08919-1

Nome: \_\_\_\_\_

Materiais: Bloco de parafina

Exame Requestado: Mutações EGFR

Termo de responsabilidade: 12005664

Nº Processo: 99361130

Nº Episódio: 12077886

Médico/Hospital Requirente: Dr. José Vilchez/Hospital Distrital de Santarém, EPE

Telefone: 243 300 220

Fax: 243 300 220

Data de entrada: 24-07-2012

**Resumo da amostra**  
Presença de mais de 20% de células tumorais na presente amostra.  
O médico Patologista, Prof. Doutor Fernando Schwert

**Técnica molecular:**  
A pesquisa de mutações no gene EGFR para os exões 18, 19, 20 e 21 foi efectuada através da técnica de PCR (Polymerase Chain Reaction) com sequenciação directa dos produtos de PCR em DNA obtido a partir de células tumorais.  
A técnica utilizada permite a detecção de 10% de ADN tumoral, o que corresponde a uma amostra com um mínimo de 20% de células tumorais. As mutações do gene EGFR são encontradas em cerca de 11% dos casos de carcinoma do pulmão de não pequenas células.

**Resultados:**  
Neste caso foi encontrada a mutação c.2573T>G no exão 21 do gene EGFR que leva à substituição de um aminoácido na posição 858 da proteína (p.Leu858Arg). Esta mutação já foi previamente descrita em doentes com carcinoma de não pequenas células do pulmão, tendo-se verificado que estes doentes tendem a responder positivamente ao tratamento com inibidores do EGFR. Não foram encontradas mutações dos exões 18, 19 ou 20 do gene EGFR.

O analista responsável: Dr. Luís Caires

O responsável pela análise: Prof. Doutor José Carlos Machado

Este laboratório possui Acreditação pelo Colégio Americano de Patologistas e um Sistema de Gestão que se encontra certificado de acordo com a NP EN ISO 9001:2008

Advancing Excellence

Endereço de E-mail: info@ipatimup.pt

Telefone: 22 8217200 Fax: 22 8217279 e-mail: info@ipatimup.pt

Presence of more than 20% of tumor cells in the sample

In this case, was found the mutation c.2573T> G in exon 21 of the EGFR gene leading to an amino acid substitution at position 858 of the protein (p.Leu858Arg). This mutation has been previously described in patients with metastatic non-small cell lung cancer, and it was found that these patients tend to respond positively to treatment with EGFR inhibitors. There were no mutations in exons 18, 19 or 20 of EGFR



## Mutation frequency and morphological control

	Mut	Wild-type	Total
Without control	463 (38%)	763 (62%)	1226
With control	309 (43%)	408 (57%)	717

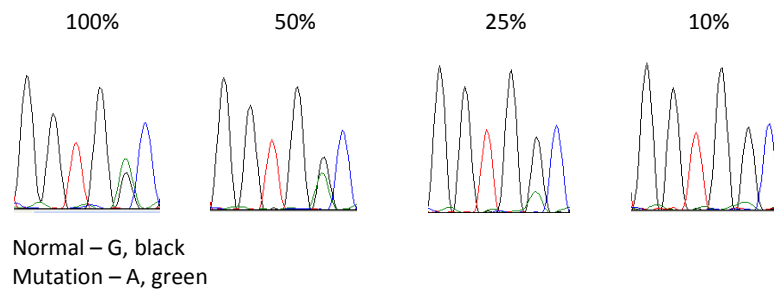
P<0.05

## Mutation frequency and tumour cell content

	Mut	Wild-type	Total
<20% tumour cells	7 (26%)	20 (74%)	27 (4%)
>20% tumour cells	296 (44%)	378 (56%)	674 (96%)

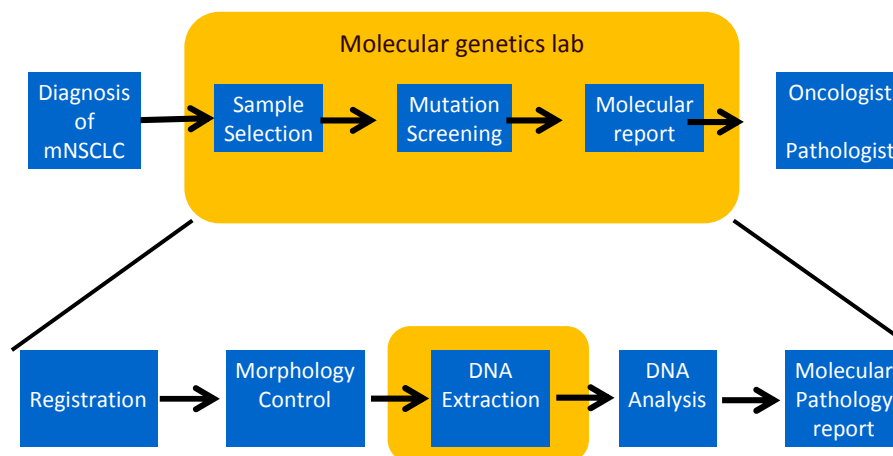
P<0.05

## % of tumour cells and mutation detection sensitivity



Method sensitivity set at 20% of tumoral cells

## MUTATION STATUS TESTING WORKFLOW

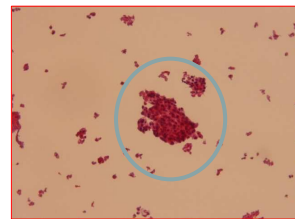
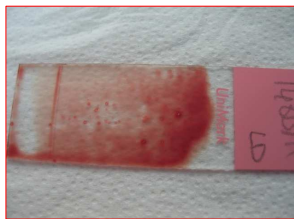


### 1. Slide Selection and Assessment

- . Testing can be performed regardless of the stain used
- . For cases with a high tumor content (>20%) the marking of areas of tumors is unnecessary. (1 cell = 6 pg DNA)

### 2. Removing the Coverslip

- . 48-72hs in xylene or substitute



**Enrichment by macrodissection if necessary.**

### 3. Collecting the Tissue

- . Once the coverslip is removed, the slide is re-soaked in xylene to remove residual mounting medium. No destaining step and no rehydration .
- . Cellular material is collected by scraping the entire slide with a flat single blade and collecting all the material into a small clump on a distal corner of the slide.
- . With a pipette tip push the clump of tissue into the Eppendorf tube.



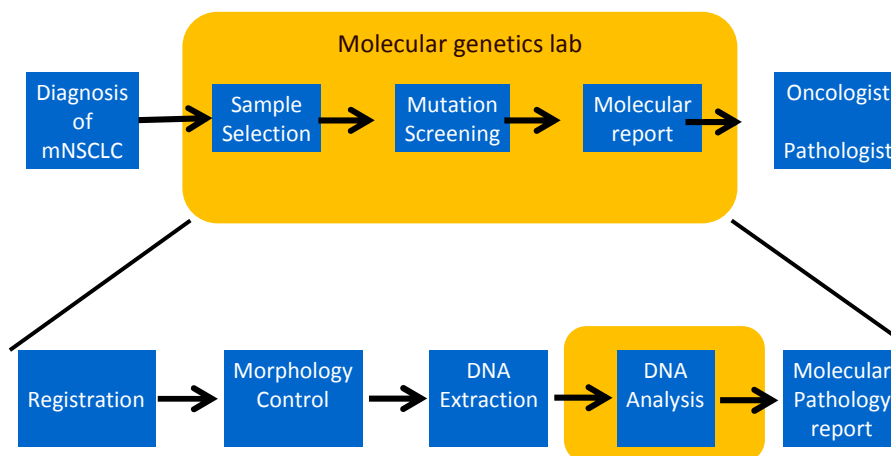
### 4. Tissue Lysis and DNA Extraction

- . Lysis with 200 µl of ATL buffer and 20-40µl of proteinase K
- . The mixture is vortexed gently and incubated at 56 °C
- . Lysates are purified according Qiagen Dneasy protocol
- . Evaluate DNA quantity and quality used a Nanodrop spectrophotometer
- . Average DNA yield from a single stained cytologic slide 7ng/µl.

## OUR experience

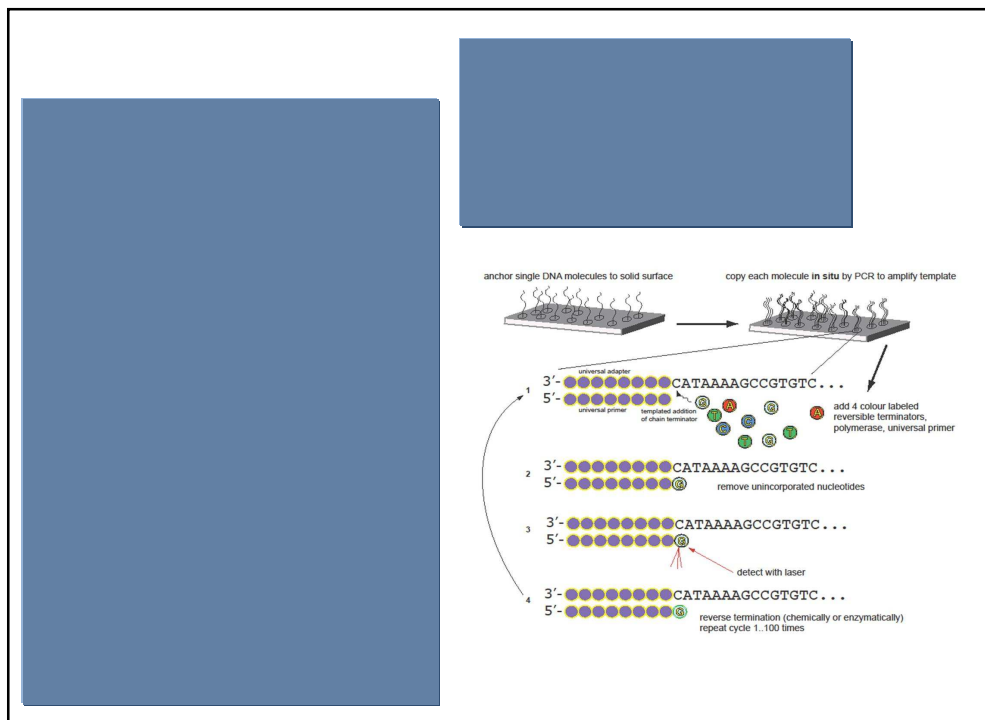
- > 3000 cases analyzed
- Mutation analysis success rate > 95%
- In LBC and cell-blocks higher success rates
- DNA quality/purity highly limitant

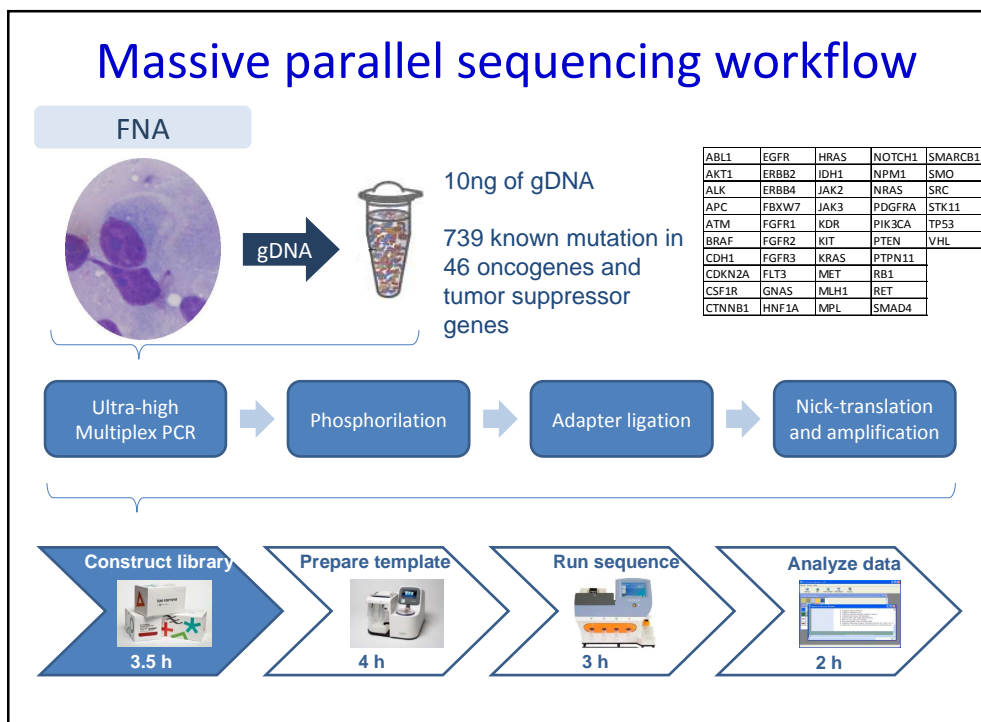
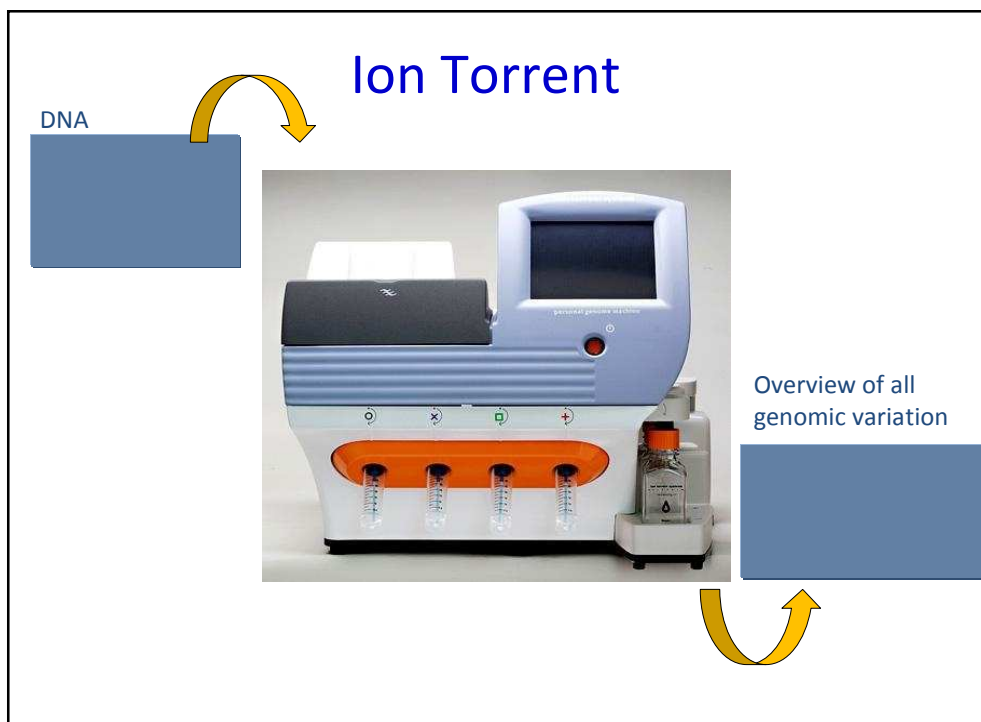
## MUTATION STATUS TESTING WORKFLOW



## Methods for mutation screening

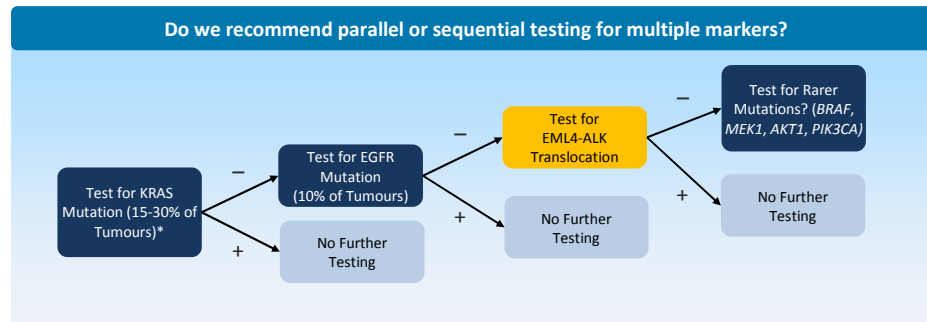
<ul style="list-style-type: none"> <li>. Allele-specific PCR</li> <li>. HRM</li> <li>. ARMS PCR</li> <li>. Others...</li> </ul>	<p>More demanding in DNA quantity and quality</p> <p>More sensitive</p>
<ul style="list-style-type: none"> <li>. Fragment analysis</li> <li>. SSCP</li> <li>. Sanger Sequencing</li> <li>. Others...</li> </ul>	<p>Less demanding in DNA quantity and quality</p> <p>Less sensitive</p>







## What should be tested?



A major clinical challenge is prospectively determining the status of multiple clinically relevant genes in tumor DNA before starting therapy.

**Multi-test single assay!**

Horn, L. et al. JCO 2009

## KEY POINTS TO USE MOLECULAR TECHNIQUES IN CYTOLOGY

- Collect good and well-preserved material.
- Validate in large scale molecular studies on cytological material.
- Control the cases morphologically.

Schmitt FC. Cytopathology 2011

## ANCILLARY TESTING

### MAIN APPLICATIONS

- LUNG CYTOLOGY
- EFFUSIONS
- THYROID CYTOLOGY
- GI/PANCREAS CYTOLOGY
- LYMPH NODE CYTOLOGY
- SOFT TISSUE CYTOLOGY
- BREAST CYTOLOGY
- METASTASIS

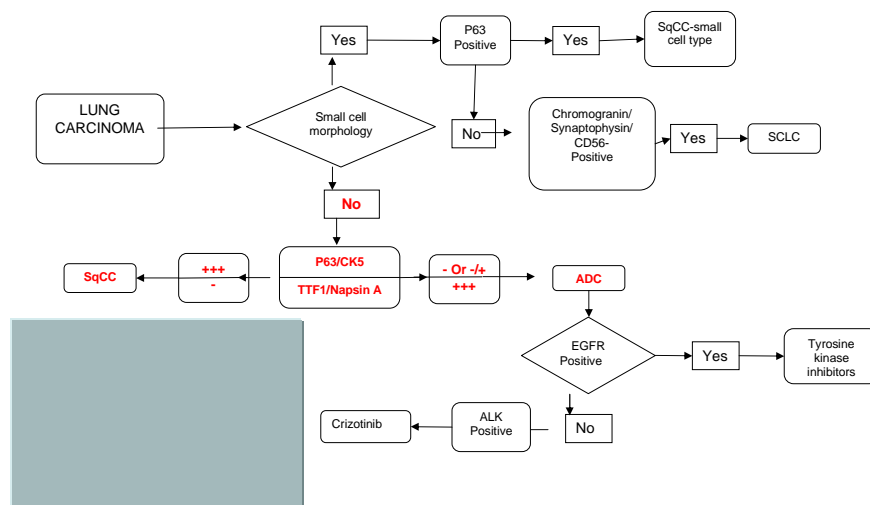


## Why to sub-classify NSCLC in cytology/small biopsies?

- TKis are used as first line therapy in patients with advanced adenocarcinoma with EGFR mutations.
- Patients with adenocarcinoma or NSCLC-NOS are more responsive to pemetrexed than those with SQCC.
- Bevacizumab has been associated with life-threatening haemorrhage in patients with lung SQCC.

## Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD<sup>1,2</sup> and Helena Barroca, MD<sup>3</sup>



Cancer Cytopathology 2011

International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

*Journal of Thoracic Oncology* • Volume 6, Number 2, February 2011

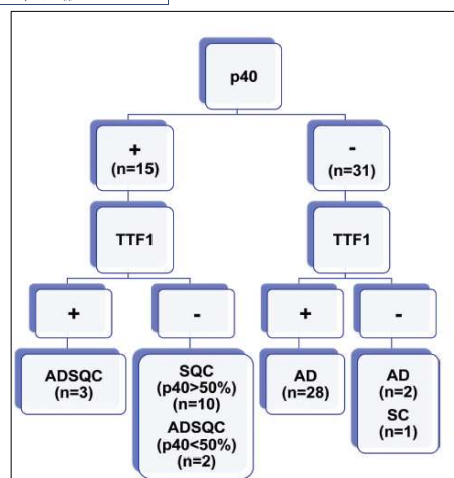
## PATHOLOGY CONSIDERATIONS FOR GOOD PRACTICE

- Small biopsy and cytology samples should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

$\Delta$ Np63 (p40) and Thyroid Transcription Factor-1 Immunoreactivity on Small Biopsies or Cellblocks for Typing Non-small Cell Lung Cancer  
A Novel Two-Hit, Sparing-Material Approach

Giuseppe Pelosi, MD, MSc,\*† Alessandra Fabbri, MD,\* Fabrizio Bianchi, DSc, PhD,‡  
Patrick Maisonneuve, Eng,§ Giulio Rossi, MD,|| Mattia Barbaretti, MD,\* Paolo Graziano, MD,¶  
Alberto Cavazza, MD,\*\* Natasha Reikhtman, MD, PhD,†† Ugo Pastorino, MD,‡‡  
Paolo Scanzigatta, MD,‡‡ and Mauro Papotti, MD§§

*Journal of Thoracic Oncology* • Volume 7, Number 2, February 2012



This minimalist IHC-based model of p40 and TTF1 on biopsy/cellblock samples was effective to correctly subtype most cases of lung cancer.

Review

ACTA  
CYTOLOGICA

Acta Cytologica 2013;51:1-9  
DOI: 10.1159/000342946

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Published online December 6, 2012

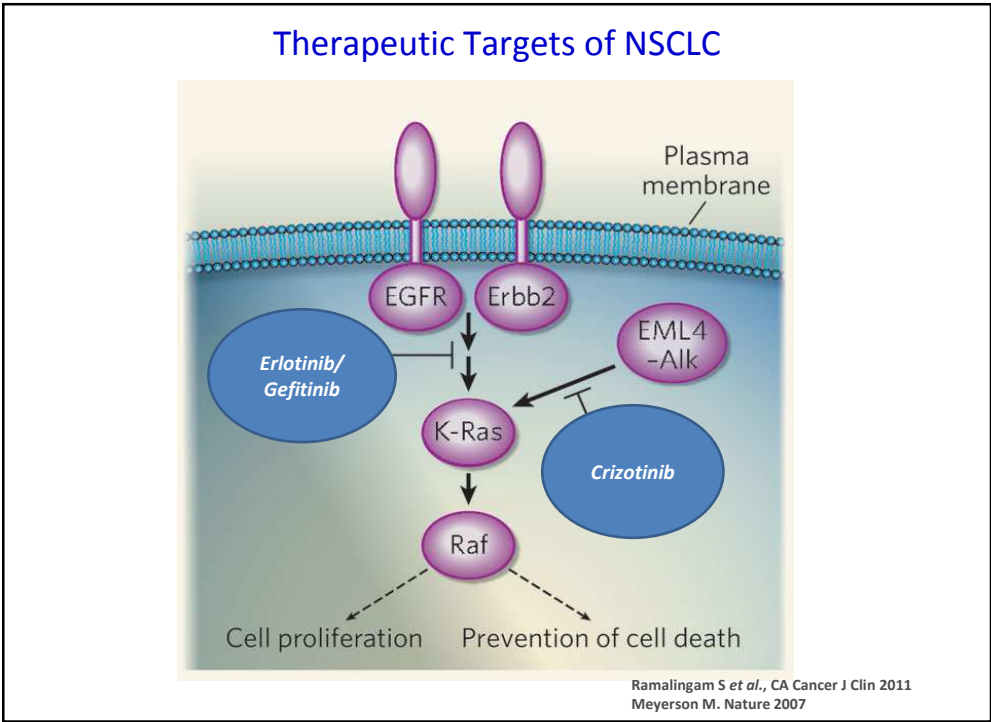
**p40: A p63 Isoform Useful for Lung Cancer Diagnosis – A Review of the Physiological and Pathological Role of p63**

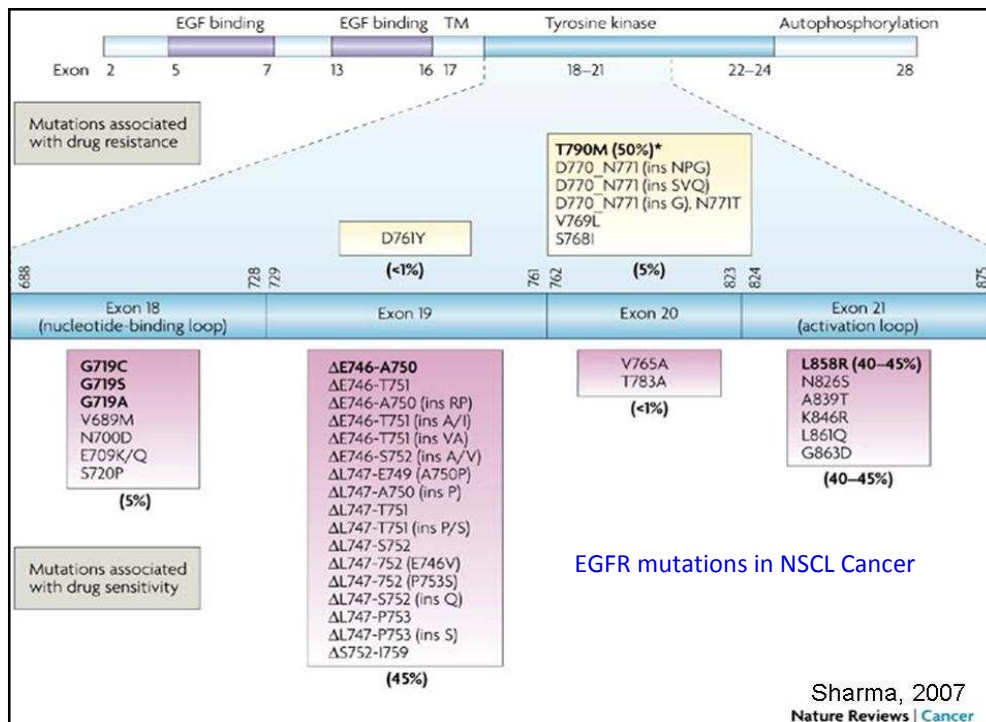
Ana Rita Nobre<sup>a,b</sup> André Albergaria<sup>a,c</sup> Fernando Schmitt<sup>a,c</sup>

**Table 1.** Distribution of TTF-1, p63, and p40 in lung cancer

	Histological diagnosis					SE	SP	PPV	NPV	Ref.
	ADC	SCC	LCC/SG	ADSC	LCL					
TTF-1	115/150 (77)	0/50 (0)	n.a.	n.a.	n.a.	0.77	1.00	1.00	0.59	45
p63	27/150 (18)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.82	0.65	1.00	
p40	0/150 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1+/p63+	26/180 (14)	0/50 (0)	n.a.	n.a.	n.a.	0.14	1.00	1.00	0.25	
TTF-1+/p63-	115/180 (64)	0/50 (0)	n.a.	n.a.	n.a.	0.64	1.00	1.00	0.43	
TTF-1-/p63+	4/180 (2)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.98	0.93	1.00	
TTF-1-/p63-	35/180 (19)	0/50 (0)	n.a.	n.a.	n.a.	0.19	1.00	1.00	0.26	
TTF-1+/p40+	0/180 (0)	0/50 (0)	n.a.	n.a.	n.a.	0.00	1.00	0.00	0.22	
TTF-1+/p40-	141/180 (78)	0/50 (0)	n.a.	n.a.	n.a.	0.78	1.00	1.00	0.56	
TTF-1-/p40+	0/180 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1-/p40-	39/180 (22)	0/50 (0)	n.a.	n.a.	n.a.	0.00	0.78	0.00	0.74	
TTF-1	26/30 (93)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.93	1.00	1.00	0.83	46
p63	9/30 (30)	10/10 (100)	1/1 (100)	5/5 (100)	n.a.	1.00	0.70	0.53	1.00	
p40	5/30 (17)	10/10 (100)	0/1 (0)	5/5 (100)	n.a.	1.00	0.83	0.67	1.00	
TTF-1+/p63+	8/30 (27)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.27	1.00	1.00	0.31	
TTF-1+/p63-	20/30 (67)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.67	1.00	1.00	0.50	
TTF-1-/p63+	1/30 (3)	10/10 (100)	1/1 (100)	2/5 (40)	n.a.	1.00	0.97	0.91	1.00	
TTF-1-/p63-	1/30 (3)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
TTF-1+/p40+	4/30 (14)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.13	1.00	1.00	0.28	
TTF-1+/p40-	24/30 (80)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.80	1.00	1.00	0.63	
TTF-1-/p40+	1/30 (3)	10/10 (100)	0/1 (0)	2/5 (40)	n.a.	0.53	0.97	0.91	0.76	
TTF-1-/p40-	1/30 (3)	0/10 (0)	1/1 (100)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
p63	74/237 (31)	81/81 (100)	n.a.	n.a.	82/152 (54)	1.00	0.69	0.52	1.00	47
p40	7/205 (3)	81/81 (100)	n.a.	n.a.	0/152 (0)	1.00	0.97	0.92	1.00	
TTF-1	51/66 (77)	0/24	3/12	0/1	n.a.	0.77	1.00	1.00	0.62	49
p63	13/66 (20)	24/24 (100)	7/12	1/1 (100)	n.a.	1.00	0.80	0.65	1.00	
p40	1/29 (3)	15/15 (100)	3/12 (25)	1/1 (100)	n.a.	1.00	0.97	0.94	1.00	
Napsin-A	11/29 (38)	0/15 (0)	1/12 (8)	0/1 (0)	n.a.	0.38	1.00	1.00	0.45	

Integrative data of the expression of TTF-1, p63, and p40 in subtypes of lung cancer and comparison of the sensitivity and specificity of these markers for SCC. Values in parentheses are percentages. ADSC = Adenosquamous carcinoma; LCC = large cell carcinoma; LCL = large cell lymphoma; n.a. = not assessed; NPV = negative predictive value; PPV = positive predictive value; SG = sarcomatoid carcinoma; SE = sensitivity; SP = specificity; Ref. = reference.



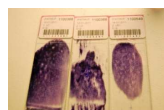


## EGFR and ALK on Lung Cancer

### Our data – 2012

#### Type of Material

- 60% - cell blocks
- 20% - liquid-media
- 10% - stained smears





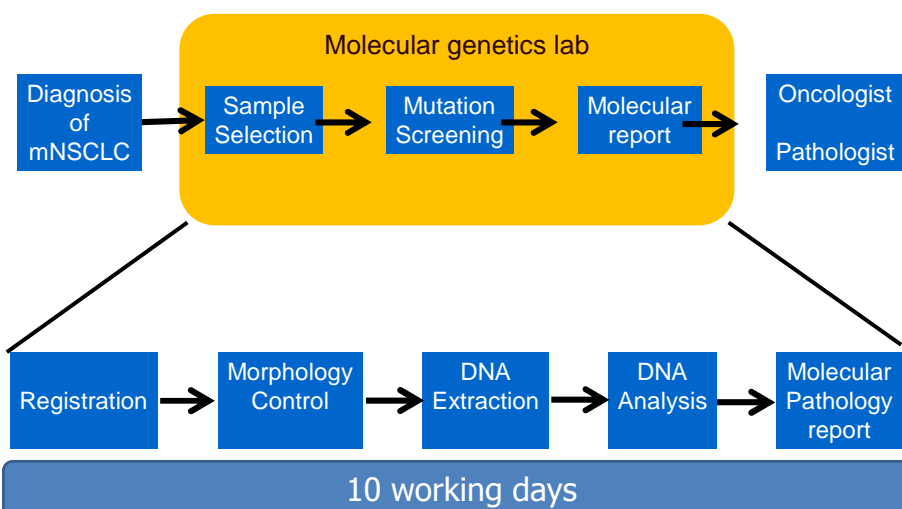
## EGFR and ALK on Lung Cancer

### Our data (2011-2012)

*EGFR mutation(n=928)*

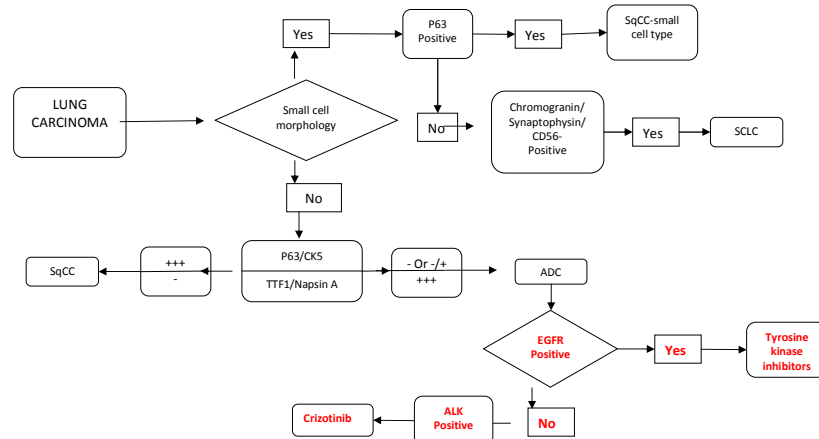
EGFR mutation	HISTOLOGY	CYTOLOGY	TOTAL
Positive	153	27	180 (19%)
Negative	630	116	746 (80%)
Inconclusive	0	2	2 (1%)
TOTAL	783	145	928 (100%)

## EGFR mutation status testing workflow



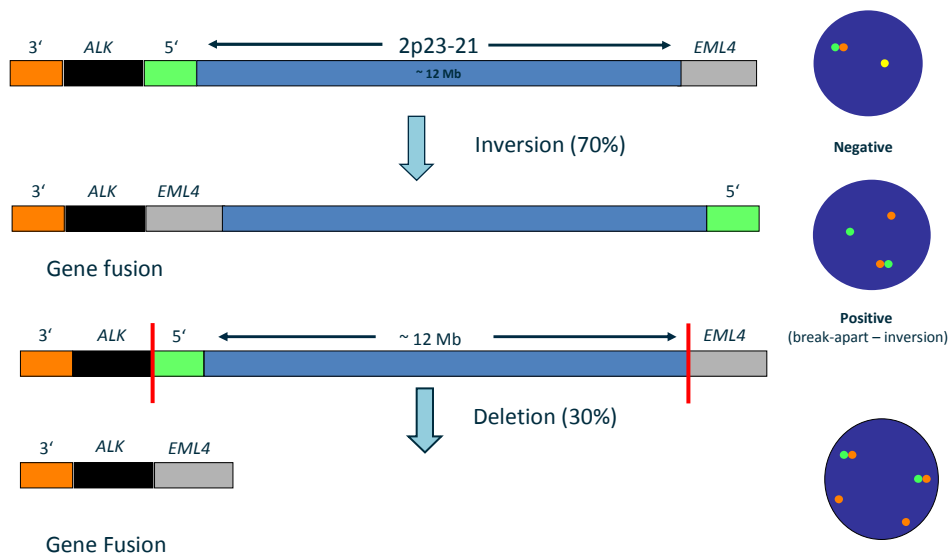
## Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD<sup>1,2</sup> and Helena Barroca, MD<sup>3</sup>



Cancer Cytopathology 2011

## Mechanisms of EML4-ALK gene fusion



Savic S and Bubendorf L, Acta Cytol 2012; 56: 611-621

## Practical aspects of FISH testing for EML4-ALK

- At least 50 tumour cells should be counted for accurate results.
- FISH is considered positive when at least one set of orange and green signals is > 2 signal diameters apart or there is a single orange signal without a corresponding green signal in addition to fused (normal signals).
- A sample is considered negative for ALK rearrangement if there are <5 positive cells (<10%) and positive if there are >25 positive cells (>50%).
- A sample is considered equivocal if 10-50% of cells are positive. In this case a 2<sup>nd</sup> reader should evaluate the slide and if the average of 2 readings contains at least 15% of positive cells the case is considered positive.

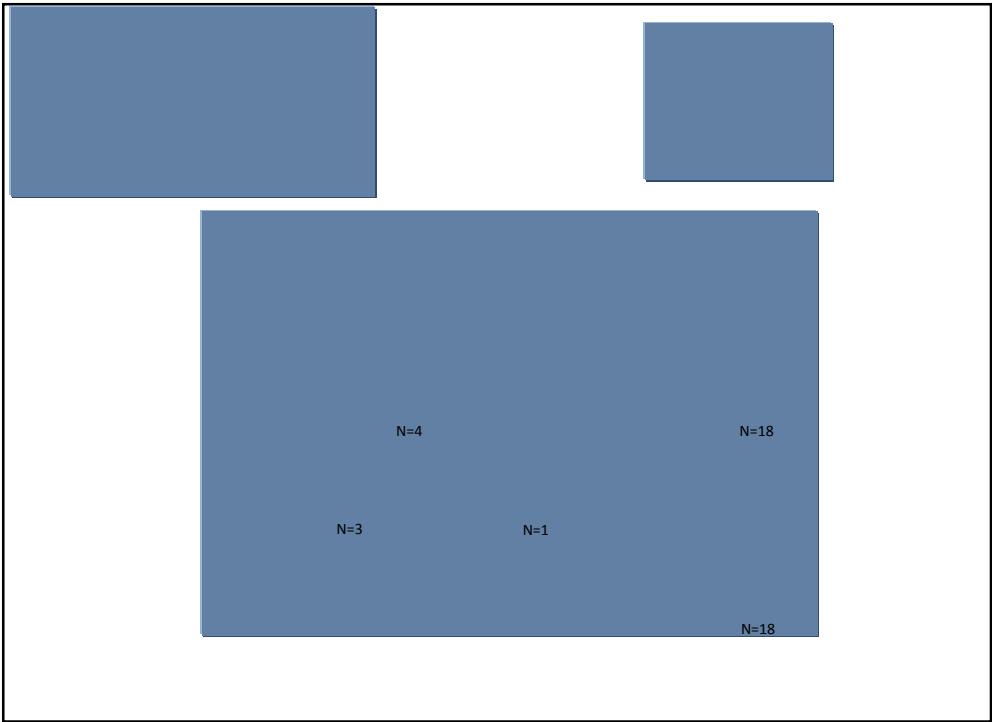
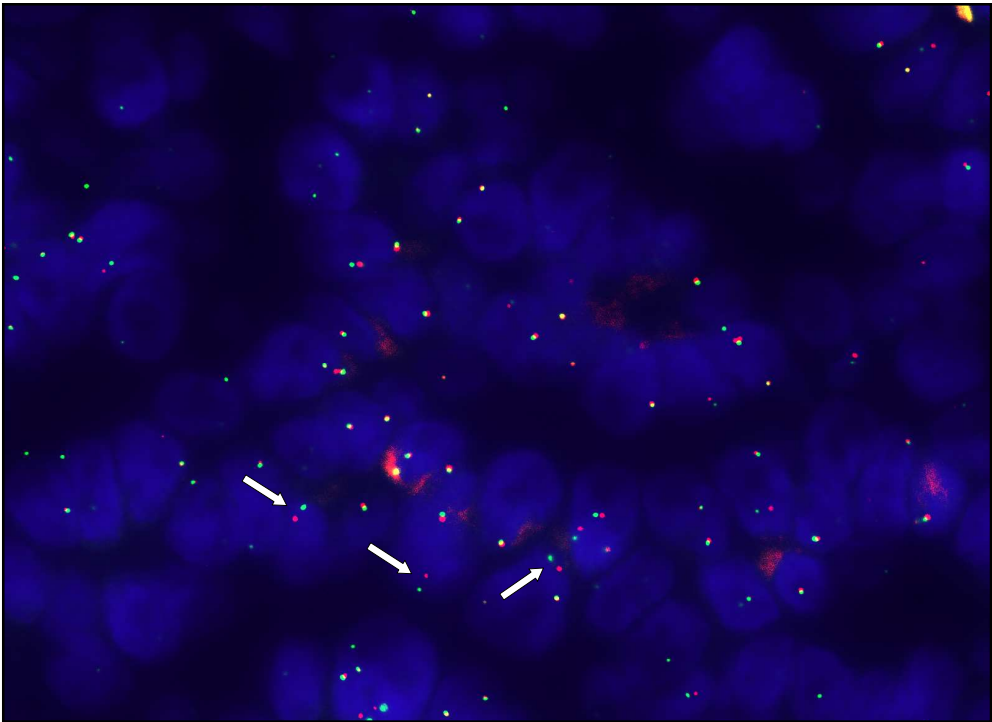
Savic S and Bubendorf L, Acta Cytol 2012; 56: 611-621

## EGFR and ALK on Lung Cancer Our data – 2011-2012 *FISH for ALK (n=143)*

ALK	HISTOLOGY	CYTOLOGY	TOTAL
Positive	6	2	8 (6%)
Negative	95	17	112 (78%)
Inconclusive	13*	10**	23 (16%)
TOTAL	114	29	143 (100%)

\* 11% of total

\*\* 34% of total



**nature  
medicine**

Feb 2012

## RET, ROS1 and ALK fusions in lung cancer

Kengo Takeuchi<sup>1,2</sup>, Manabu Soda<sup>3</sup>, Yuki Togashi<sup>1,2</sup>,  
Ritsuro Suzuki<sup>4</sup>, Seiji Sakata<sup>1</sup>, Satoko Hatano<sup>1</sup>, Reimi Asaka<sup>1,2</sup>,  
Wakako Hamanaka<sup>2</sup>, Hironori Ninomiya<sup>2</sup>, Hirofumi Uehara<sup>5</sup>,  
Young Lim Choi<sup>6</sup>, Yukitoshi Satoh<sup>5,7</sup>, Sakae Okumura<sup>3</sup>,  
Ken Nakagawa<sup>3</sup>, Hiroyuki Mano<sup>3,6</sup> & Yuichi Ishikawa<sup>2</sup>

## KIF5B-RET fusions in lung adenocarcinoma

Takashi Kohno<sup>1,15</sup>, Hitoshi Ichikawa<sup>2,15</sup>, Yasushi Totoki<sup>3</sup>,  
Kazuki Yasuda<sup>4</sup>, Masaki Hiramoto<sup>4</sup>, Takao Nanno<sup>4</sup>,  
Hiromi Sakamoto<sup>2</sup>, Koji Tsuta<sup>5</sup>, Koh Furuta<sup>5</sup>, Yoko Shimada<sup>1</sup>,  
Reika Iwakawa<sup>6</sup>, Hideaki Ogiwara<sup>1</sup>, Takahiro Oike<sup>6</sup>, Masato Enari<sup>7</sup>,  
Aaron J Schetter<sup>8</sup>, Hirokazu Okayama<sup>8</sup>, Aage Haugen<sup>9</sup>, Vidar Skaug<sup>9</sup>,  
Suenori Chiku<sup>10</sup>, Itaru Yamanaka<sup>11</sup>, Yasuhito Arai<sup>3</sup>,  
Shun-ichi Watanabe<sup>12</sup>, Ikuo Sekine<sup>13</sup>, Seishi Ogawa<sup>14</sup>, Curtis C Harris<sup>8</sup>,  
Hitoshi Tsuda<sup>5</sup>, Teruhiko Yoshida<sup>5</sup>, Jun Yokota<sup>6</sup> & Tatsuhiro Shibata<sup>3</sup>

## RET fusions in lung cancer !

### Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies

Doron Lipson<sup>1,9</sup>, Marzia Capelletti<sup>2,9</sup>, Roman Yelensky<sup>1</sup>,  
Geoff Otto<sup>1</sup>, Alex Parker<sup>1</sup>, Mirna Jarosz<sup>1</sup>, John A Curran<sup>1</sup>,  
Sohail Balasubramanian<sup>1</sup>, Troy Bloom<sup>1</sup>, Kristina W Brennan<sup>1</sup>,  
Amy Donahue<sup>1</sup>, Sean R Downing<sup>1</sup>, Garrett M Frampton<sup>1</sup>,  
Lazaro Garcia<sup>1</sup>, Frank Juhn<sup>1</sup>, Kathy C Mitchell<sup>1</sup>, Emily White<sup>1</sup>,  
Jared White<sup>1</sup>, Zac Zwirko<sup>1</sup>, Tamar Peretz<sup>1</sup>, Hovav Nechushtan<sup>1</sup>,  
Lior Soussan-Gutman<sup>4</sup>, Jhingook Kim<sup>5</sup>, Hidefumi Sasaki<sup>6</sup>,  
Hyeon Ryul Kim<sup>7</sup>, Seung-il Park<sup>7</sup>, Dalia Ercan<sup>2</sup>,  
Christine E Sheehan<sup>8</sup>, Jeffrey S Ross<sup>1,8</sup>, Maureen T Cronin<sup>1</sup>,  
Pasi A Jänne<sup>2</sup> & Philip J Stephens<sup>1</sup>



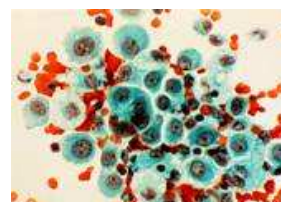
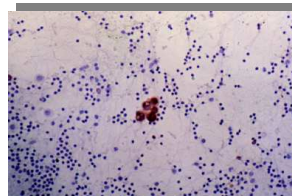
### A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing

Young Seok Ju,<sup>1,2</sup> Won-Chul Lee,<sup>1,3</sup> Jong-Yeon Shin,<sup>1,4</sup> Seungbok Lee,<sup>1,3</sup>  
Thomas Bleazard,<sup>1</sup> Jae-Kyung Won,<sup>5</sup> Young Tae Kim,<sup>6,7</sup> Jong-Il Kim,<sup>1,3,4,8</sup>  
Jin-Hyoung Kang,<sup>9</sup> and Jeong-Sun Seo<sup>1,2,3,4,8,10</sup>

Dec 2011

## Effusion Cytology

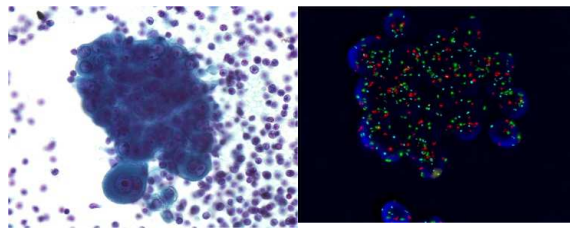
- Cytology is the first approach to search neoplastic cells in effusions. However the cytological examination has limited sensitivity.
- The main causes are: presence of scarce neoplastic cells and the d.d. between neoplastic cells and reactive mesothelial cells.
- Many innovative techniques have been used to improve the search of neoplastic cells in effusions.



## 9p21 Deletion in malignant mesothelioma

	Total n	9p21 deletion	
		hetero-	homozigous
Savic et al.	52	15%	58%
Onofre et al.	33	36%	49%
Illei et al.	13	-	92%
Flores-Staino et al.	21	-	57%

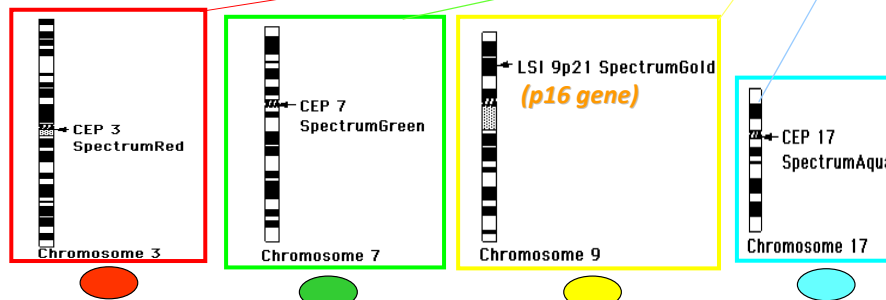
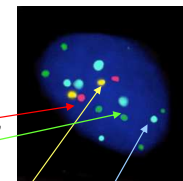
Specificity: 100% (0/116 benign effusion)



## Utility of Multiprobe FISH in Bladder Cancer

•The UroVysion probe set was developed to detect the most clinically-relevant, recurring chromosome abnormalities in TCC

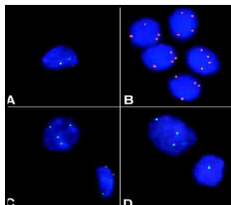
•UroVysion looks at the ploidy status for chromosomes 3, 7, 17 and the 9p21 locus





## INDICATIONS FOR FISH ANALYSIS IN URINE CYTOLOGY

- Atypical urinary cytology
- Control after intravesical BCG treatment
- Upper urinary tract cytology
- Surveillance after transurethral resection
- Hematuria in patients with an increased risk of UC



## Detection of bladder carcinoma recurrence in urine cytology

	Cytology	FISH
Sensitivity	63.8 %	80.4 %
Specificity	86 %	85.3 %
Efficacy	73.5 %	82.5 %

M.M ALMEIDA, 2008

## FISH ANALYSIS IN URINE CYTOLOGY



FISH-negative result with 2 signals for each of the 4 probes in all cell nuclei



FISH showing polyploidy in a reactive umbrella cell



FISH-positive result with tumor cell nuclei showing increased copy number (polysomy) of the chr3 (blue), 7 (red), but a normal copy number of 17 (green). There is a loss (deletion) of 9p21 (gold, 0–1 signals).

## Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD<sup>1,2</sup> and Helena Barroca, MD<sup>3</sup>

### Ancillary Studies in FNA/Schmitt and Barroca

**Table 2.** Molecular Markers and Techniques With Practical Application in Solid Tumors Sampled by Fine-Needle Aspiration

Solid Tumor Type	Molecular Markers and Techniques
Lung	<b>SCLC:</b> Chromogranin, CD56, synaptophysin by ICC <b>NSCLC:</b> P63/CK5/6 and TTF1/Napsin-A by ICC <sup>a</sup> ; EGFR mutation status by sequencing mutation or FISH <sup>b</sup> ; EML4-ALK translocation by FISH <sup>b</sup>
Thyroid	<b>Medullary carcinoma:</b> TTF1; calcitonin; chromogranin; CEA by ICC <sup>a</sup> <b>Follicular/papillary carcinoma:</b> CK19, galectin-3, and HBME-1 by ICC <sup>a</sup> ; BRAF, RAS, PAX8/PPAR and RET/PTC <sup>a</sup> See Table 1
Kidney	<b>Pancreatic ductal tumors:</b> KOC, loss of SMAD4 by ICC <sup>a</sup>
Gastrointestinal tumors	<b>Pancreatic endocrine tumors:</b> Chromogranin, CD56, synaptophysin by ICC <sup>a</sup> <b>Pancreatic solid pseudopapillary tumors:</b> Vimentin, alpha-1-antitrypsin, CD10, beta-catenin and progesterone receptor, chymotrypsin, and trypsin by ICC <sup>a</sup> <b>Cystic lesions:</b> Amylase, CEA, and K-RAS mutations <sup>a</sup> <b>GISTs:</b> DOG and CKIT by ICC <sup>a</sup> ; CKIT and PDGFR mutation <sup>a</sup> <b>Colon cancer:</b> KRAS <sup>b</sup>
Soft tissue	<b>Small round cell tumors:</b> CD56, NSE, chromogranin, synaptophysin, CD99, FLI1, EMA, caveolin, BCL2, miogenin, MYO D1, WT1 by ICC <sup>a</sup> ; N-Myc, del1p, ploidy, search for characterizing specific translocations <sup>b</sup> <b>Other sarcomas:</b> Specific immunomarkers and characterizing specific translocations (see text) <sup>a,b</sup>

Cancer Cytopathology 2011

## THYROID CYTOLOGY

- There are two critical points in thyroid FNA:
  - The rates of inadequate material
  - Indeterminate cases in which cytology does not allow a definitive diagnosis of benignity or malignancy.

## Thyroid FNA and ancillary techniques

### Immunocytochemistry

TPO antibody  
Cyclin-dependent kinase inhibitor  
p27kip1  
Dipeptidyl peptidase IV (CD 26)  
Galectin-3  
HBME-1  
Cytokeratins  
.....

### Molecular cytogenetics and molecular genetics

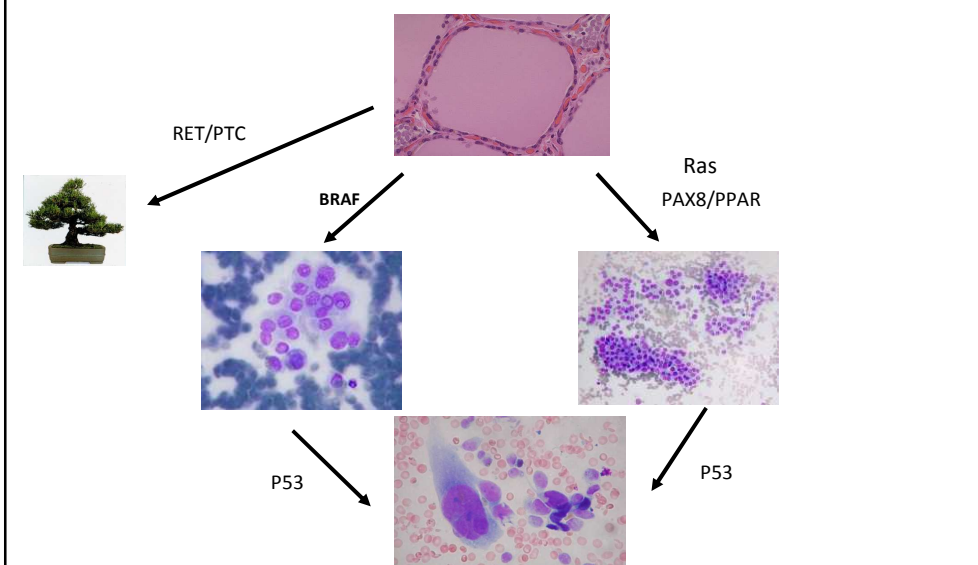
BRAF, RET/PTC, RAS or PAX-PPAR gamma mutations



### Cytometry

DNA pattern  
% S-phase cells

## Genomic alterations in follicular cell-derived carcinomas



## BRAF Mutations

- Most frequent genetic event in PTC (36-83%)
- BRAF mutations are not randomly distributed by PTC histotypes. Hotspot BRAF V<sup>600E</sup> clusters to PTC cases of papillary architecture.
- BRAF mutation is highly specific for malignancy in FNA aspirates, however the sensitivity is low mainly because BRAF mutation is less frequent in the FVPTC, one of the most problematic entities on FNA.

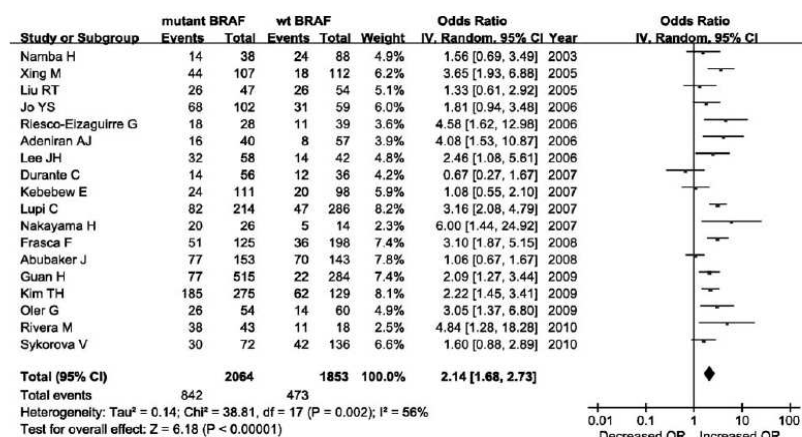
**Impact of Mutational Testing on the Diagnosis and Management of Patients with Cytologically Indeterminate Thyroid Nodules: A Prospective Analysis of 1056 FNA Samples**

Tariq E. Nikiforov, H. Paul Chow, Steven P. Hodak, Sally E. Carty, Sharon O. Udelsman, Robert L. Ferris, Lindean Yip, Raja R. Seshulu, Michael E. Tisler, Michael T. Tisler, Christopher Cypke, Michael T. Johnson, Andrew P. Stewart, and Marina N. Nikiforova

Atypia of Undetermined Significance (AUS) (n=247)			
	Histology Malignant (n=35)	Histology Benign (n=212)	
Mutation Positive (n=25)	16 RAS, 5 BRAF, 1 PAX8/PPAR $\gamma$	3 RAS (FA)	Sensitivity: 63%
Mutation Negative (n=222)	13 (11PTCFV, 2PTC)	209	Specificity: 99%
Follicular Neoplasm (n=214)			
	Histology Malignant (n=58)	Histology Benign (n=156)	
Mutation Positive (n=38)	29 RAS, 2 BRAF, 2 PAX8/PPAR $\gamma$	5 RAS (FA)	Sensitivity: 57%
Mutation Negative (n=176)	25 (16PTCFV, 3PTC, 6FTC)	151	Specificity: 97%
Suspicious for Malignant Cells (n=52)			
	Histology Malignant (n=28)	Histology Benign (n=24)	
Mutation Positive (n=20)	10 BRAF, 7 RAS, 1 PAX8/PPAR $\gamma$ , 1 RET	1 RAS (FA)	Sensitivity: 68%
Mutation Negative (n=32)	9 (7PTC, 2PTCFV)	23	Specificity: 96%

Nikiforov Y et al., 2011

**BRAF<sup>V600</sup> mutation is associated with prognostic factors and poor clinical outcome in PTC**



## Prognostic Role of BRAF Mutations on Thyroid FNA

- BRAF + predicted disease persistent/recurrence, so BRAF status can modify surgical approach.
- This mutational parameter can be used as a pre-operative risk stratification for papillary carcinoma on FNA allowing a correct surgical approach without further instrumental procedures.
- BRAF mutation has a role in the risk stratification of Papillary carcinoma, including papillary microcarcinoma.

## Veracyte Affirma Gene Expression Classifier (GEC)

- Gene expression from mRNA on thyroid FNA washings
- The algorithm uses expression of 167 genes to classify the aspirates in benign or suspicious.
- There are 25 genes that initially filter out rare neoplasms (melanoma, RCC, Breast carcinoma, MTC, parathyroid, HCT).
- There are 142 genes in the main classifier. These genes are related with multiple cell functions.



Performance across the Primary Data Set of Indeterminate Nodules (N=265)		
GEC result	Malignant reference standard (N=85)	Benign reference standard (N=180)
Suspicious	78	87
Benign	7	93
Sensitivity, 92% (84–97); specificity, 52% (44–59); PPV, 47% (40–55); NPV, 93% (86–97); prevalence of malignant lesions, 32%		
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (N=129, 48.7%)		
GEC result	Malignant reference standard (N=31)	Benign reference standard (N=98)
Suspicious	28	46
Benign	3	52
Sensitivity, 90% (74–98); specificity, 53% (43–63); PPV, 38% (27–50); NPV, 95% (85–99); prevalence of malignant lesions, 24%		
Follicular or Hürthle-Cell Neoplasm or Suspicious for Follicular Neoplasm (N=81, 30.6%)		
GEC result	Malignant reference standard (N=20)	Benign reference standard (N=61)
Suspicious	18	31
Benign	2	30
Sensitivity, 90% (68–99); specificity, 49% (36–62); PPV, 37% (23–52); NPV, 94% (79–99); prevalence of malignant lesions, 25%		
Suspicious for Malignancy (N=55, 20.8%)		
GEC result	Malignant reference standard (N=34)	Benign reference standard (N=21)
Suspicious	32	10
Benign	2	11

N ENGL J MED 367:8 NEJM.ORG AUGUST 23, 2012

## Conclusions

AUS >> NPV 95%  
FN/SFN >> NPV 94%

Probability of malignancy post-test: 5-6%

Similar for nodules cytologically benign on FNA

SM >> NPV 85%

Probability of malignancy post-test: 15%

Maybe useful in deciding whether to perform hemithyroidectomy or TT

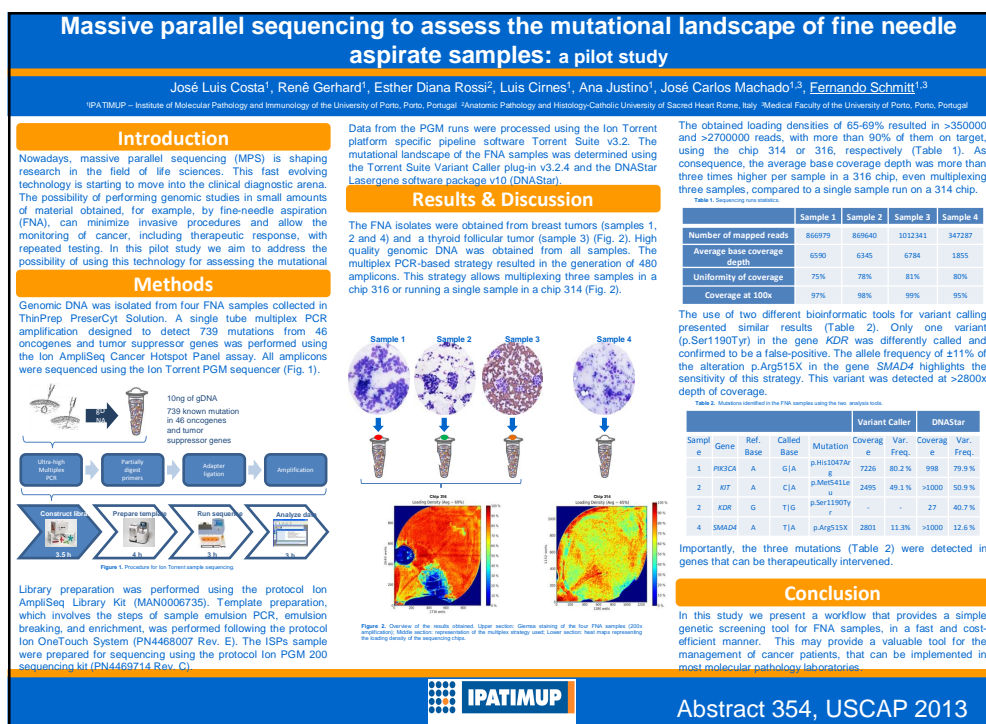
Nodules cytologically benign > specificity of 70% (13/44 benign "suspicious on GEC")

GEC should not be used in the analysis of samples with benign cytologic features.

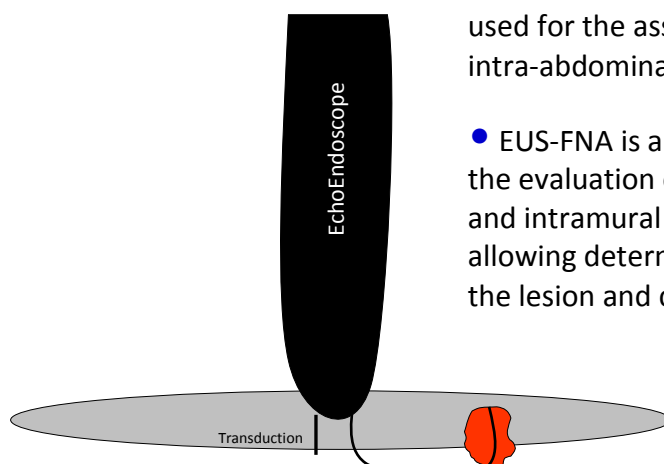
Nodules with indeterminate cytologic features and benign findings in the GEC

Recommend watchful waiting in lieu of diagnostic surgery.

N ENGL J MED 367:8 NEJM.ORG AUGUST 23, 2012

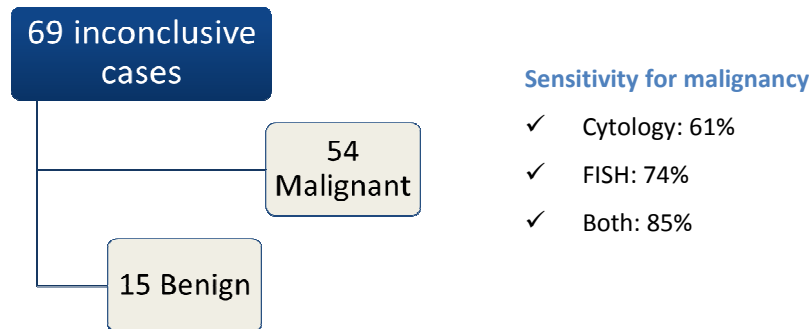


## Endoscopic Ultrasound-guided Fine Needle Aspiration (EUS-FNA)



- EUS-FNA has been increasingly used for the assessment of diverse intra-abdominal tumours.
- EUS-FNA is a key component of the evaluation of both extramural and intramural structures of GI tract, allowing determination of origin of the lesion and cytological sampling.

## EUS-FNA with rescue fluorescence in situ hybridization for the diagnosis of pancreatic carcinoma in patients with inconclusive on-site cytopathology results.



➤ FISH detected an additional 13 cases of pancreatic adenocarcinoma missed by cytology.

Kubiliun N et al. Gastrointest Endosc 2011

- Like urinary samples, a positive pancreatic UFISH result is defined as a significant number of cells with polysomy, trisomy, or loss of the 9p21 locus.
- A polysomic UFISH test result in an FNA sample with atypical cells is virtually diagnostic of malignancy.
- A negative UFISH result does not rule out malignancy but may prompt the search for other causes of a sonographic mass.
- UFISH appears to have high sensitivity and specificity for malignancy but is a supplement test to morphology, not a replacement for it.

## Pancreatic Cystic Lesions

### Ancillary tests

- Amylase level.
- Tumour markers (CEA, CA 125, KOC).
- Molecular techniques (KRAS, P53, LOH)

#### Molecular Analysis of Pancreatic Cyst Fluid

A Comparative Analysis With Current Practice of Diagnosis

Jian Shen, MD, PhD<sup>1</sup>; William R. Brugge, MD<sup>2</sup>; Christopher J. DiMaio, MD<sup>2</sup>; and Martha B. Pitman, MD<sup>1</sup>

Cancer Cytopathology June 25, 2009

- *KRAS*
- LOH
- DNA quantity/quality

#### Molecular Diagnosis

#### Criteria

1. Benign non-mucinous	1. DNA quantity/quality low to moderate; <i>AND</i> 2) <i>KRAS</i> gene point mutation not present; <i>AND</i> 3) LOH, <2 genomic loci
2. Benign mucinous	2. DNA quantity/quality: high; <i>OR</i> 2) <i>KRAS</i> gene point mutation present; <i>OR</i> 3) LOH, ≥2 genomic loci
3. Malignant (in situ or invasive carcinoma)	3. <i>KRAS</i> gene point mutation, high amplitude (>75%); <i>OR</i> 2) ≥2 more LOH, high amplitude (>75%)

## Gastrointestinal Stromal Tumors

- GISTs are frequently discovered on endoscopy performed for other reasons and are characterized by a bulging of the GI wall with intact, normal, overlying mucosa.
- EUS allows identification of the tumor and collection of material for diagnosis and molecular analysis by EUS-guided FNA.
- GISTs are characterized by the presence of activating mutations of CKIT and PDGFR TK receptors. Detection of these mutations are useful for diagnosis confirmation and to predict likelihood of Imatinib response. Exon 11 mutants respond better.



Cancer Cytopathology 2011

### Molecular Analysis of *c-Kit* and *PDGFRA* in GISTs Diagnosed by EUS

Ana L. Gomes, BSc,<sup>1</sup> Ricardo H. Bardales, MD,<sup>2\*</sup> Fernanda Milanezi, MD,<sup>1,3</sup> Rui M. Reis, PhD,<sup>1</sup> and Fernando Schmitt, MD, PhD<sup>1,3,4</sup>

Ann J Gastroenterol 2011; 117:1004-1010  
DOI: 10.1099/mitecs/jebacm11010

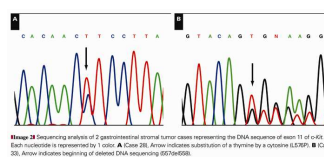
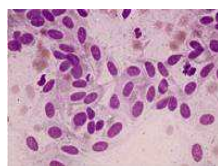


Table 2: Mutation analysis results for *c-Kit* gene in cell-blocks obtained from aspirates

Tumours	<i>c-Kit</i> status				
	Wild type	Exon 9	Exon 11	Exon 13	Exon 17
<b>GIST</b> (n = 33)	42.5 %	3.0 %	57.6 %	-	-
<b>non-GIST</b> (n = 18)	100%	-	-	-	-

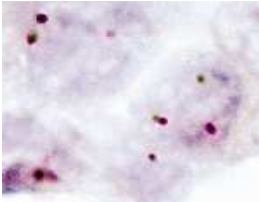
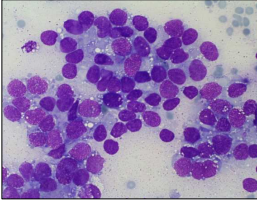
Possible use and role of molecular techniques in fine-needle aspiration cytology (FNAC) practice

Fernando Schmitt  
Helena Barroca

DIAGNOSTIC HISTOPATHOLOGY 17:7 © 2011 Elsevier Ltd.

Chromosomal translocations in soft tissue sarcomas

Tumour type	Cytogenetics
Ewing sarcoma/PNET	t(11;22)(q24;q12) t(21;22)(q22;q21) t(7;22)(p22;q12) t(2;22)(q33;q12) t(17;22)(q12;q12)
Desmoplastic small round cell tumour	t(11;22)(p13;q12)
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21)
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) t(1;13)(p36;q14)
Myxoid liposarcoma	t(12;16)(q13;p11)
Synovial sarcoma	t(X;18)(p11;q11)
Clear cell sarcoma	t(12;22)(q13;q12)
Alveolar soft-part sarcoma	t(X,17)(p11.2;q25)
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)
Congenital fibrosarcoma/Mesoblastic nephroma	t(12;15)(p13;q25)



t(11;22)(q24;q12)

Ewing Sarcoma/PNET

MDM2 amplification in FNA of dedifferentiated liposarcoma

CYTOJOURNAL 2010; 7: 5.

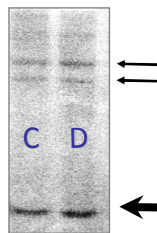
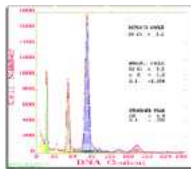
Second TASTE Workshop, 20th April 2013

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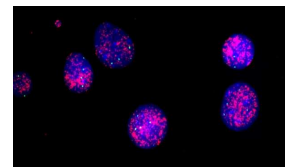
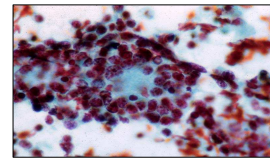
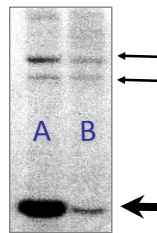
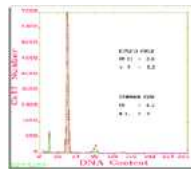


## NEUROBLASTOMAS

Aneuploid  
N-myc amplification -  
Good response to therapy



Diploid  
N-myc amplification +  
1p deletion  
Limited response to therapy



Barroca H, Schmitt F. Acta Cytol 45: 169-72, 2001

### Molecular techniques in cytopathology practice

F C Schmitt,<sup>1,2</sup> A Longatto-Filho,<sup>3,4</sup> A Valent,<sup>5</sup> P Vielh<sup>5</sup>

*J Clin Pathol* 2008;**61**:258-267. doi:10.1136/jcp.2006.044347

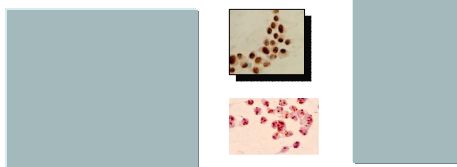
### Possible use and role of molecular techniques in fine-needle aspiration cytology (FNAC) practice

Fernando Schmitt  
Helena Barroca

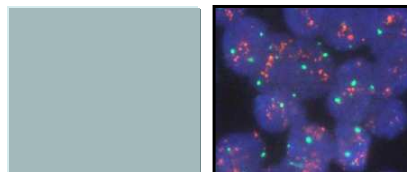
DIAGNOSTIC HISTOPATHOLOGY 17:7 © 2011 Elsevier Ltd.

***In breast cancer, molecular cytopathology can be used***

#### • In primary tumours



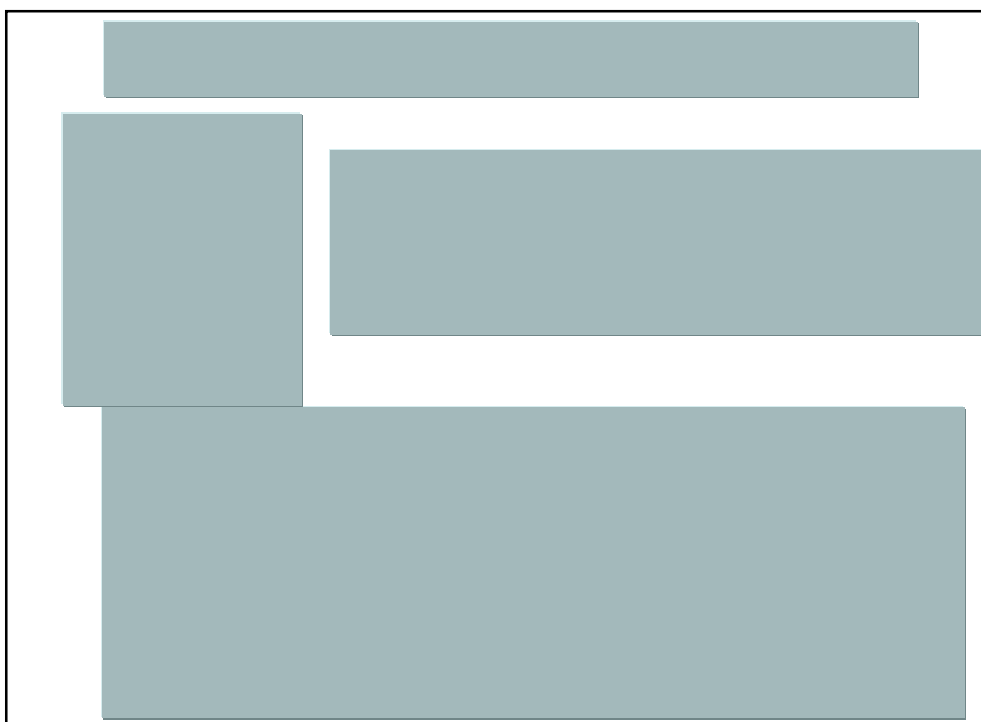
#### • In metastatic tumours



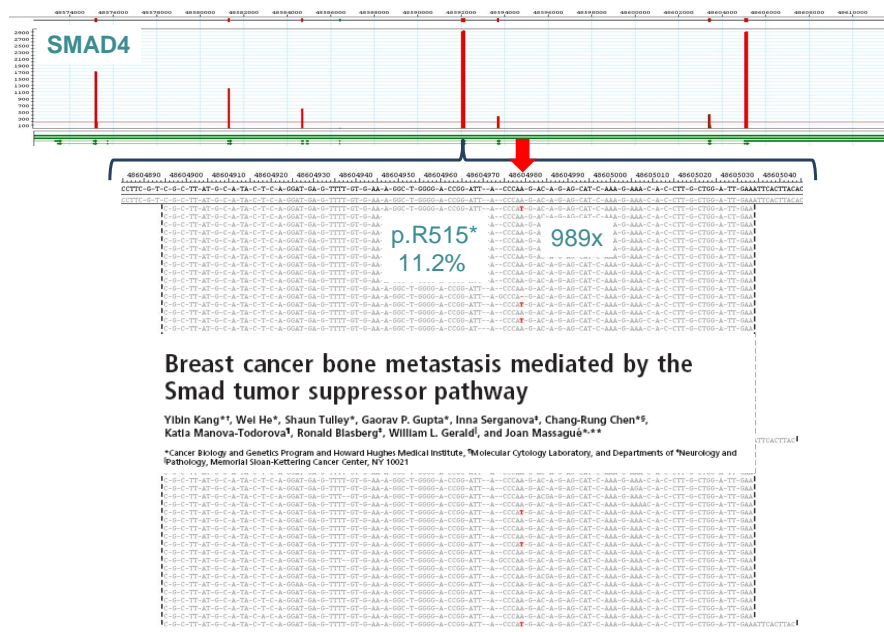
**Predictive Biomarkers and Personalized Medicine****Evaluation of a 30-Gene Paclitaxel, Fluorouracil, Doxorubicin, and Cyclophosphamide Chemotherapy Response Predictor in a Multicenter Randomized Trial in Breast Cancer**

Adel Tabchy<sup>1</sup>, Vicente Valero<sup>1</sup>, Tatiana Vidaurre<sup>5</sup>, Ana Lluch<sup>6</sup>, Henry Gomez<sup>5</sup>, Miguel Martin<sup>6</sup>, Yuan Qi<sup>2</sup>, Luis Javier Barajas-Figueroa<sup>7</sup>, Eduardo Souchon<sup>4</sup>, Charles Coutant<sup>1</sup>, Franco D. Doimi<sup>5</sup>, Nuha K. Ibrahim<sup>1</sup>, Yun Gong<sup>3</sup>, Gabriel N. Hortobagyi<sup>1</sup>, Kenneth R. Hess<sup>2</sup>, W. Fraser Symmans<sup>3</sup>, and Lajos Pusztai<sup>1</sup>

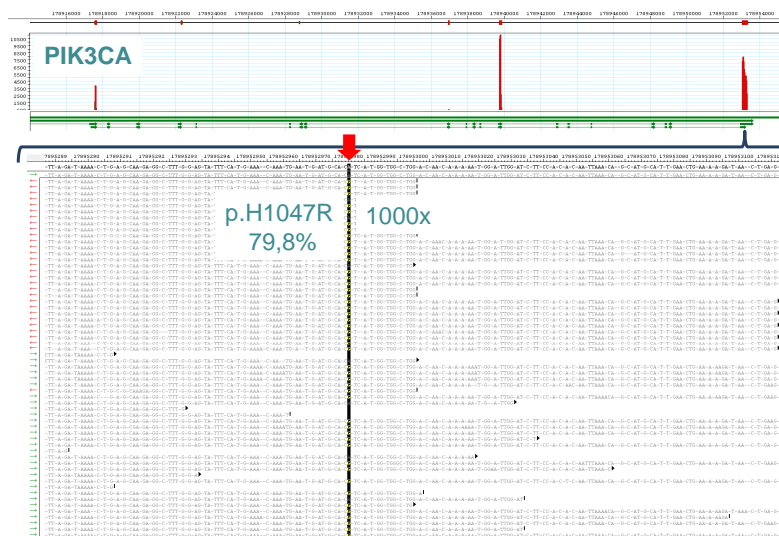
- 273 patients were randomly assigned to receive either paclitaxel followed by FAC (n=138), or FAC x 6 (n=135) neoadjuvant chemotherapy. All patients underwent a **pretreatment FNA** of the tumor for gene expression profiling and treatment response prediction.
- Gene expression profiling for prospective response prediction was feasible in this international trial. The 30-gene predictor can identify patients with greater sensitivity to T/FAC chemotherapy.

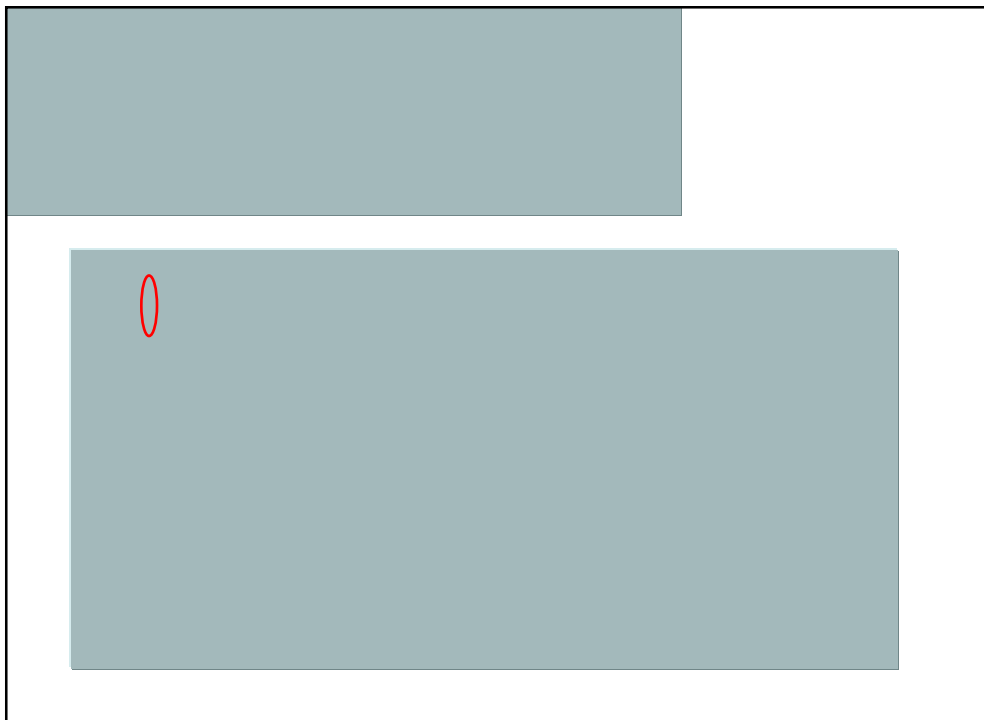


Ion Torrent PGM in the diagnostic setting

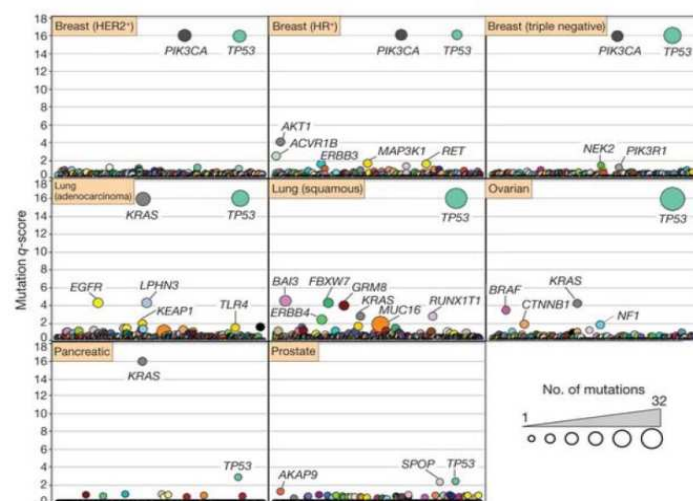


## This case





## Patterns of significantly mutated genes across common cancer subtypes



Kan et al, Nature 2010

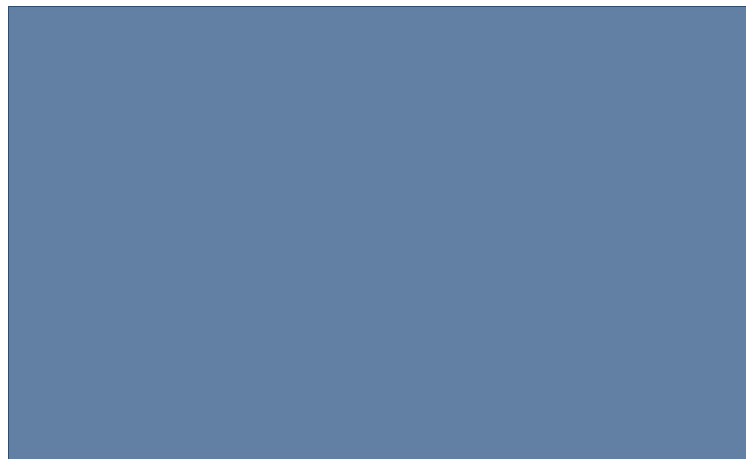
## METASTATIC DISEASE? AND NOW Be sure to treat **the** present disease



**Primary BC**  
**HER-2 negative**

**FNA from liver metastases HER-2 positive**

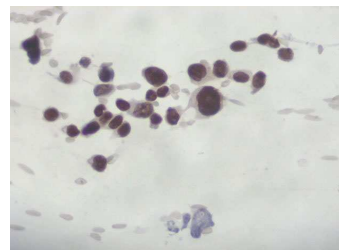
Schmitt FC, unpublished data, 2012



## ER/PR ASSESSMENT IN BREAST FNAs

### Estimation of Hormone Receptor Status in Fine-Needle Aspirates and Paraffin-Embedded Sections From Breast Cancer Using the Novel Rabbit Monoclonal Antibodies SP1 and SP2

Guillermo Cano, M.D.,<sup>1</sup> Fernanda Milanezi, M.D.,<sup>2</sup> Dina Leitão, B.Sc.,<sup>2,3</sup>  
 Sara Ricardo, B.Sc.,<sup>2</sup> Maria José Brito, M.D.,<sup>1</sup>  
 and Fernando Carlos Schmitt, M.D., Ph.D.,<sup>2,3\*</sup>

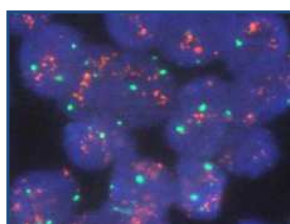


Diagn. Cytopathol. 2003;29:207–211.

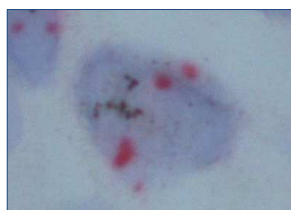
**Table III.** Accuracy, Sensitivity, and Specificity of Estrogen Receptor (ER) Immunocytochemical Assay Using the Rabbit Monoclonal Antibody SP1 in Fine-Needle Aspirate Specimens (FNA) and Formalin-Fixed Specimens (FF) Compared With ER Detection Using the Mouse Monoclonal Antibody 6F11 in Formalin-Fixed Specimens (FF)

Reaction	FF-6F11 N (%)		
	Accuracy	Sensitivity	Specificity
FNA-SP1	38/40 (95)	22/24 (91.7)	16/16 (100)
FF-SP1	40/40 (100)	24/24 (100)	16/16 (100)

## ISH FOR HER2 IN BREAST FNA



- ISH can be performed successfully in the majority of cases on archival cytological slides, and the results are reliable and accurate.



- Good concordance between HER-2 amplification in FNA samples and whole histological sections, using single or dual probes.

Gu M *et al.* Acta Cytol 2005  
 Ricardo S *et al.* J Clin Pathol 2007



## FISH studies comparing primary breast cancers and their matched distant metastases

Source	Patients (n)	Discordance between primary and metastases	Type of Material
Gancberg et al., 2002	68	7%	Histology
Bozetti et al., 2003	14	0	Histology
Houssanni et al., 2011	105	7.6%	Histology
Wilking et al., 2011	147	9.5%	FNA
Schmitt et al., 2012	30	10%	FNA

## And now?

*HER2 status should be repeated, especially if unknown, orig*

## *KRAS and BRAF mutation analysis can be reliably performed on aspirated cytological specimens of metastatic colorectal carcinoma*

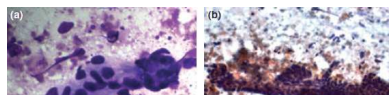
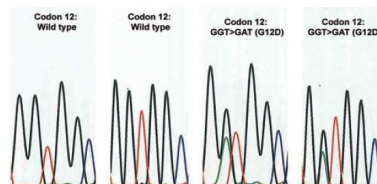


Table 2. Clinical findings and summary of comparative mutation results

No.	Race, gender and age	Cytology specimen type and location	Source of paired histological sample	Location of paired histological sample	Mutation analysis of <i>KRAS</i> codons 12,13 and 61 and <i>BRAF</i> codon 609	
1	Chinese male/68	FNA lung	Primary tumour	Sigmoid colon	Cytology – WT for <i>KRAS</i>	Histology – G12D for <i>KRAS</i>
2	Chinese female/71	FNA lung	Primary tumour	Rectal	Cytology – WT for <i>KRAS</i> and <i>BRAF</i>	Histology – WT for <i>KRAS</i> and <i>BRAF</i>
3	Chinese female/60	FNA lung	Primary tumour	Rectal	Cytology – WT for <i>KRAS</i> and <i>BRAF</i>	Histology – WT for <i>KRAS</i> and <i>BRAF</i>
4	Chinese male/72	FNA lung	Primary tumour	Sigmoid colon	Cytology – G12C for <i>KRAS</i>	Histology – G12C for <i>KRAS</i>
5	Chinese female/91	FNA lung	Primary tumour	Rectal	Cytology – G12D for <i>KRAS</i>	Histology – G12D for <i>KRAS</i>
6	Chinese female/53	FNA liver	Primary tumour	Ascending colon	Cytology – G12D for <i>KRAS</i>	Histology – G12D for <i>KRAS</i>
7	Chinese female/70	FNA intra abdominal mass	Resected metastasis	Peritoneal nodule	Cytology – WT for <i>KRAS</i> and <i>BRAF</i>	Histology – WT for <i>KRAS</i> and <i>BRAF</i>
8	Chinese male/58	FNA supra-clavicular lymph node	Primary tumour	Sigmoid colon	Cytology – WT for <i>KRAS</i> and <i>BRAF</i>	Histology – WT for <i>KRAS</i> and <i>BRAF</i>

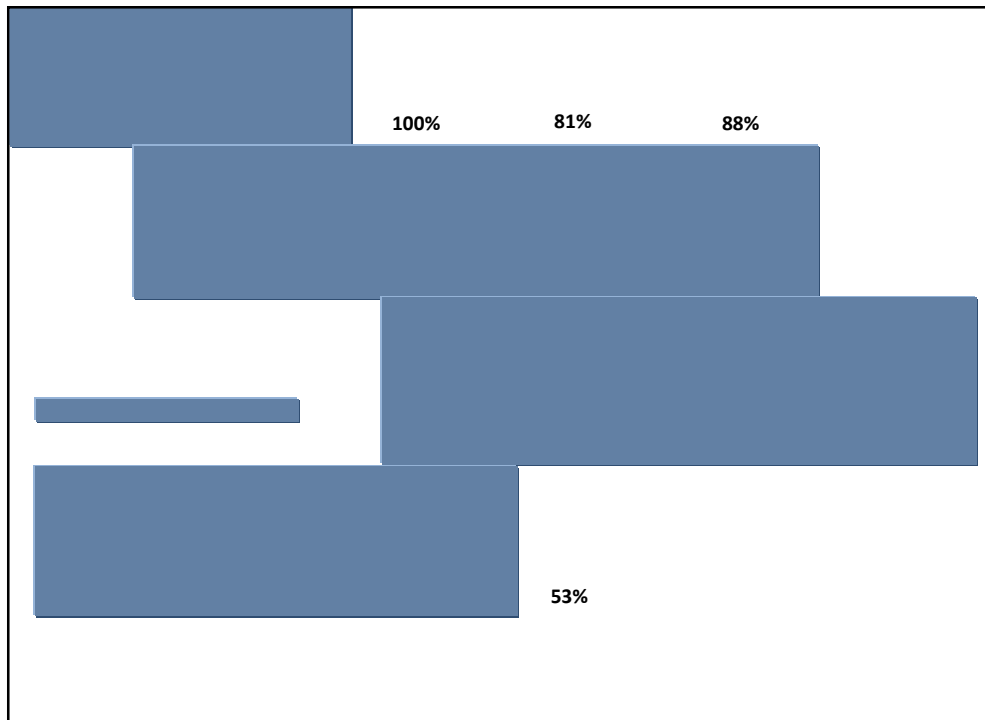
FNA, fine needle aspiration; WT, wild type.



Pang N *et al.* Cytopathology 2011

## *Metastatic melanoma Significant unmet medical need*

- Melanoma is a tumour that is frequently diagnosed in a metastatic site by FNA.
- These patients have a poor prognosis (8-10 month median overall survival).
- In the last 3 decades there were no advances in therapy.
- 50% of melanoma cases have BRAF mutation and recently a inhibitor (RG7204) was described and tested with success.
- BRAF mutation testing will be a routine in melanoma cases.
- FNA is a suitable method to obtain melanoma cells from metastatic sites for test BRAF.



## Conclusions

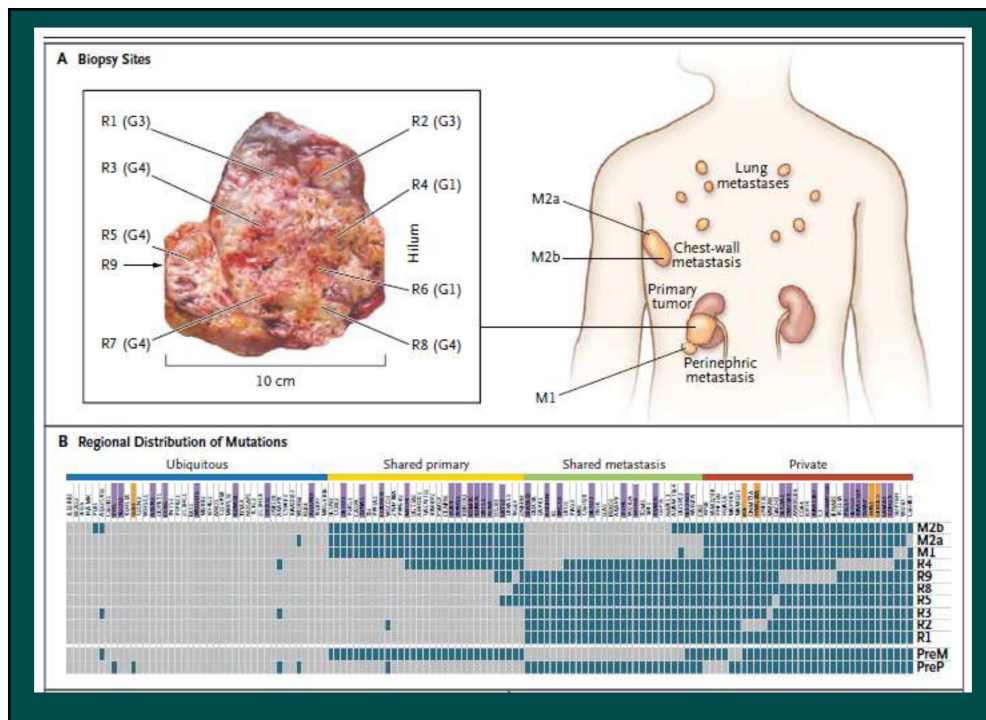
- Small samples obtained by minimally invasive methods exploited morphological and molecular information are the model biopsies of the future.
- Various techniques using different types of cytological samples and preparations have shown promising results, with similar or higher accuracy and sensitivity when compared with surgical specimens.
- Presently, with the rapid development of personalized treatment, tumors should be tested for available biomarkers and every effort should be made to spare the tissue for molecular testing.



The Second TASTE Workshop, Brussels 2013

## **"2D and 3D" large-format breast pathology**

Tibor Tot  
Uppsala University  
Laboratory Medicine Dalarna  
Sweden



## IMPAKT Meeting Brussels 2012

- Intratumoral genetic heterogeneity is so obvious that it may compromise individual therapy efforts.
- There is no genetic test with clinical utility.
- The efforts in molecular classification of breast cancer have failed; ER and HER2 are the only clinically useful biomarkers.

## Breast cancer 2013

- **Morphological parameters will remain essential in the era of molecular pathology.**

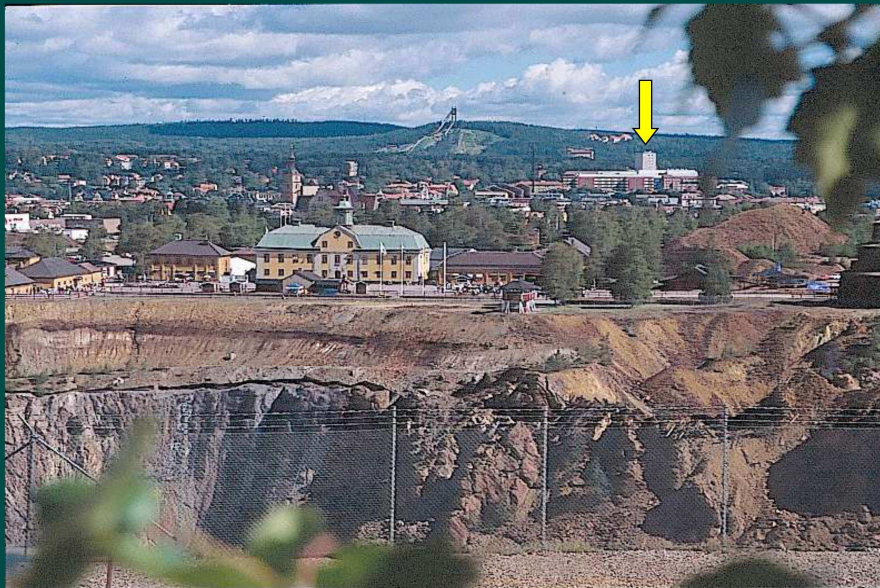
## Breast cancer 2013

- **Early breast cancer is the most common cancer stage in countries with ongoing screening.**
- These tumors tend to have favourable molecular characteristics, but
- **complex subgross morphology.**



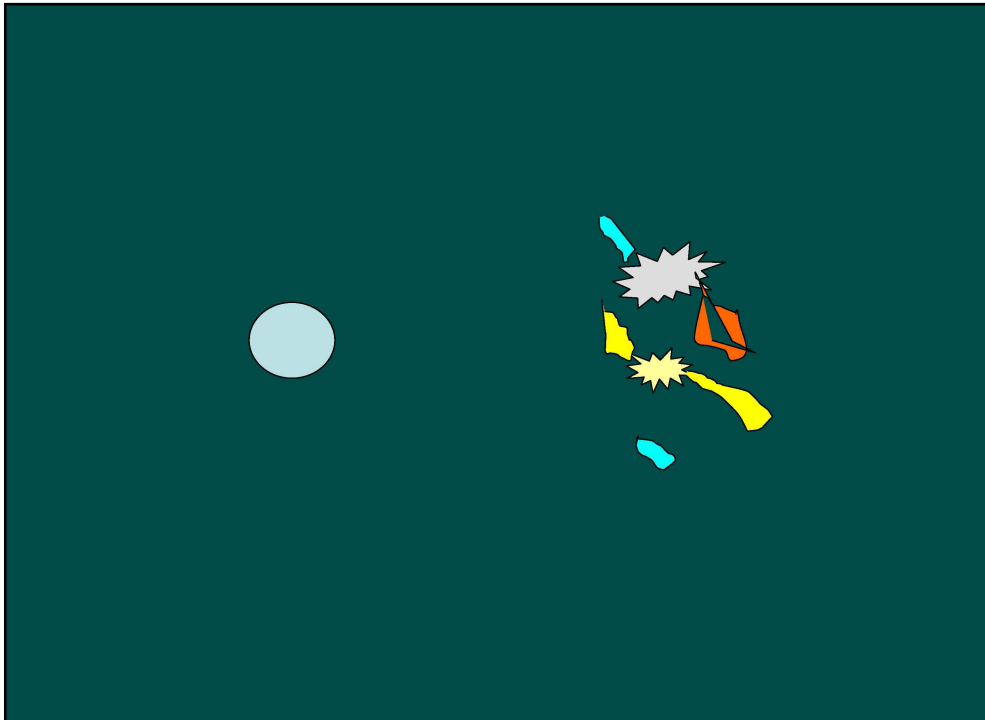
## Breast cancer 2012

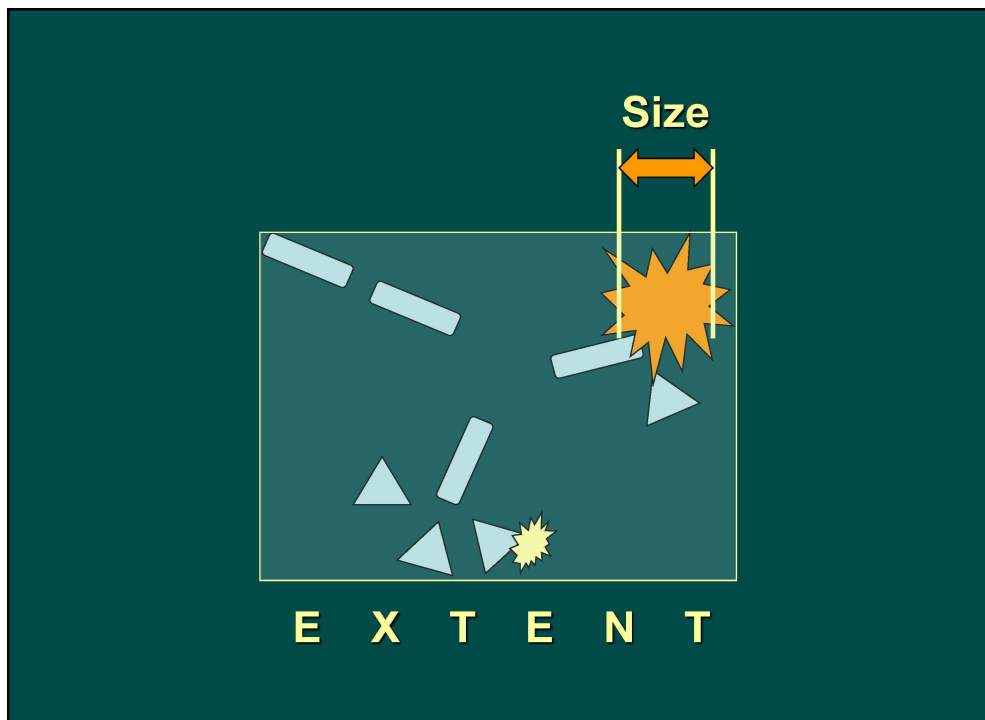
- **Proper characterization of early breast cancer is still based on assessing "classical" pathological parameters, not on assessing genetic portraits.**





**Radiological – pathological correlation is essential  
in diagnosing breast carcinoma**



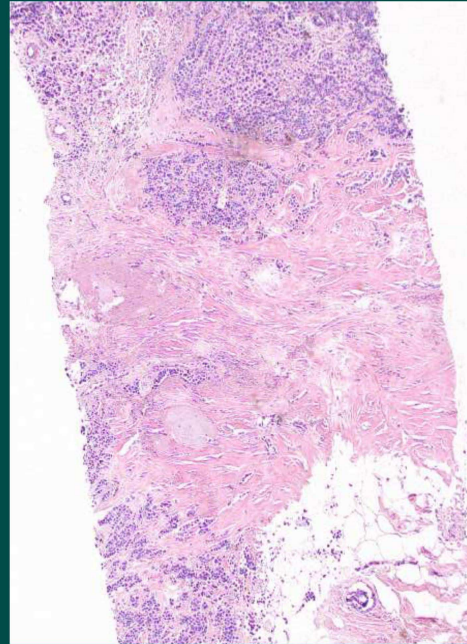
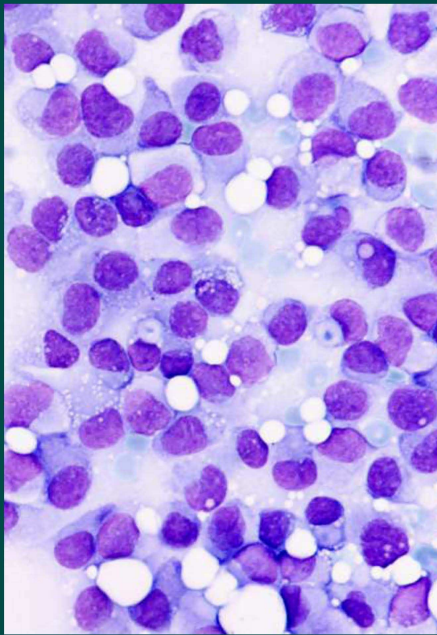


When describing malignant lesions in the breast, the following morphologic parameters should be listed (independent of the used imaging method):

- the distribution of the lesions (as unifocal, multifocal or diffuse) separately for invasive and in situ lesions,
- the extent of the disease (representing the whole area including all the invasive, in situ and intravascular malignant structures),
- the size of the tumor corresponding to the largest diameter of the largest individual invasive tumor focus,
- evidence for intratumoral or intertumoral heterogeneity.

## Practical approach: preop

- The need for neoadjuvant therapy
- Type of surgical intervention:  
the breast
- Type of surgical intervention:  
the axilla

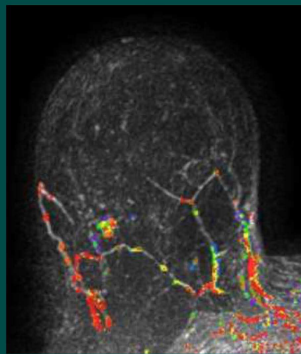




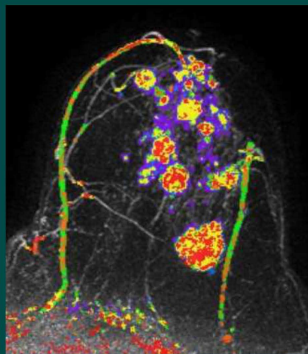
**It is far not sufficient  
to preoperatively verify malignancy;**

**lesion distribution,  
disease extent,  
localization,  
tumor size,  
tumor stage**

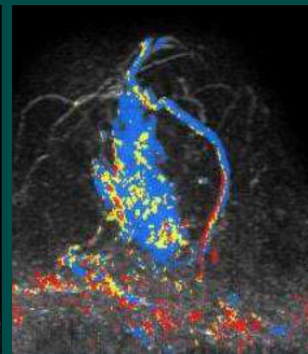
**should also be assessed  
for adequate therapeutic decision**



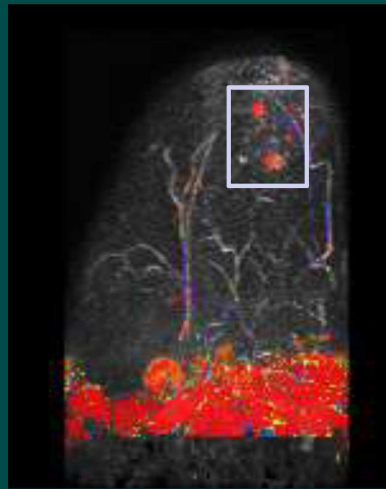
Unifocal



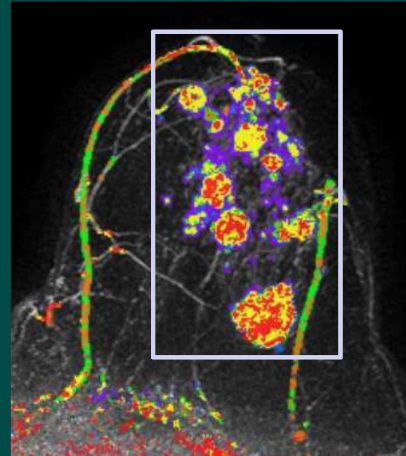
Multifocal



Diffuse



Limited extent &lt; 4 cm

Extensive  $\geq 4$ cm

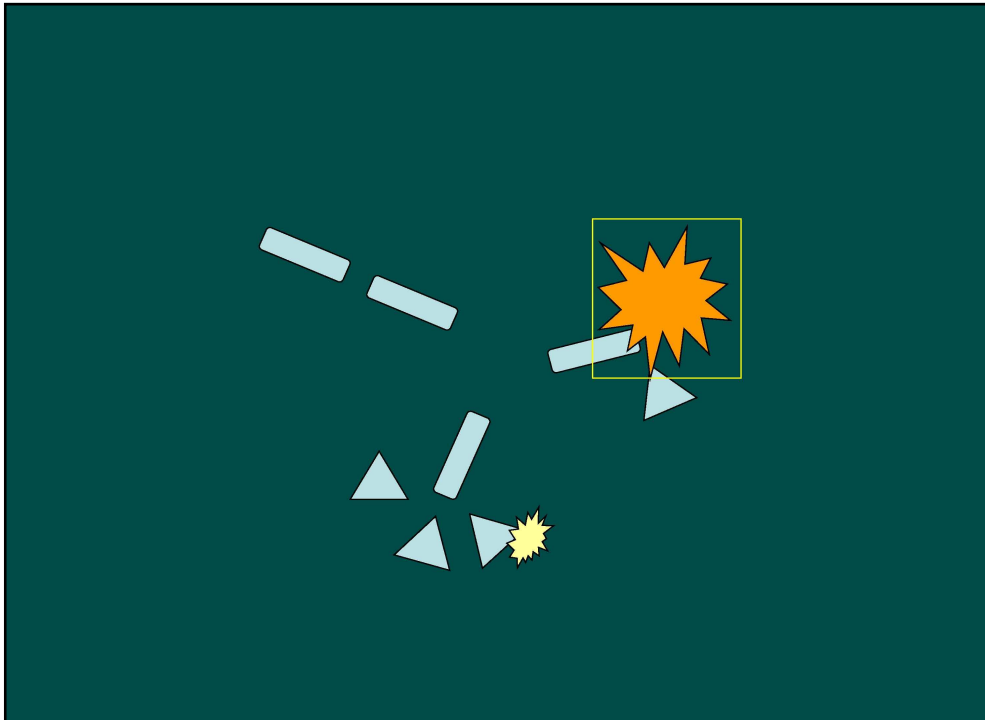
## Decisive factors for breast conservation

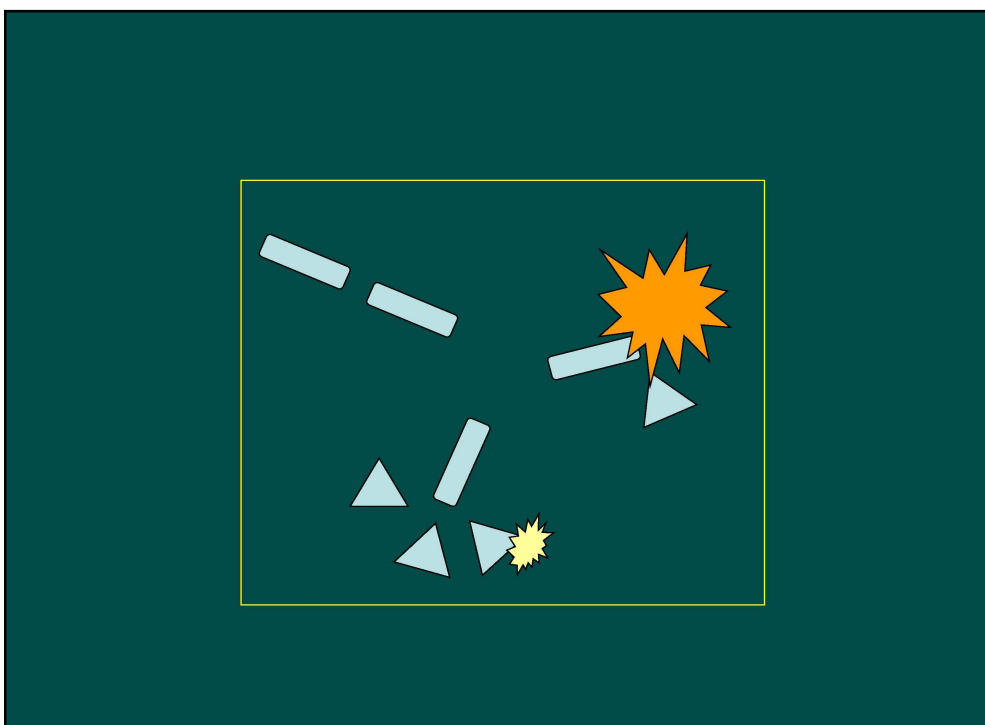
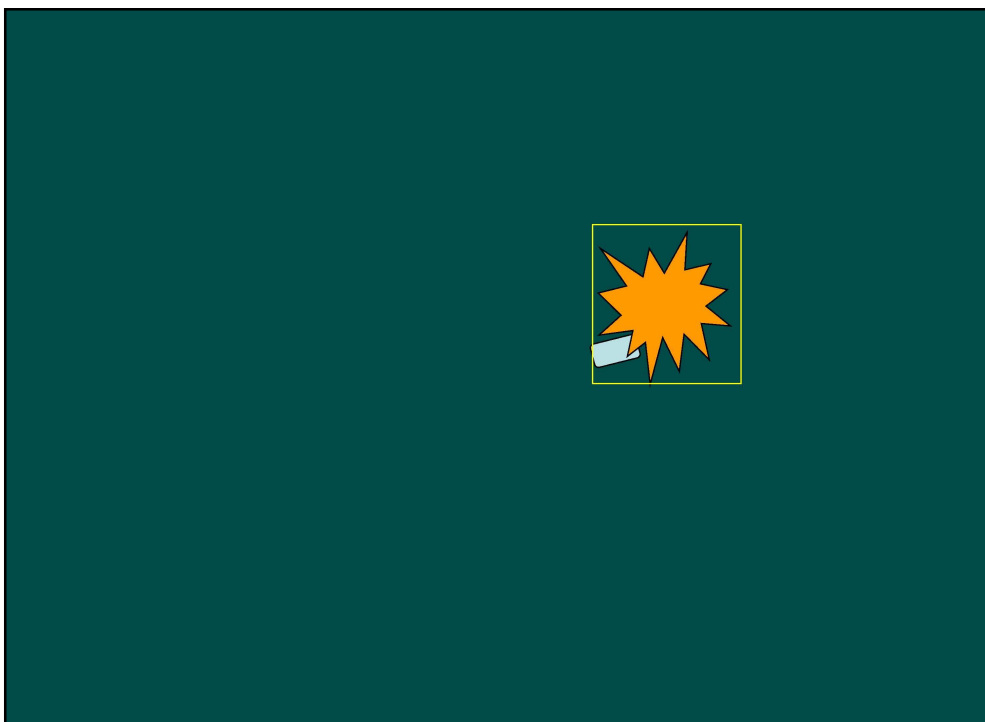
- **Disease extent**
  - **Tolerability of radiotherapy**
  - **Contraindications**
  - **Patient's preference**
- 
- + **Localisation of the lesion**
  - + **Breast volume**



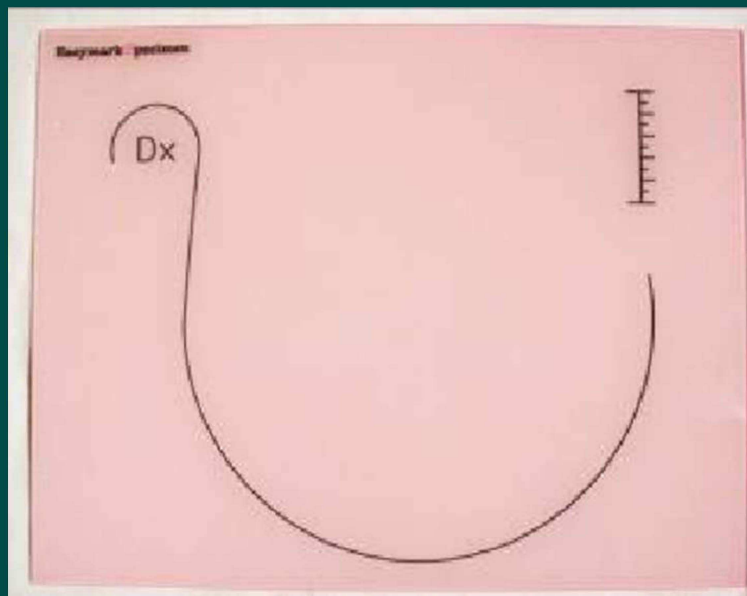
## Practical approach: postop

- Check the correctness of the preoperative findings
- Check the correctness/effects of the treatment
- Provide prognostic parameters
- Provide predictive parameters

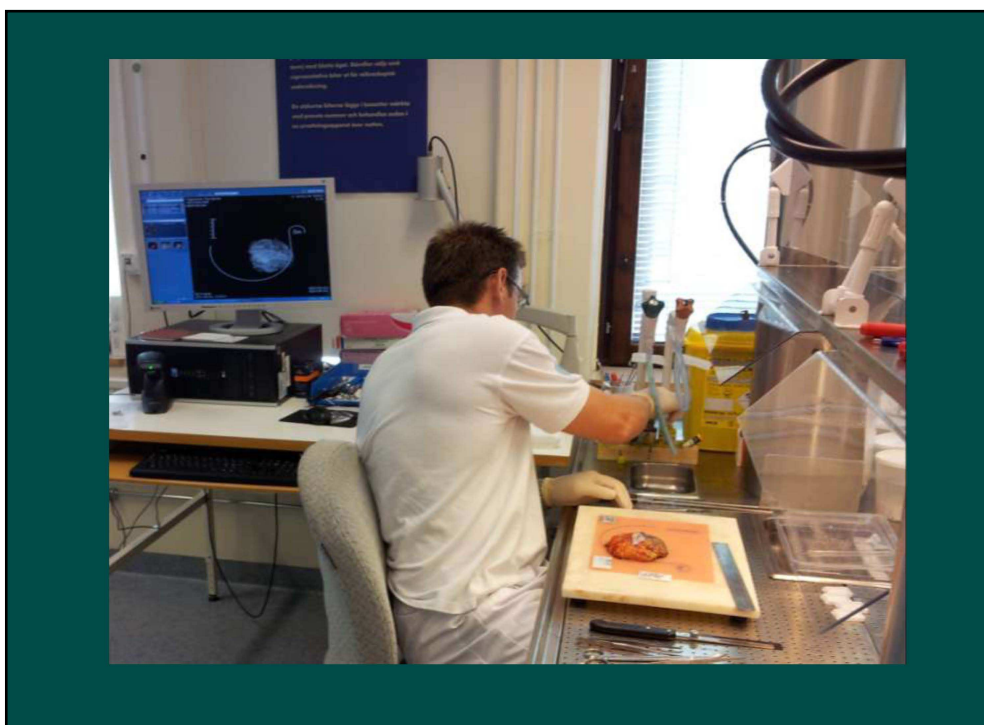
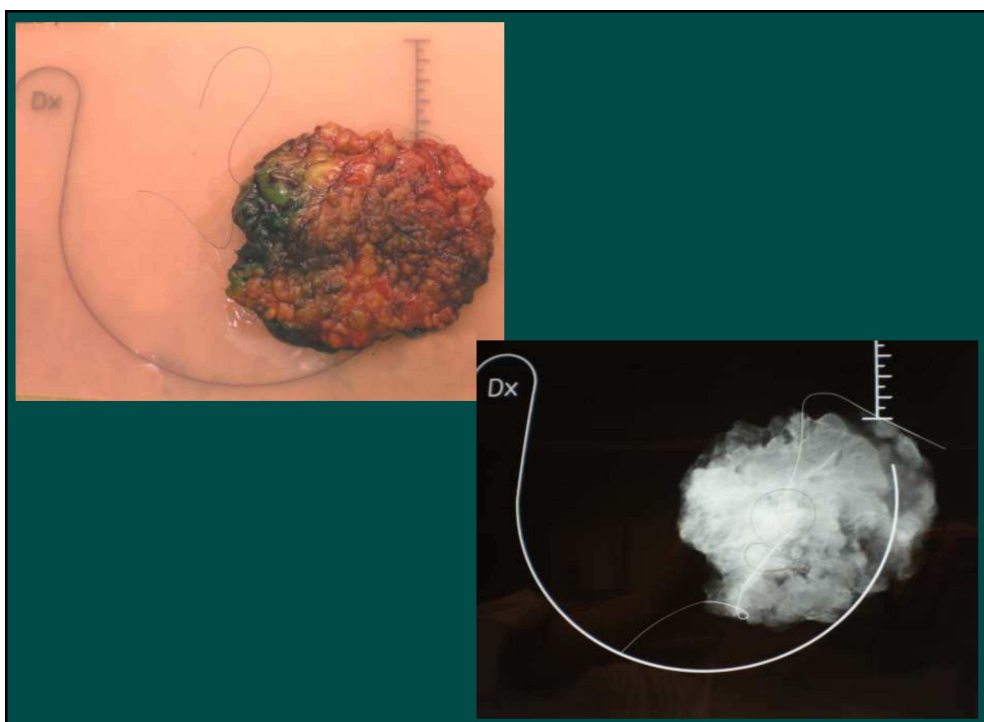


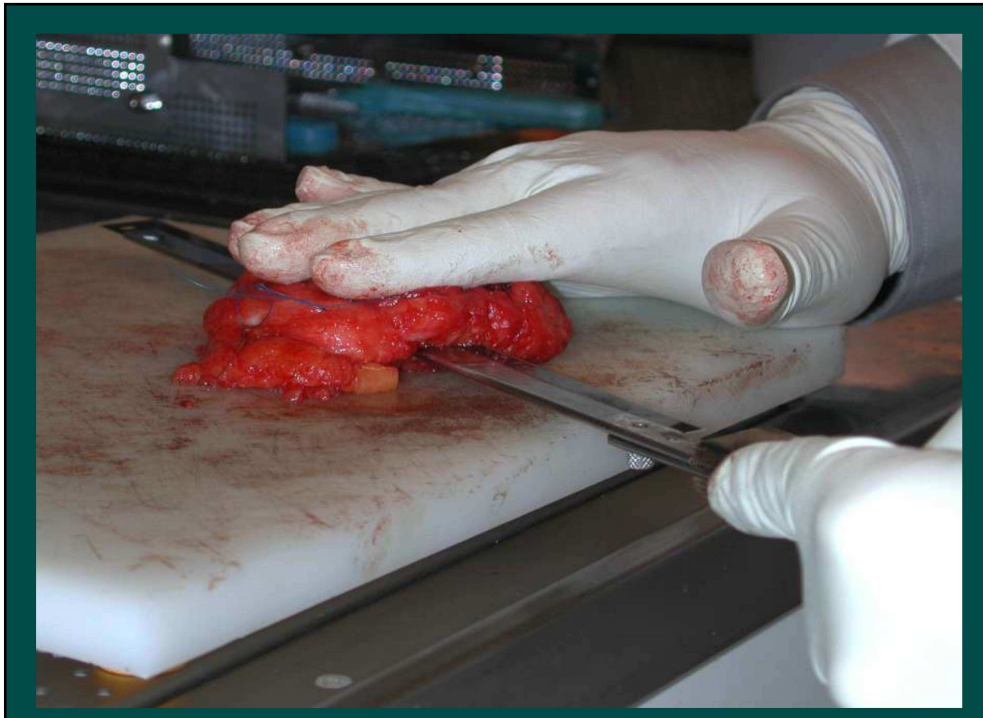


## Large section histology

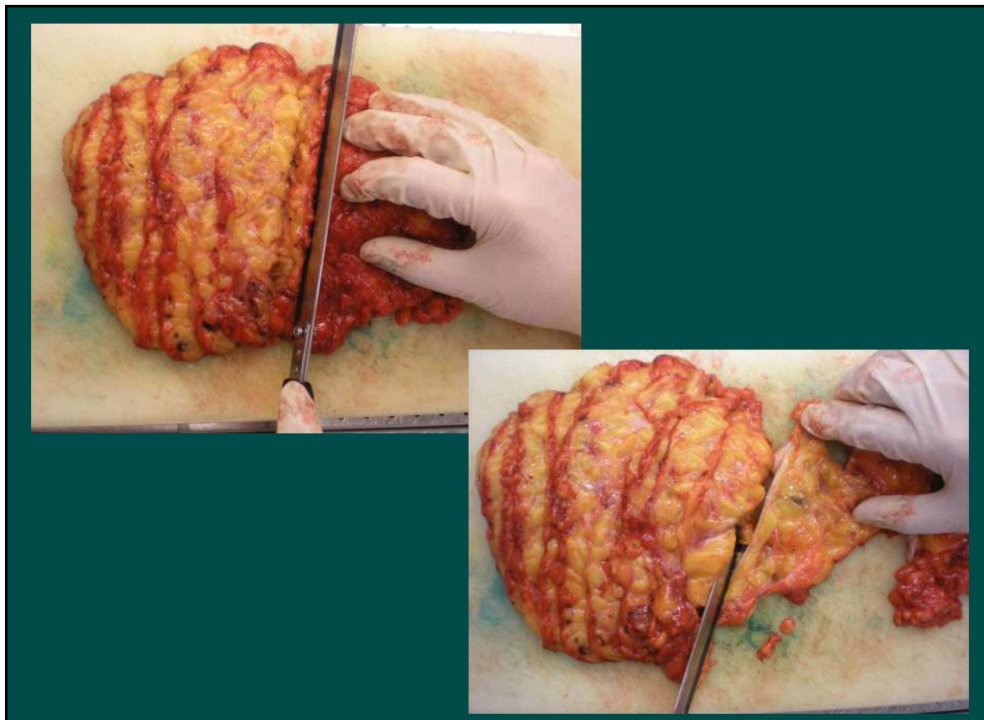


The EasyMarkSpecimen® accessory ("Dieterloop")

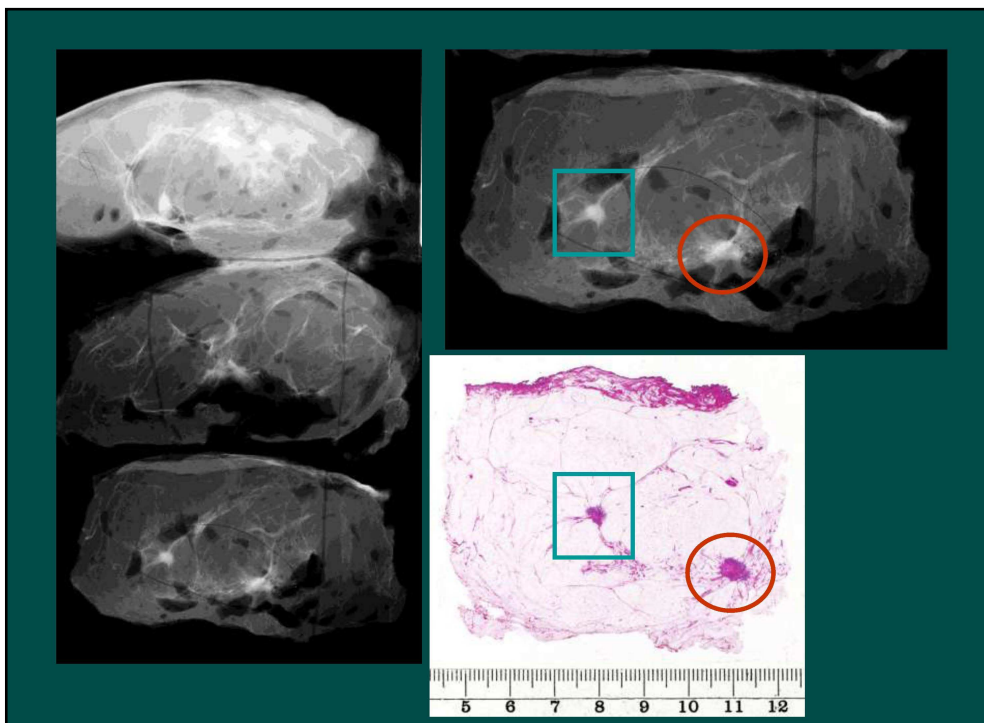
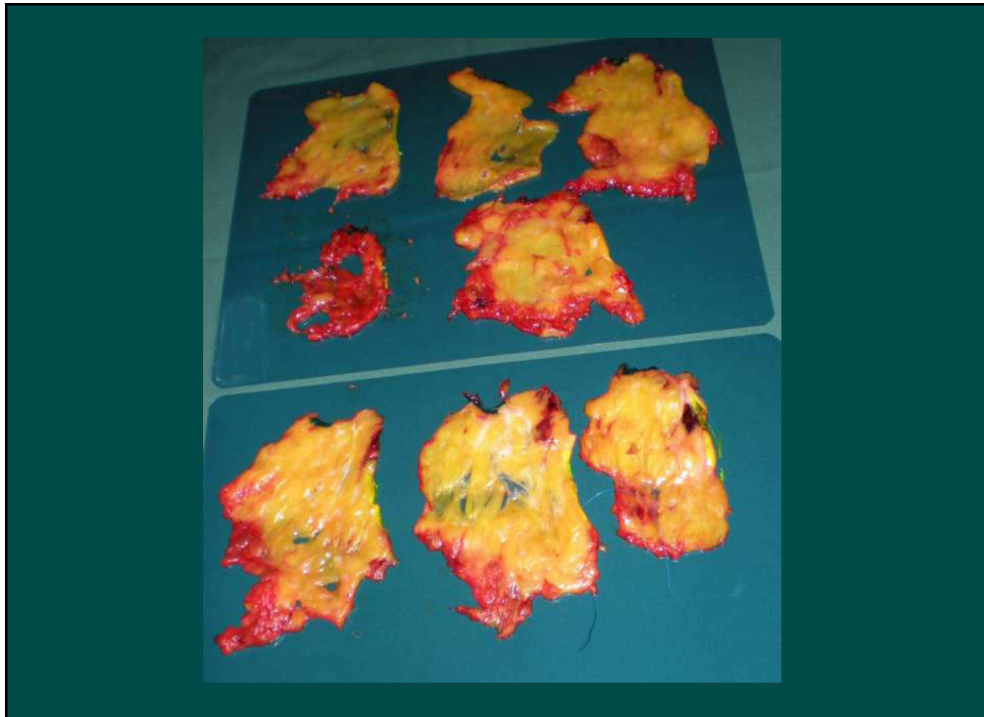






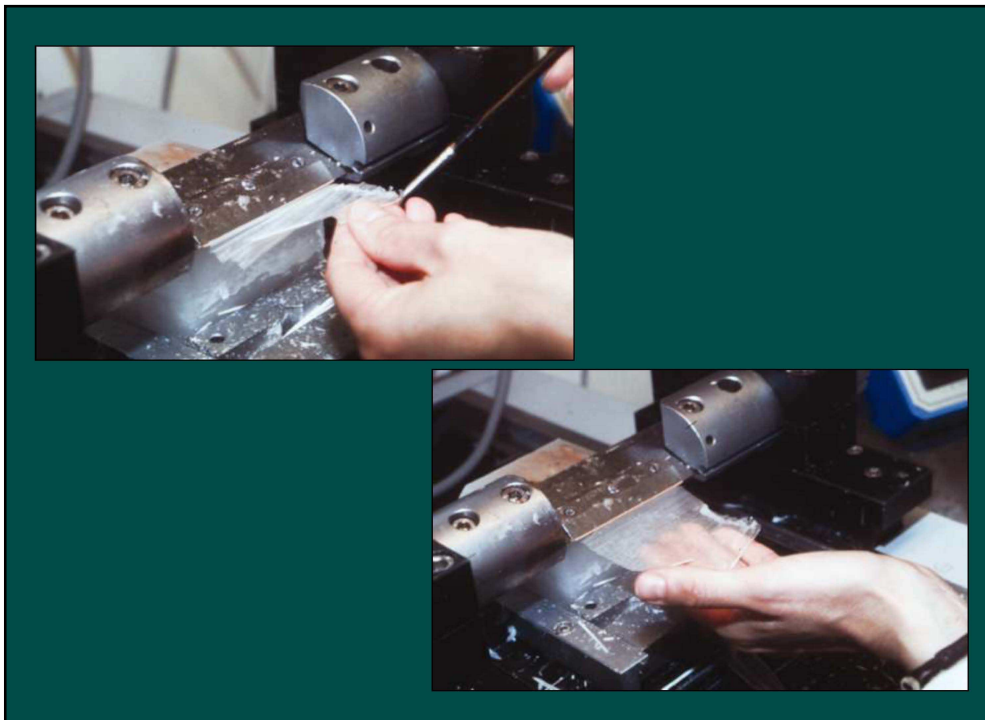




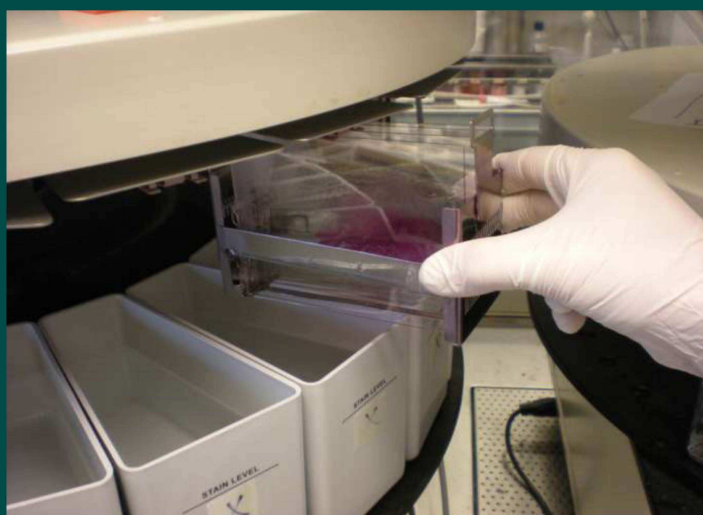




















Visualising 80 cm<sup>2</sup> tissue surface:  
 2 large sections blocks = 5 megacassetts = 45 traditional blocks

	Minimal Conventional sampling, 7 small blocks	Average Conventional sampling, 12 small blocks	“Optimal” Conventional sampling, 22 small blocks	Average large-section case 1.6 large blocks in 3 levels + 3 small blocks
Maximum surface area, cm <sup>2</sup>	35	60	110	110
Typing and grading	Standard	Standard	Standard	Standard
Receptor status	Standard	Standard	Standard	Standard
Documenting Histol. tumor size	Possible in 50% of cases	Often	Standard	Standard
Assessing disease extent	Not possible	Not possible	Standard	Standard
Assessing lesion distribution	Not possible	Often	Standard	Standard
Assessing surgical Margins	Partial	Partial	Complete in one plane	Complete in one plane

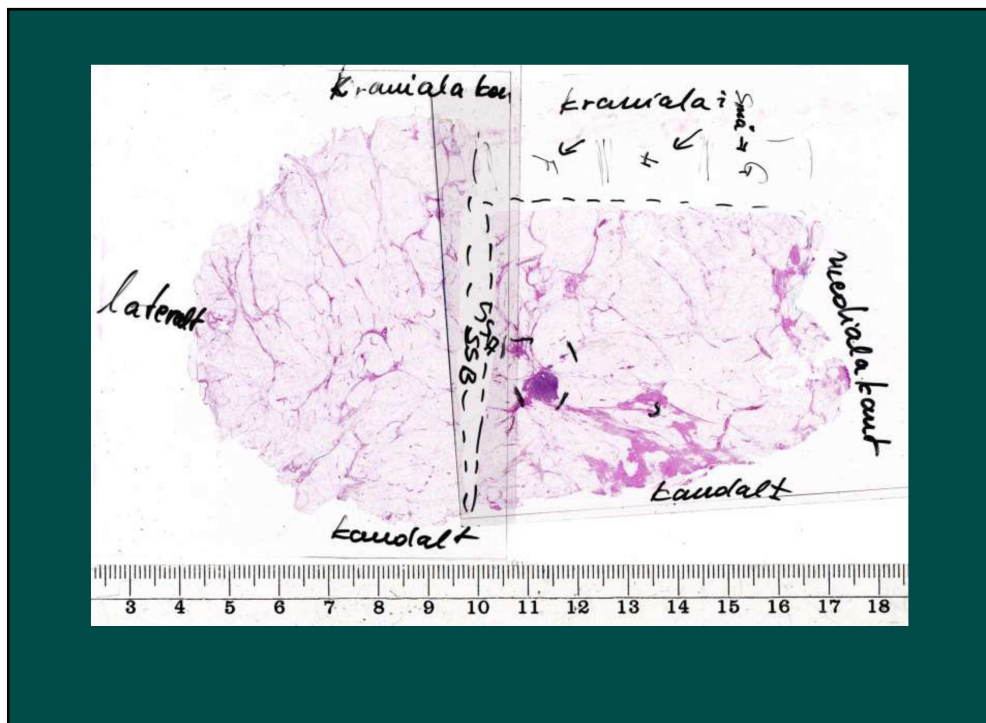
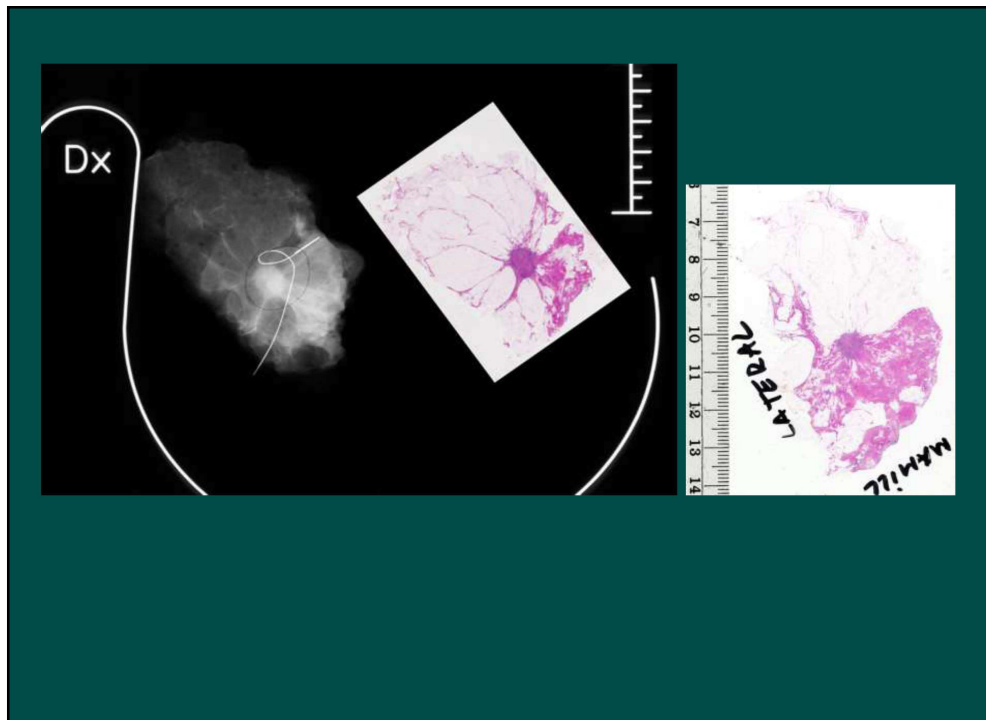
	<b>Minimal</b> Conventional sampling, 7 small blocks	<b>Average</b> Conventional sampling, 12 small blocks	<b>“Optimal”</b> Conventional sampling, 22 small blocks	Average large-section case 1.6 large blocks in 3 levels + 3 small blocks
Radiological- Pathological correlation	Not possible	Not possible	<b>Very Complicated</b>	<b>Standard</b>
Cost of consumables, USD	16.4	30.4	<b>55.7</b>	<b>39.5</b>
Personnel time per case, min	38.5	66	<b>209</b>	<b>85.3</b>

**Tot T. Cost-Benefit Analysis of Using Large-Format Histology Sections in Routine Diagnostic Breast Care. The Breast, 2010**

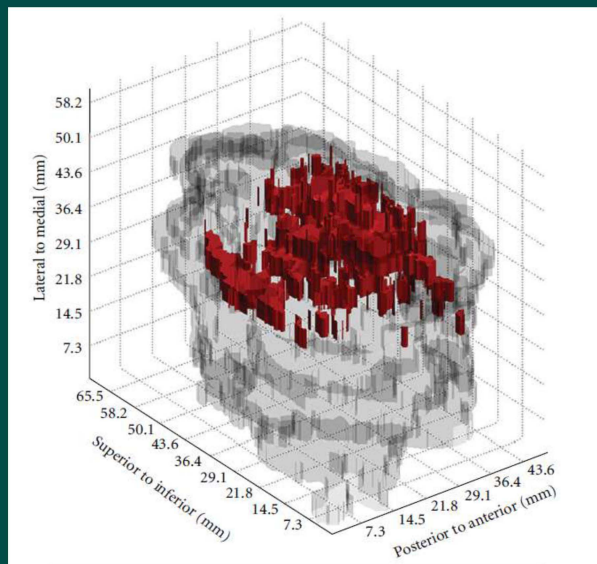
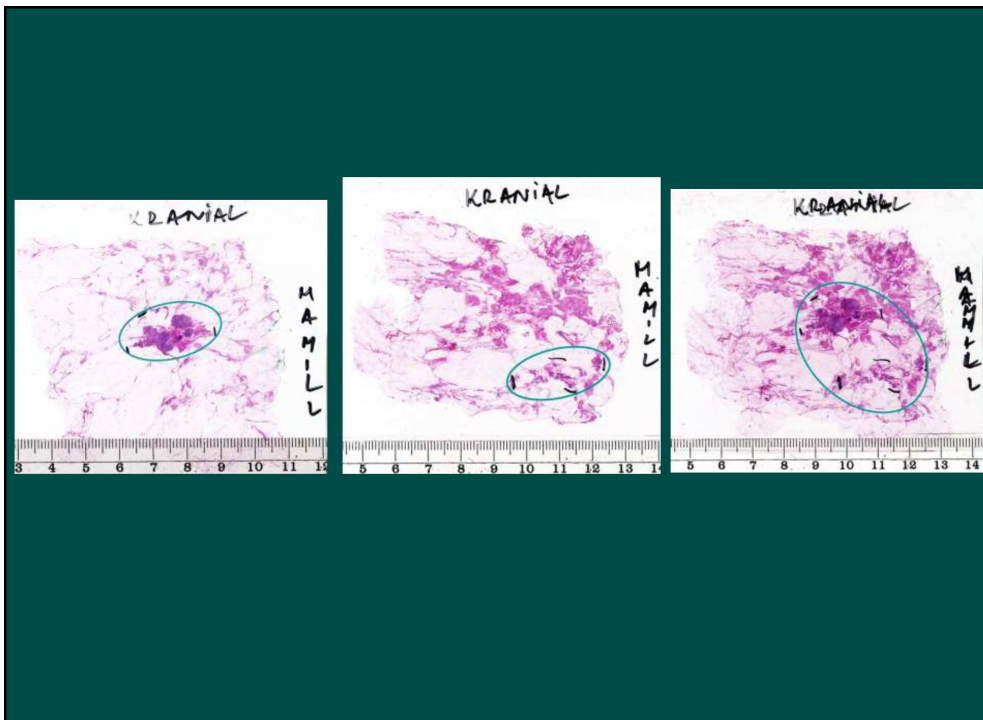
### Direct labor and material expense per finished slide, excluding amortization

- **0.4 USD/ cm<sup>2</sup>**, for Conventional slides
- **0.48 USD/cm<sup>2</sup>**, for Large-format slides

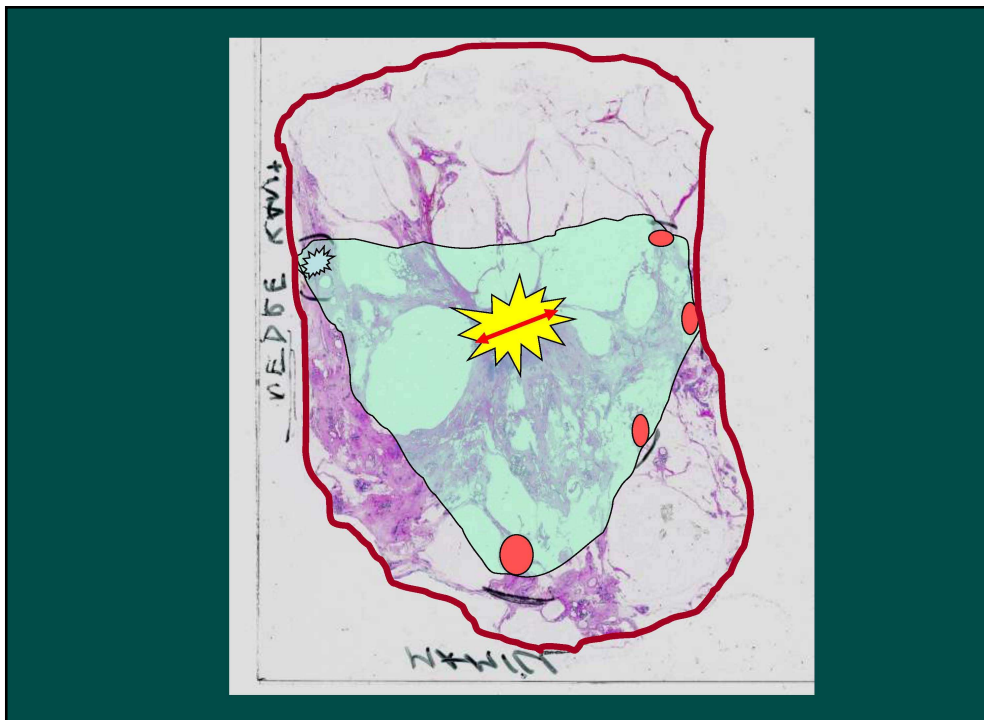
Results of F Lee Tucker, Virginia Biomedical Laboratories, USA







G. M. Clarke, M. Murray, C. M. B. Holloway, K. Liu, J. T. Zubovits, and M. J. Yaffe  
3D Pathology Volumetric Technique: A Method for Calculating  
Breast Tumour Volume from Whole-Mount Serial Section Images  
Int J Breast Cancer Special Issue 2012





### **A single large section may document**

- the tumor size
- the extent of the disease
- the distribution of the lesions
- intratumoral and intertumoral heterogeneity
- the surgical margin **at the same time,**

**which all can be directly correlated to the mammogram and specimen radiogram.**



Jackson PA, Merchant W, McCormick CJ, Cook MG. A comparison of large block macrosectioning and conventional techniques in breast pathology. Virchows Arch 425, 243-8, 1994.

	<b>Large format n = 100</b>	<b>Conventional n = 111</b>
<b>Reliable measurement of invasive component</b>	<b>100%</b>	<b>63%</b>
<b>In situ carcinoma present</b>	<b>80%</b>	<b>64%</b>
<b>Extent of the disease</b>	<b>larger</b>	<b>smaller</b>
<b>Concurrent carcinoma</b>	<b>20%</b>	<b>13.4%</b>

#### **Summary of Limitations of Conventional Pathologic Technique in the Diagnosis and Reporting of Breast Carcinomas in Breast-Conserving Surgical Specimens**

- Gross inspection and palpation has insufficient sensitivity to guide section submission of imaging - only detected neoplasia, including DCIS and multifocal invasive carcinoma
- Complete imaging data, including MRI features are not available to pathologists at time of specimen evaluation
- Spatial 3-D integrity of the specimen is lost through sectioning, section submission and histopathologic examination
- Margin evaluation is often reliant on gross inspection and palpation, even for imaging-only detected disease
- Lack of standardization of methods to measure DCIS extent, multifocality, margins
- Suboptimal case correlation between pathology and pre-surgical imaging studies

F Lee Tucker, Virginia Biomedical Laboratories, USA

DCIS extent and multifocality of invasive cancer. Comparison of 462 Consecutive Breast Conserving Surgical Specimens. Conventional Pathology without MRI (CP) vs Large Format Breast Pathology with MRI (LBP).

<b>DCIS Extent, mm</b>	<b>CP n=250 median (range)</b>	<b>LBP n=212 median (range)</b>
Histopathology	6.1 (1-24)	29.1 (1-125)
Mammography	11.0 (0-69)	12.0 (1-68)
MRI	not performed	27.0 (2-113)
<b>Multifocality, invasive cancer</b>	<b>12%</b>	<b>38%</b>

Results of F Lee Tucker, Virginia Biomedical Laboratories, USA

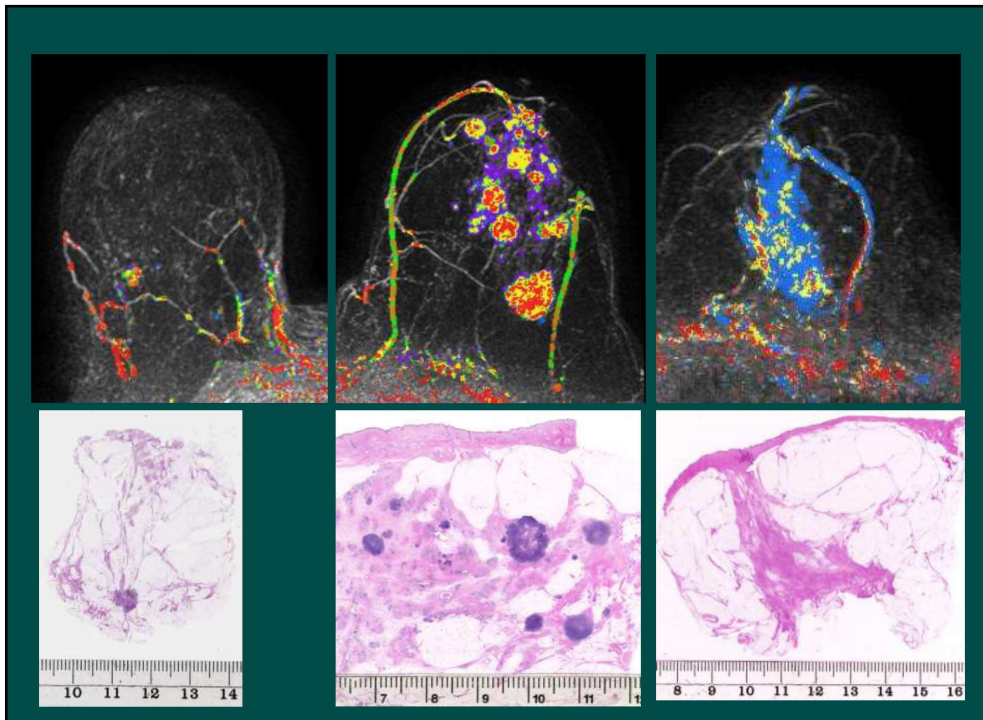
Comparison of 135 Consecutive Breast Conserving Surgical Specimens with DCIS. Conventional Pathology without MRI (CP) and Large Format Breast Pathology with MRI (LBP).

	<b>CP n=43</b>	<b>LBP n=92</b>
Re-excised after Breast Conservation	<b>14 (32%)</b>	<b>11 (12%)</b>
Volume of Breast Conservation Specimen, median (cm <sup>3</sup> )	<b>97.1</b>	<b>191.2</b>
Breast Conservation Rate	<b>65%</b>	<b>63%</b>

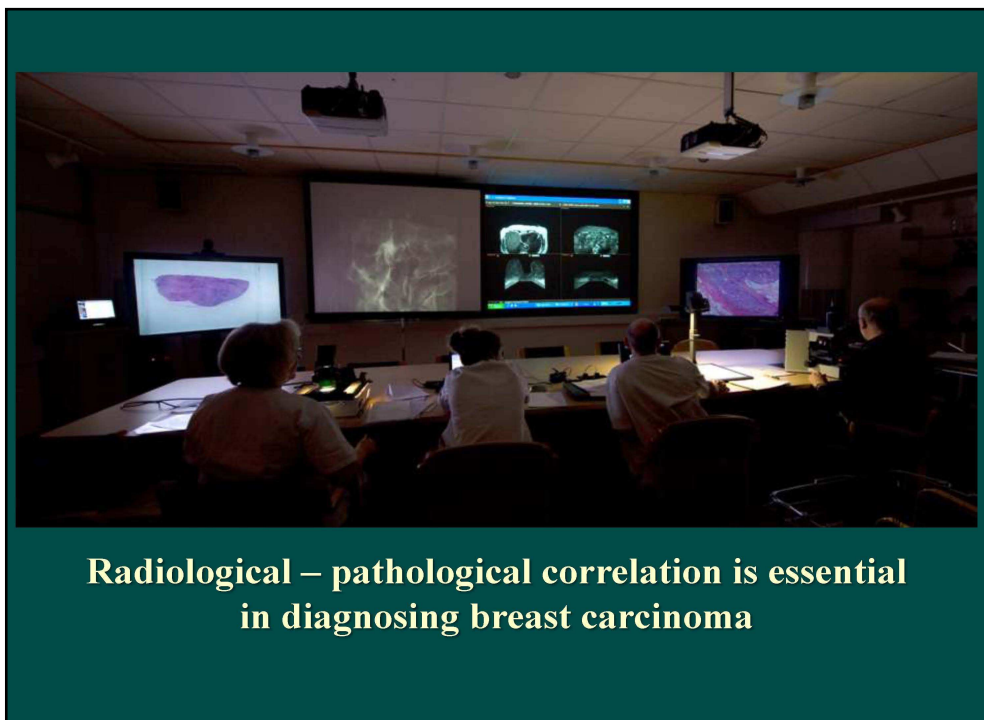
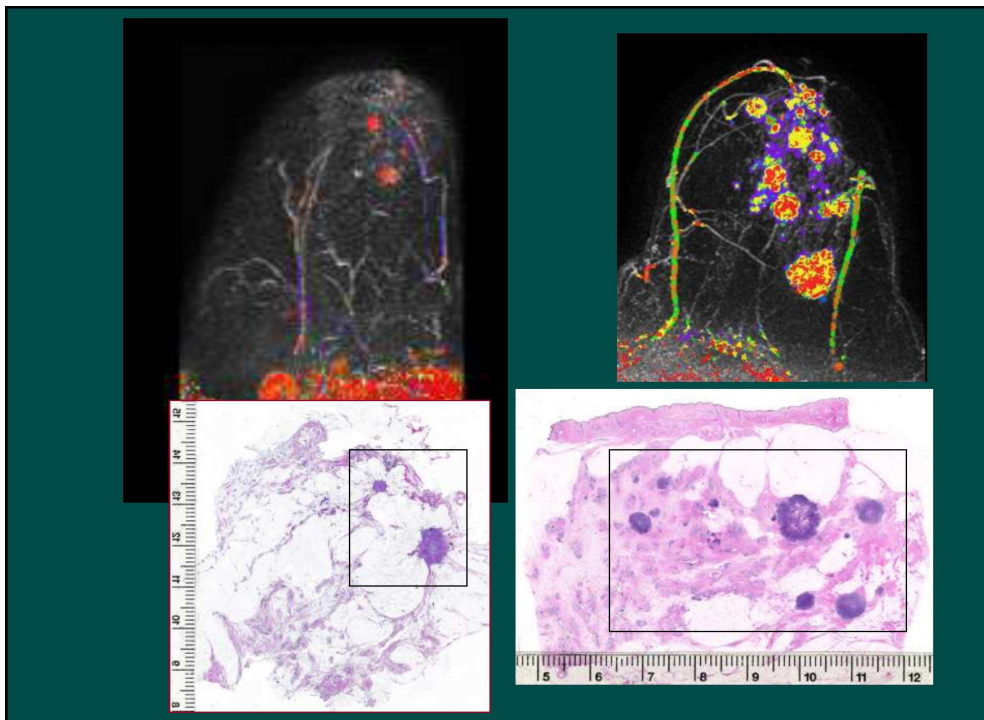
Results of F Lee Tucker, Virginia Biomedical Laboratories, USA

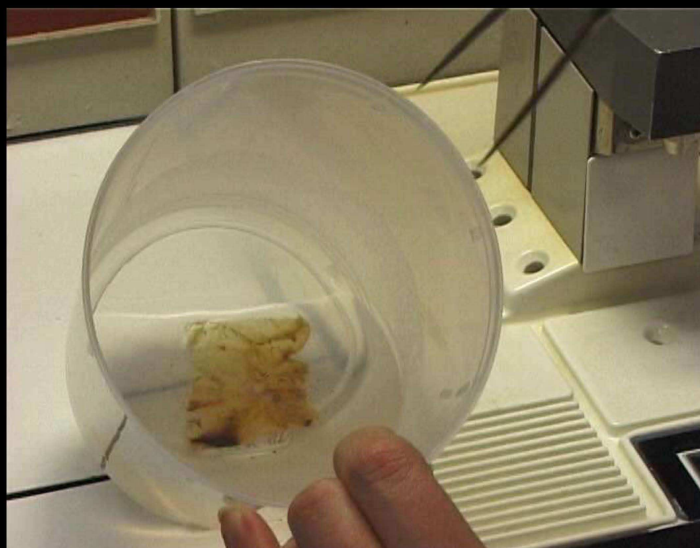
## Practical approach: postop

- Check the correctness of the preoperative findings
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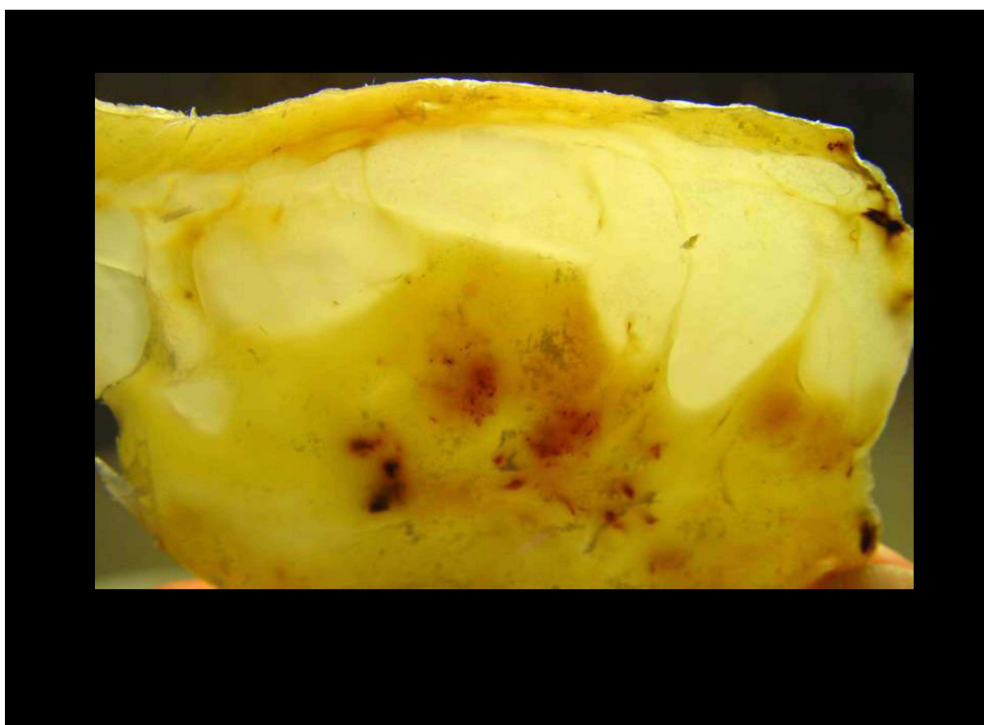
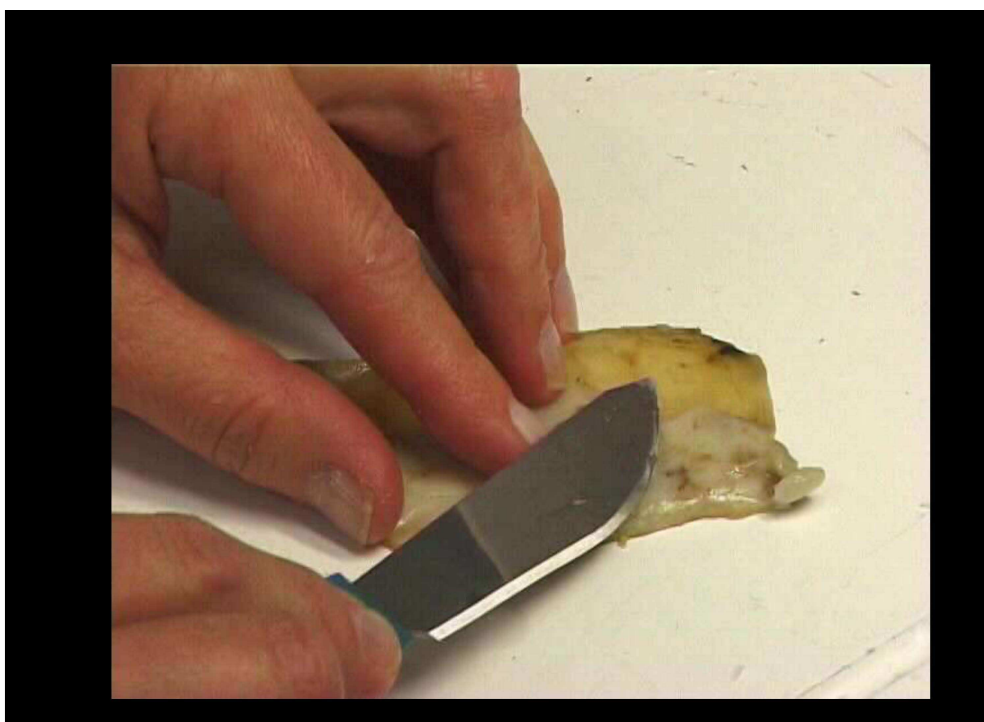


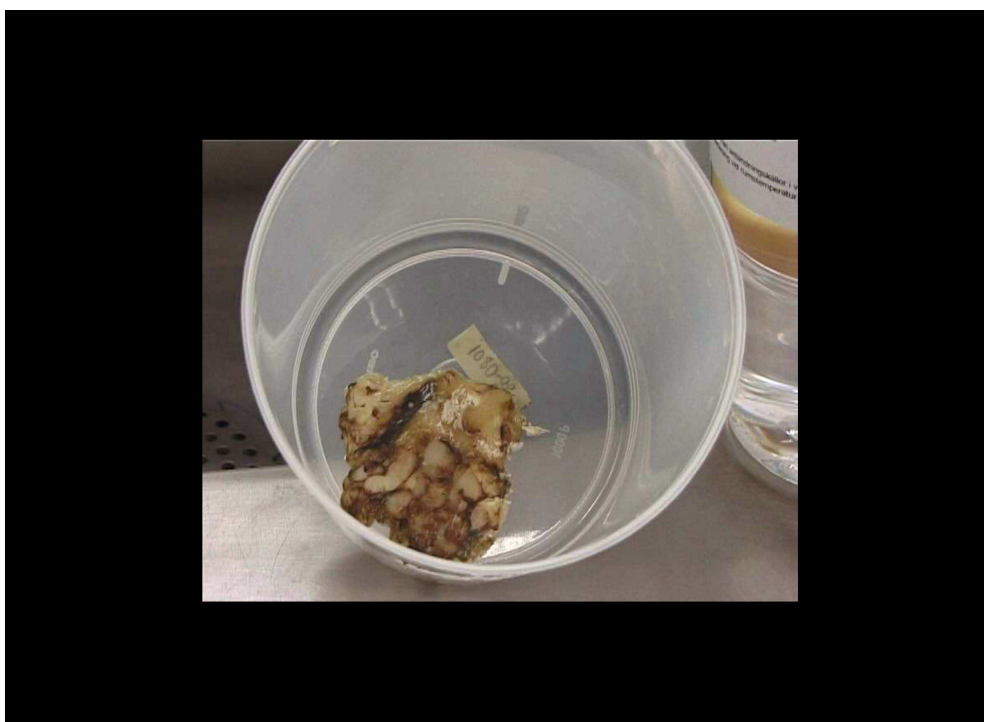


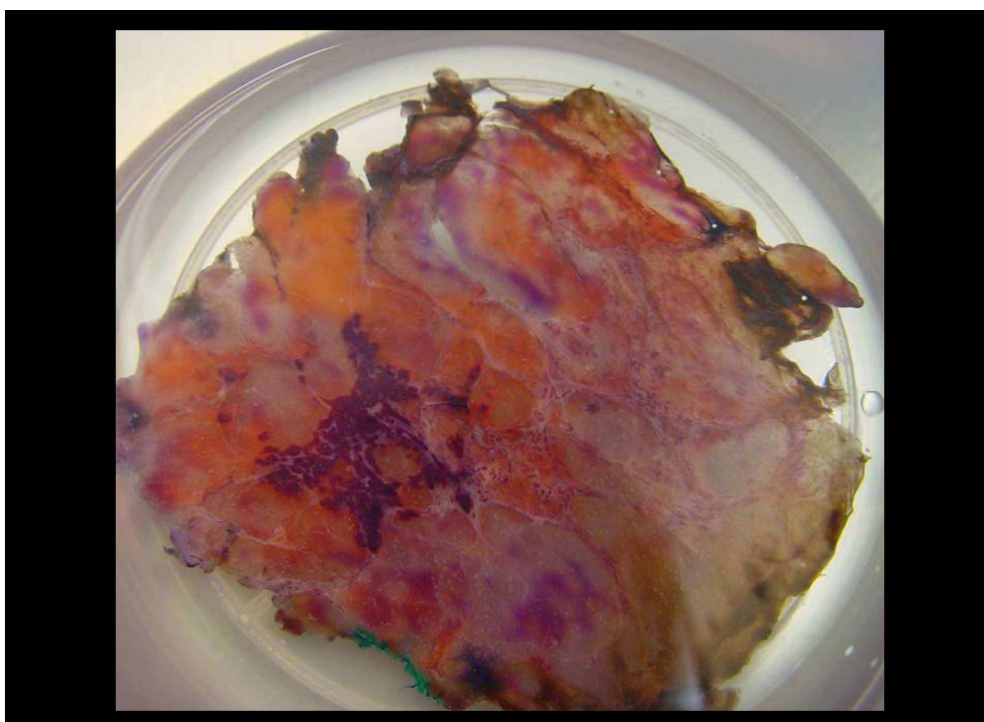




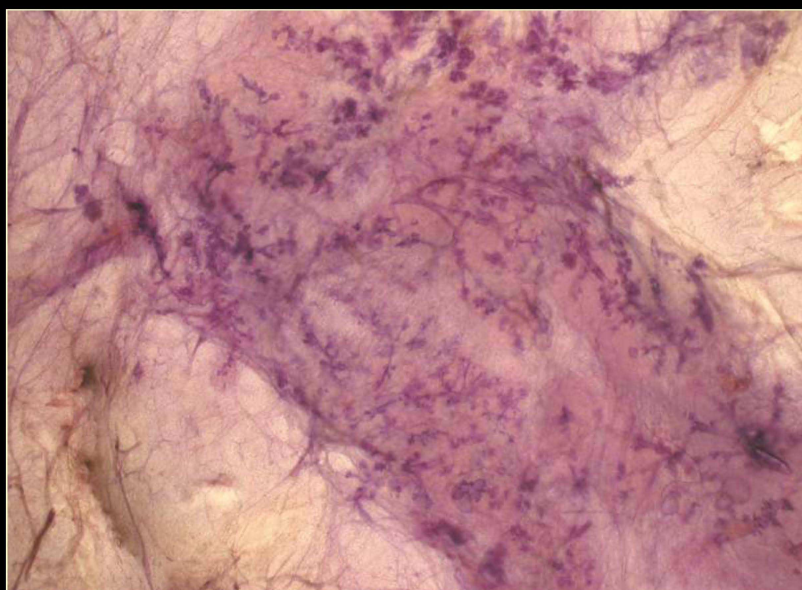


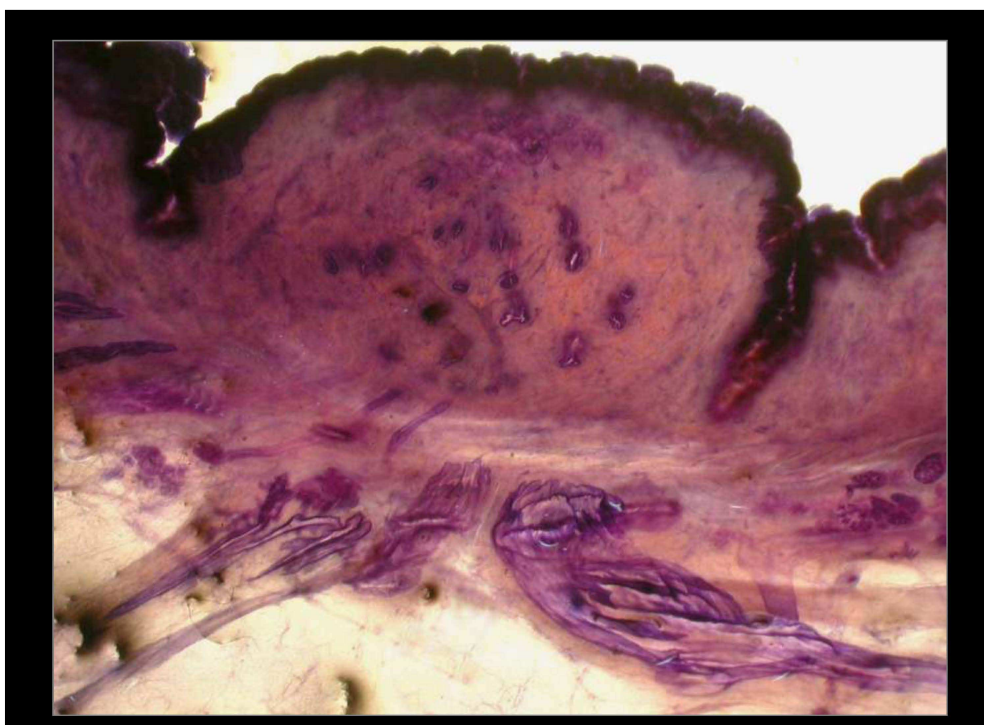
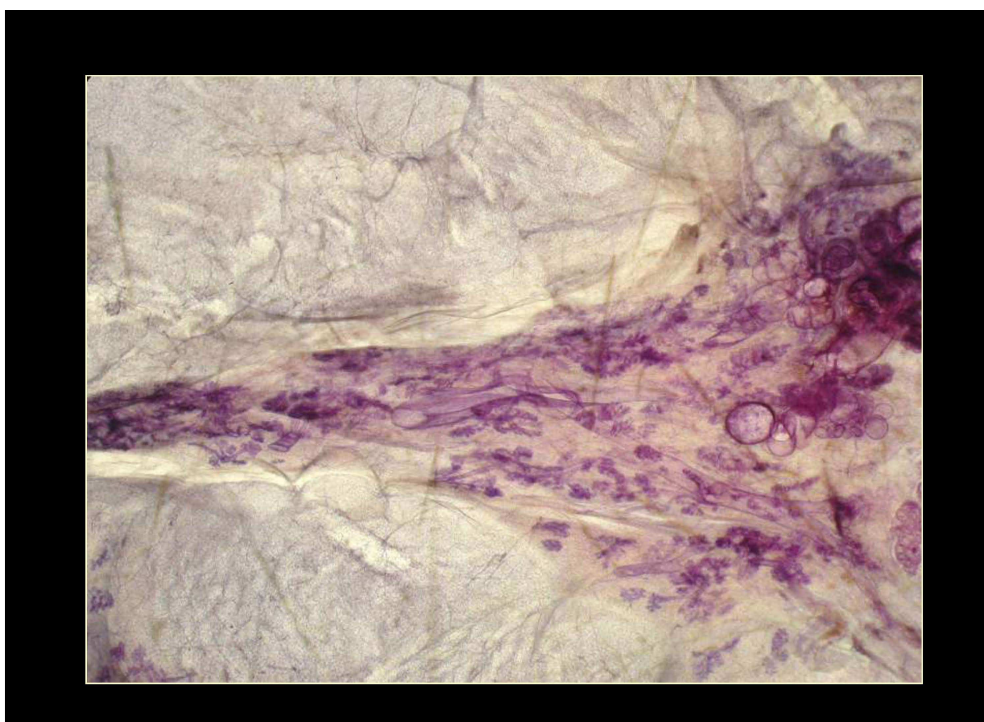


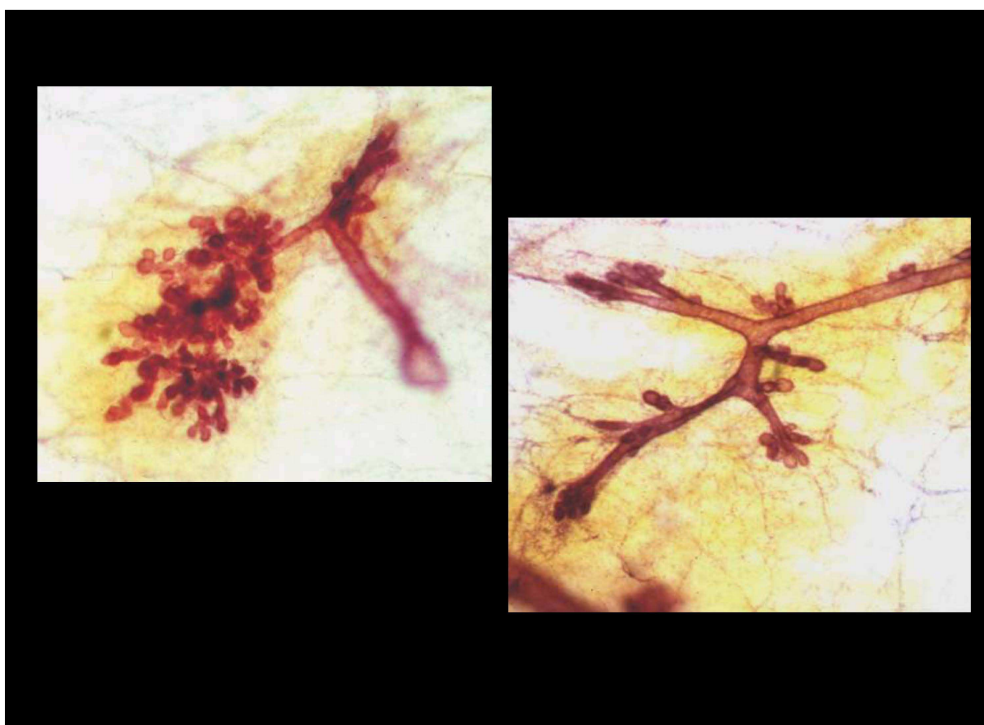
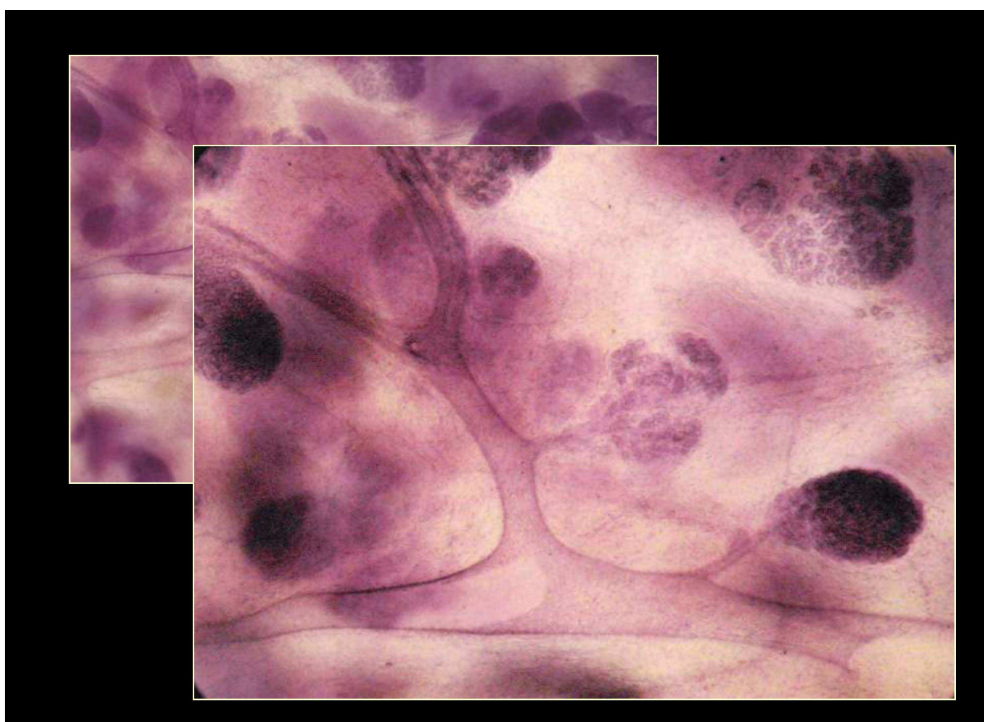




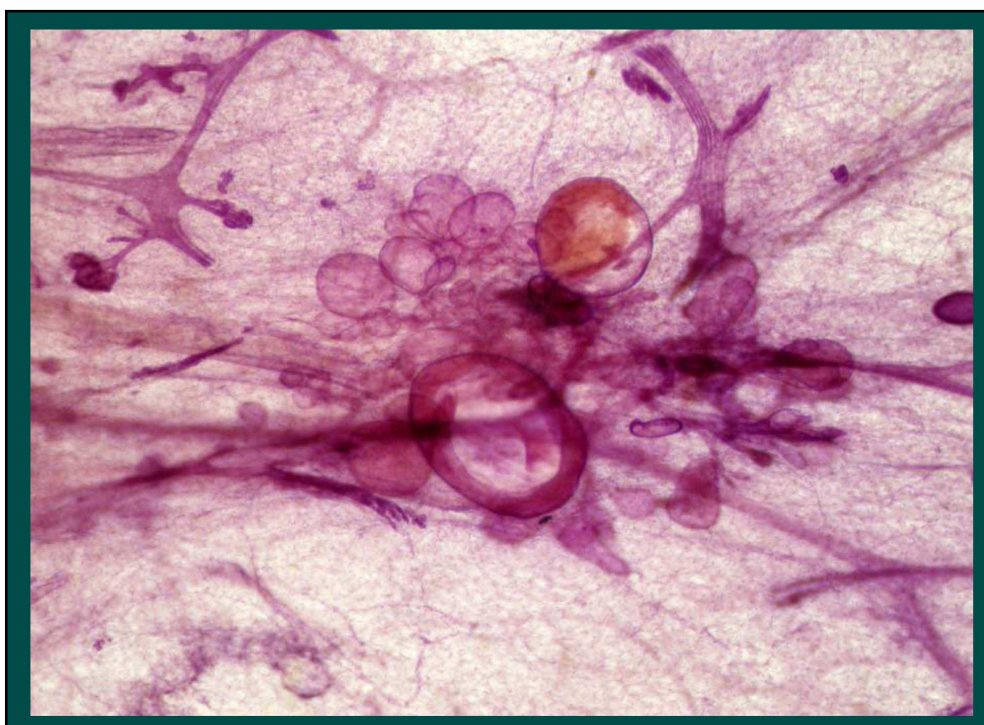
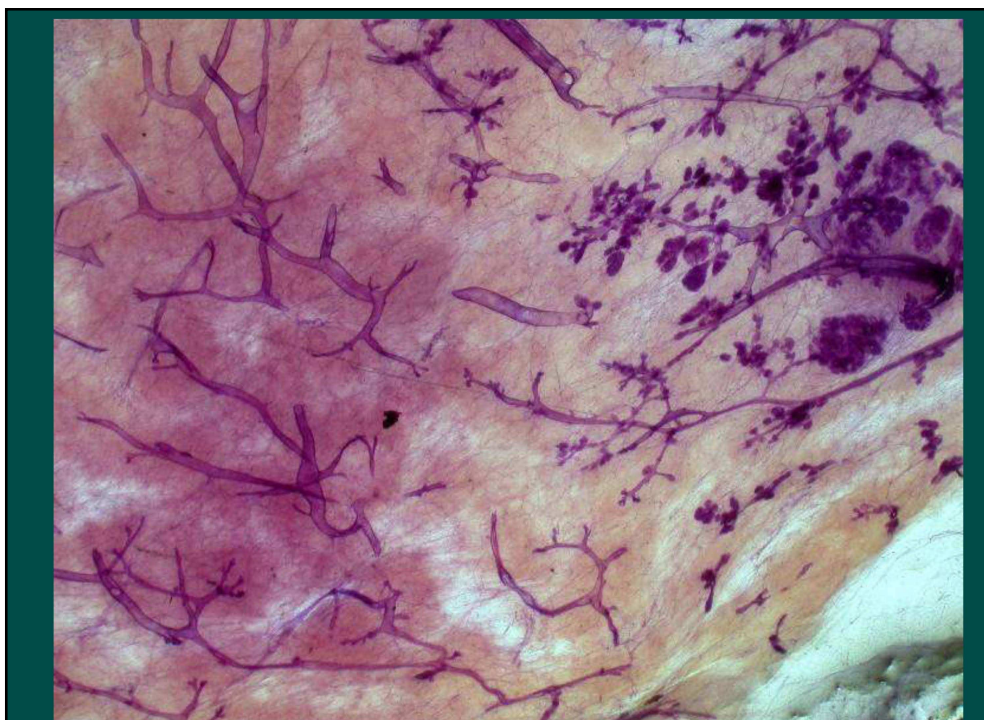


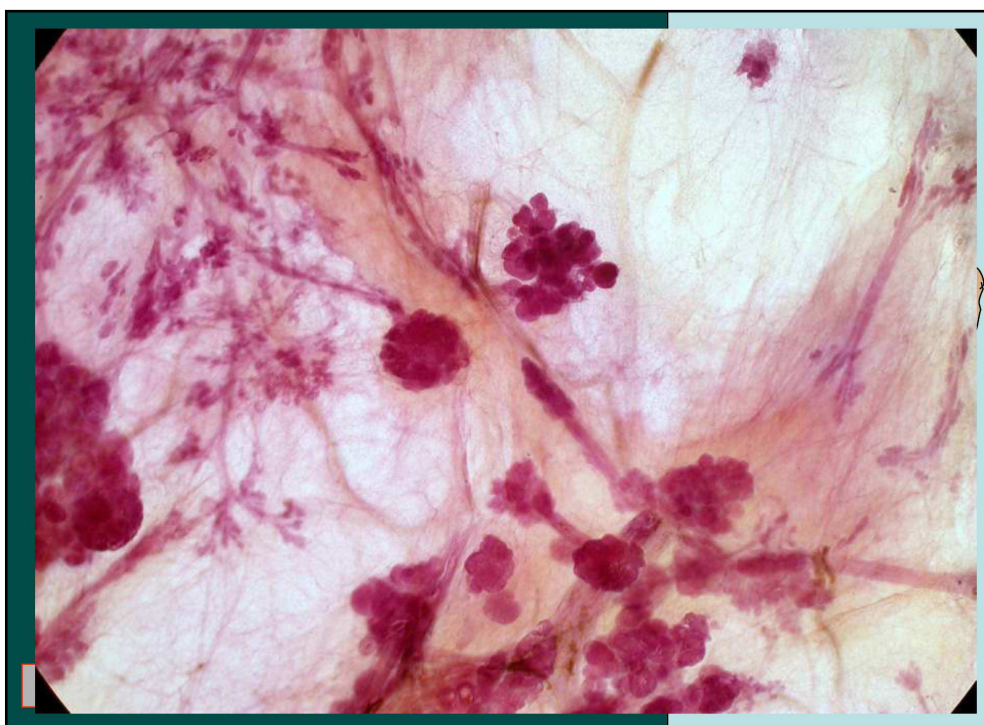
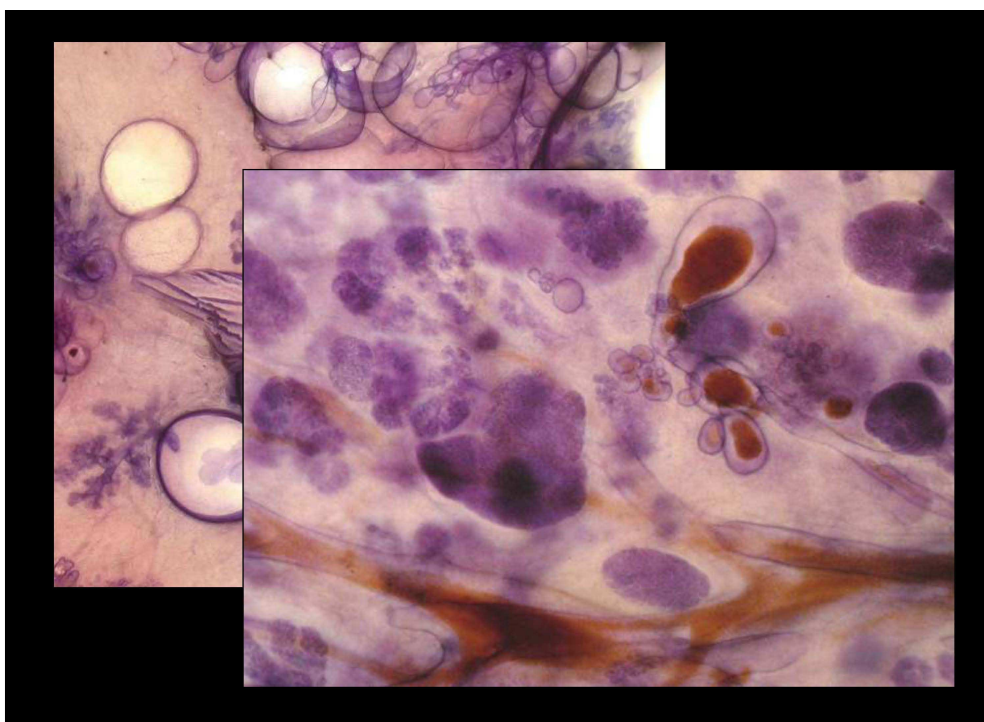




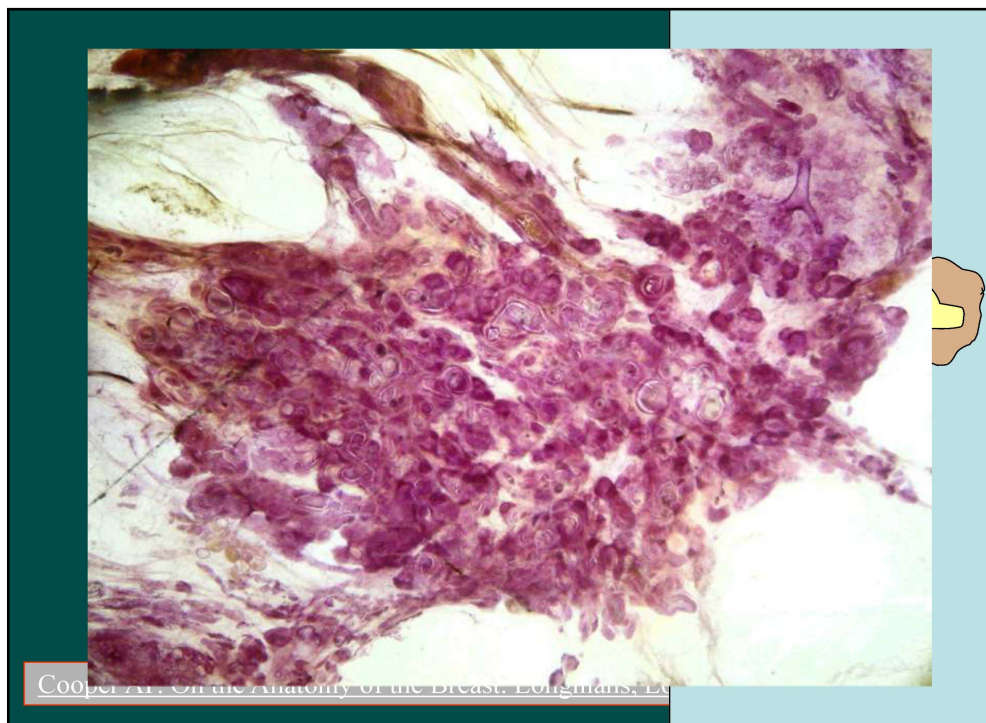
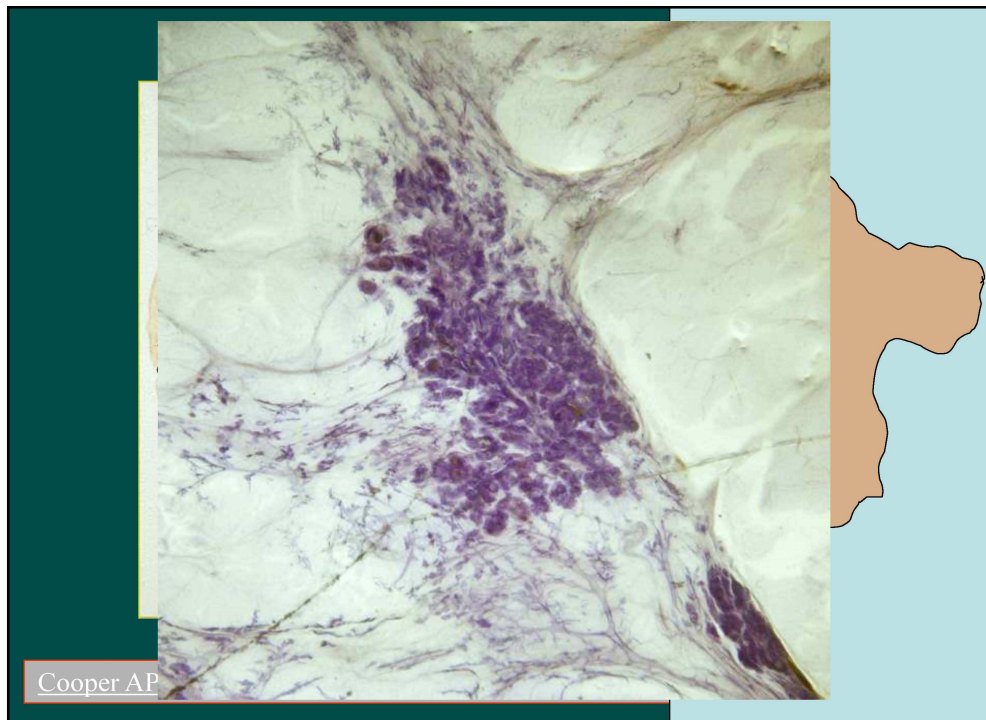












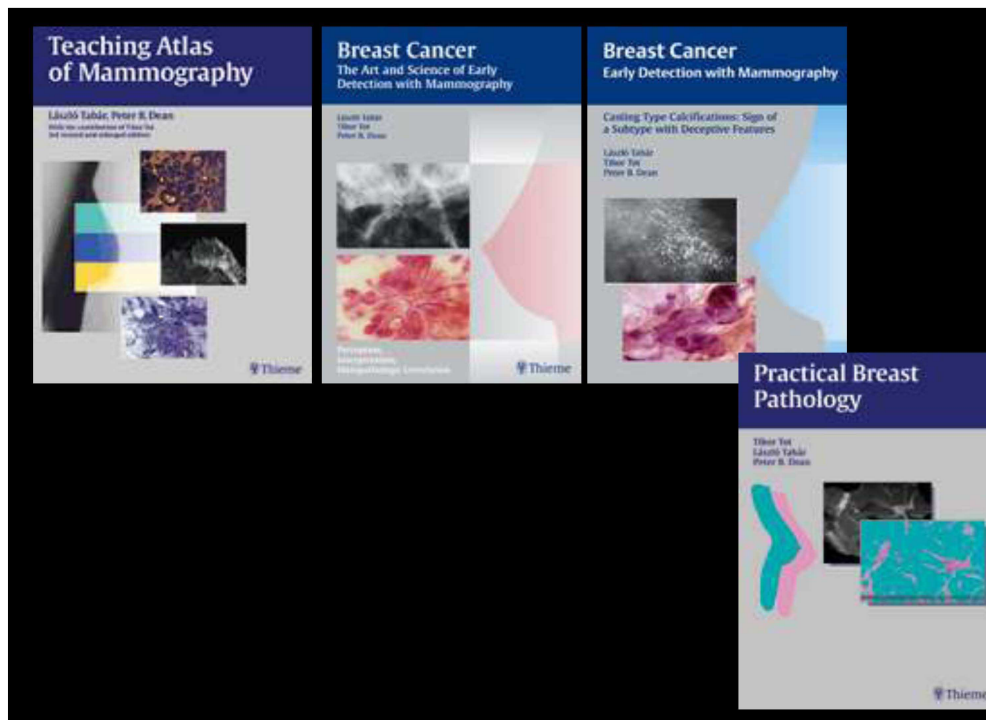


## Conclusion 1.

- Conventional histopathology is far insufficient in defining tumor size, distribution and disease extent in breast cancer in a considerable number of cases
- Modern breast imaging, especially in multimodality approach, is very accurate in assessing these parameters and represents a real challenge for pathology,

## Conclusion 2.

- Using multiple large-format histology sections represents a **prerequisite** and detailed radiological-pathological correlation the only proper way in adequate demonstration and evaluation of morphological findings in modern breast pathology.



Tibor Tot, Vincenzo Eusebi, Julio A. Ibarra

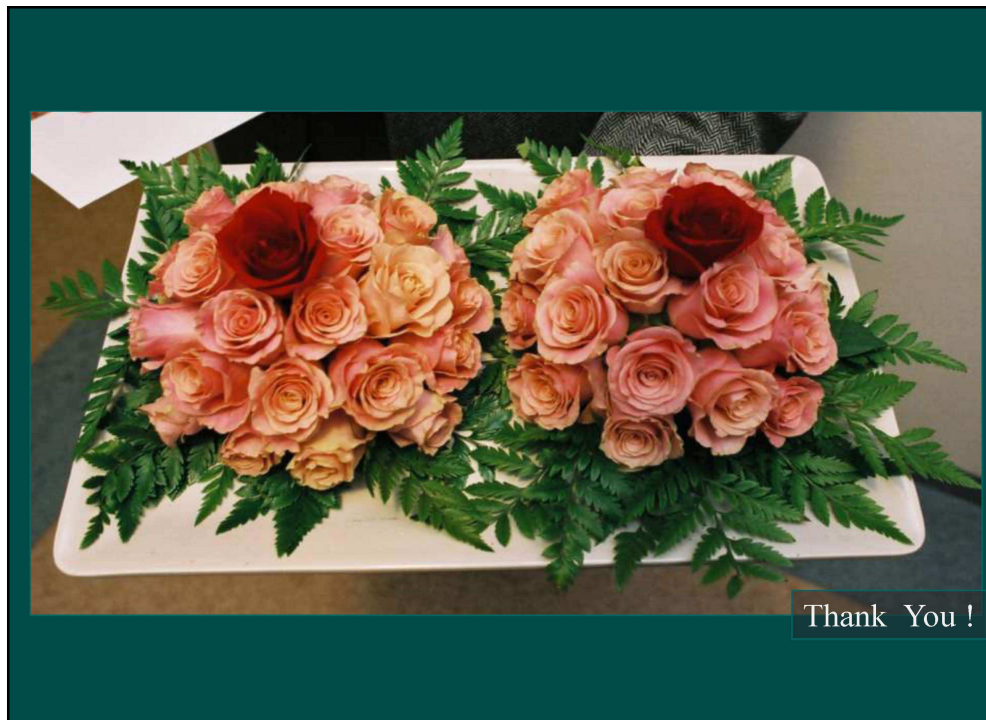
## **Large-Format Histology in Diagnosing Breast Carcinoma**

Special Issue of the International Journal of Breast Cancer, 2012

**”Interdisciplinary diagnosis and treatment of breast diseases is slowly but irrevocably becoming accepted as the new gold standard for patient care. It requires an additional investment in time and effort, which is soon repaid by smoother delivery of care and far fewer iatrogenic complications.”**

Tot T, Tabár L, Dean PB: Practical Breast Pathology, Thieme Stuttgart – New York, 2002





# A Better Path

**Olivier Poulin**  
Area Manager

Dako North America, Inc 6392 Via Real Carpinteria, CA 93013 USA	Dako Produktionsvej 42 DK-2600 Glostrup Denmark	Dako Belgium, nv Interleuvenlaan 12 B 3001 Heverlee BELGIUM
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Dako confidential information - 070812



## Contents




Agilent & Dako  
Companion Dx  
Something Big is going to  
happen

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## Agilent Technologies.....a strong foundation





### Agilent Technologies




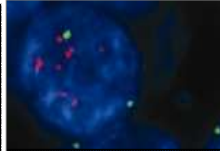
 <p><b>Electronic Measurement Group</b></p> <p>FY11 Revenue: \$3.3B</p> <ul style="list-style-type: none"> <li>Wireless technologies</li> <li>Mobile phone R&amp;D and manufacturing</li> <li>Aerospace/defense</li> <li>Low-cost instrumentation</li> </ul>	 <p><b>Chemical Analysis Group</b></p> <p>FY11 Revenue: \$1.5B</p> <ul style="list-style-type: none"> <li>Food safety, quality</li> <li>Energy research, production</li> <li>Quality of air, water, soil</li> <li>Forensics, drugs of abuse</li> </ul>	 <p><b>Life Sciences Group</b></p> <p>FY11 Revenue: \$1.8B</p> <ul style="list-style-type: none"> <li>Pharmaceutical research and manufacturing</li> <li>Genomics, proteomics, metabolomics tools for disease research</li> </ul>
<p><b>Agilent Research Laboratories</b> Enabling technology breakthroughs across Agilent</p>		

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**Agilent and Dako.....together we are stronger**




 **Agilent Technologies**

			
<b>Electronic Measurement Group</b>	<b>Chemical Analysis Group</b>	<b>Life Sciences Group</b>	<b>Diagnostics &amp; Genomics Group</b>
FY11 Revenue: \$3.3B	FY11 Revenue: \$1.5B	FY11 Revenue: \$1.8B	FY11 Revenue: \$332M
Wireless technologies	Food safety, quality	Pharmaceutical research and manufacturing	Dako heads this NEW Division of Agilent Technologies
Mobile phone R&D and manufacturing	Energy research, production	Genomics, proteomics, metabolomics tools for disease research	SureFISH Technology
Aerospace/defense	Quality of air, water, soil		Pharma, Imaging & Reagent Partnerships
Low-cost instrumentation	Forensics, drugs of abuse		

**Agilent Research Laboratories**  
Enabling technology breakthroughs across Agilent

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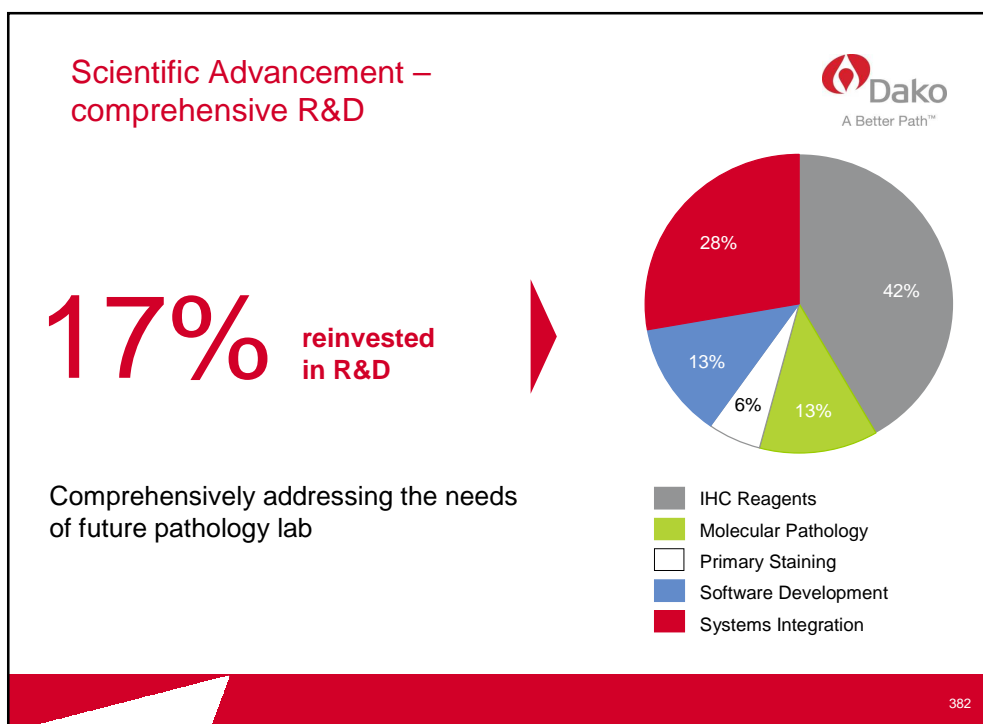
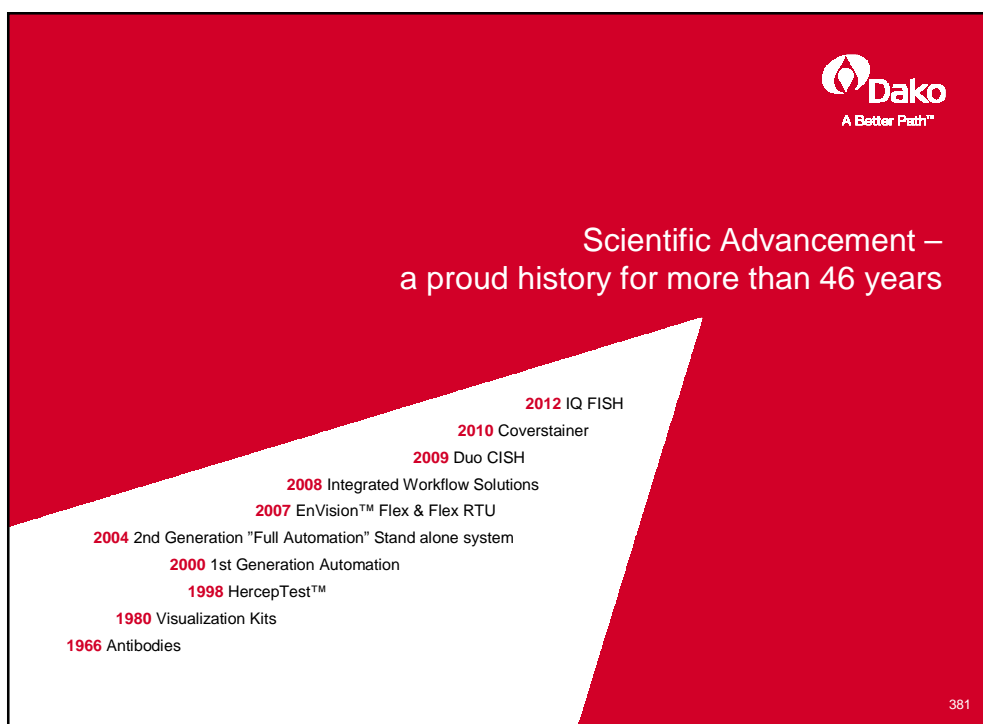
# A Better Path

Certainty


Scientific Advancement

Lasting Partnerships

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## Scientific Advancement – to deliver in 2013, we must think about 2023




### Reagent

- Quality
- Consistency
- Portfolio



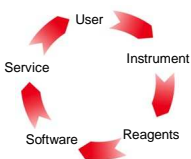
### Automation

- Automation
- Instrument features




### Solution

- Workflow
- Usability
- Efficiency
- Predictability
- Quality synergies



### Information

- Proactive information use
- On-line monitoring & QA
- Multiplex, quantitative algorithms
- Integrated reporting w. treatment



2000 ————— 2010 ————— 2020

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## Lasting Partnerships



# 15+ years

Our current  
average age of a  
customer  
relationship

*"To me, Dako is the person I can work with. High quality products and staff, highly competent and tremendously flexible – you do not find that with other big players in the market."*

Pathologist, Germany


*"Dako is part of the family. We work together as partners. I can rely on them to fix my issues."*

Lab manager UK

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**We are committed to fighting cancer - together**



**Greg Zdechlik**  
Chief Operating Officer, Diagnostics, Eli Lilly and Company

*Lilly*

“Tailored therapies are a key component of Lilly’s strategy of providing improved outcomes for individual patients. We look forward to collaborating with Dako in an effort to develop oncology companion diagnostics for patients worldwide who are waiting.”

**Lars Holmkvist**  
Senior Vice President, Agilent  
President, Diagnostic and Genomics Group  
President and CEO of Dako

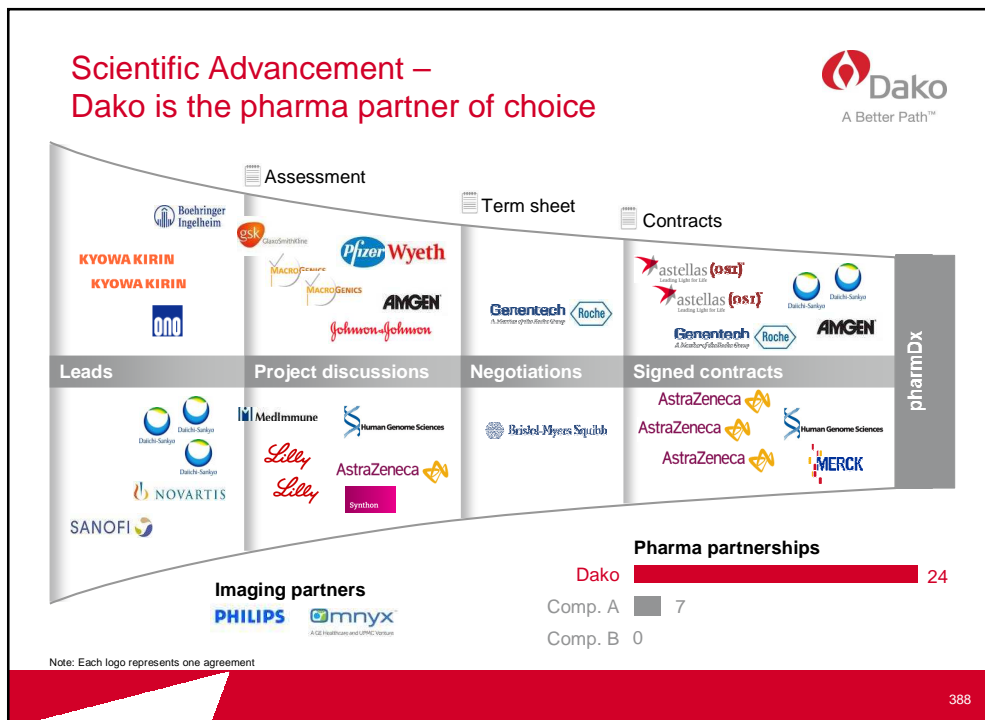
“Agilent and Dako have long histories of scientific advancement. Together, we are committed to fighting cancer, and I am excited about what we are working to accomplish for the benefit of patients worldwide.”


**Henrik Winther**  
Vice President Corporate Business Development, Dako

“At Dako, we are constantly looking for new opportunities to improve patient diagnostics. Building lasting partnerships with pharma is a cornerstone in the strategy to lead within the area of companion diagnostics.”

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**Dako**  
A Better Path™

Dako Omnis Solution  
We've been listening ...

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**Dako**  
A Better Path™

Understanding your world

"You know, it just gets more and more difficult to find well trained staff ..."

"Slide volumes really fluctuate – from day to day, and hour to hour"

"There is an increasing demand for consistency in slide quality, if we want to keep being accredited ..."

"Molecular testing keeps going up and up..."

"I am being asked to save on my budget each year, but yet the workload continues to rise ... I am challenged to improve my productivity ..."

"We are looking at how we can become more lean like in our workflow, with the focus on patient cases..."

"As we get ready for accreditation, documentation becomes even more important ..."

Source: Dako market surveys 2011-12


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Type footer here


## Dako offers a new complete staining solution in IHC and ISH

**Dako**  
A Better Path™

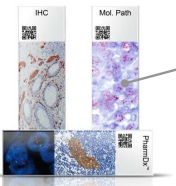
**Software**



**Instrument**




**Reagents**



**Dako Omnis Solution**

- Time
- Choice
- Better Patient Care


**Service, support and workflow consultancy**



9  
1

## Dako Omnis - adding to our heritage in advanced staining

**Dako**  
A Better Path™



**Instrument**

- Automated IHC and ISH, depar to counterstain
- Power of parallel processing
- Rack-based – 5 slides per rack
- True continuous loading
- Up to 60 slides simultaneously in process
- 60 reagent vials
- Onboard reagent temperature control

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## Dako Omnis - adding to our heritage in advanced staining

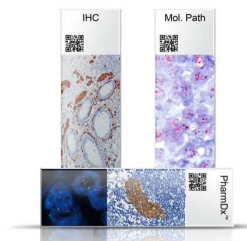


### Solution software and connectivity

- Onboard computer used for instrument operations (loading of reagents and slides, maintenance tasks)
- LAN seat concept: distributed software for entering test requests and workflow management
- Full LIS connectivity as well as Autostainer and Omnis co-existence within a laboratory handled through Test Request Distributor (TRD) component

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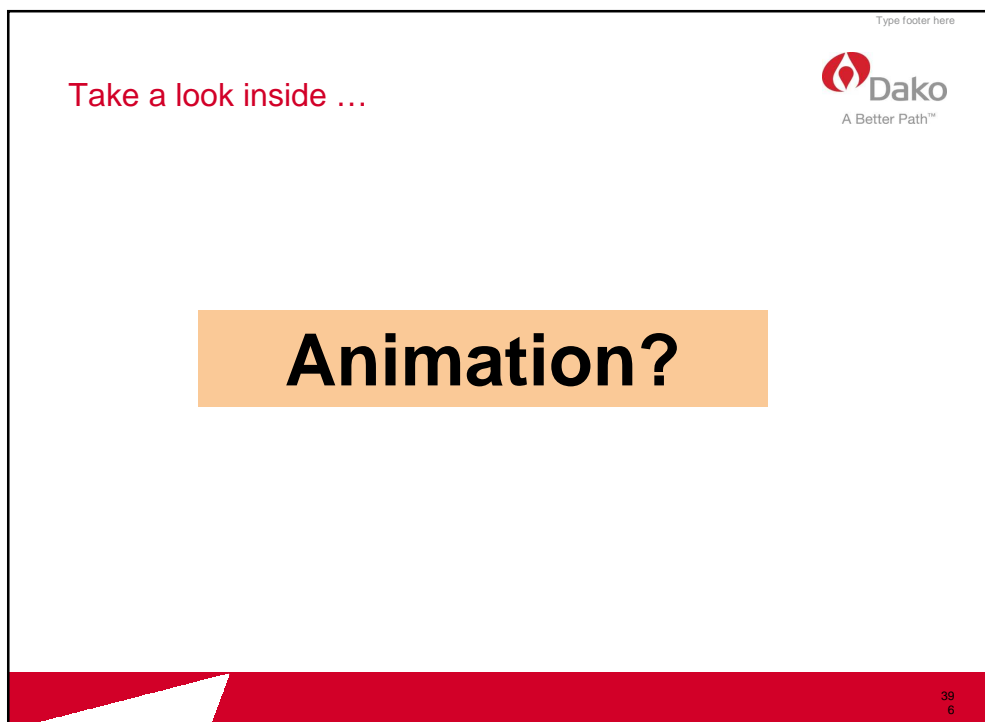
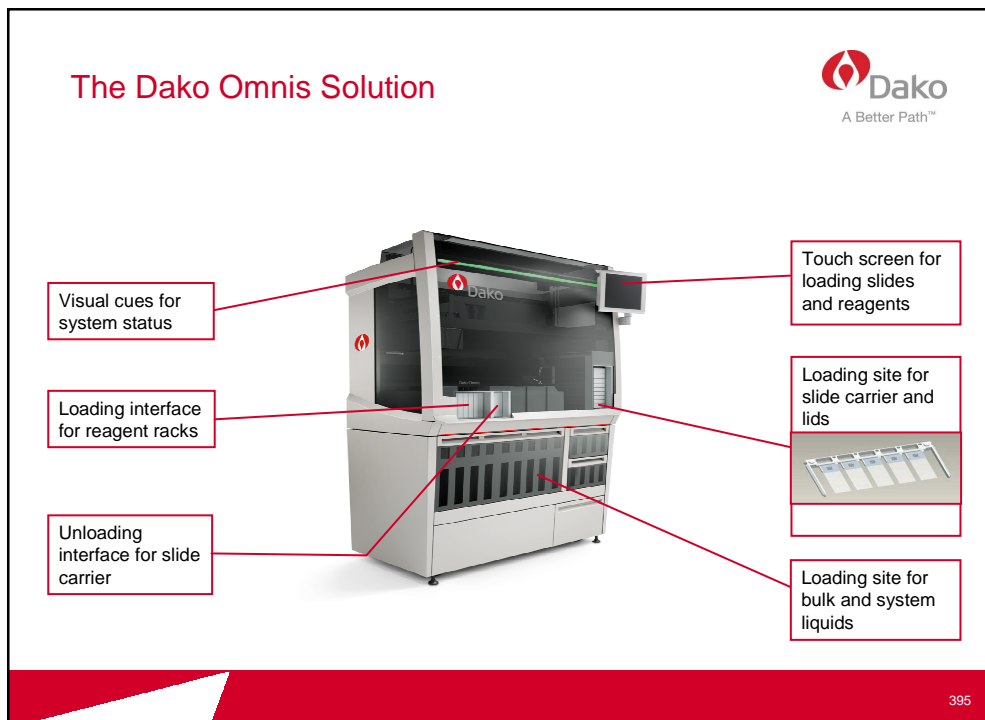
## Dako Omnis - adding to our heritage in advanced staining



### Reagents

- Validated with EnVision™ FLEX and FLEX RTU, HercepTest™ and pharmDx™ products
- HER2 IQFISH pharmDx™ as the first ISH product
- Third-party antibodies

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## Three things to know about Omnis and TIME

**Omnis sets new standards for performance...**

**0 min** waiting time to load  
**new reagents and slides**  
up to full capacity

**Major reduction** in **hands-on time**  
versus the most efficient  
staining solution currently  
on the market

**6 min** maximum daily  
**maintenance time**  
per system

**...with distinct benefits**

- **Never compromise** on when you can load your patient case
- **Don't have** staff/pathologists/clinicians/patients **waiting**
- **Eliminate non-value** adding steps common on existing systems
- **Free up** your most precious resource: **your staff**

All information based on design input specifications

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## Three things to know about Omnis and CHOICE

**Omnis sets new standards for performance...**

**Batch and continuous** flow

**60** temperature controlled  
**reagent positions** to choose  
from when you load your vials

**LAN seats** display  
**information where and when you need it** including  
information provided by your **LIS**

**...with distinct benefits**

- Omnis supports **your choice of workflow**, both when it is batch and continuous
- Have **full support for all your patient cases**, without constantly switching reagents
- LAN seats support the task at hand – **all over the lab** and in the office

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## Three things to know about Omnis and BETTER PATIENT CARE

**Dako**  
A Better Path™

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**Omnis sets new standards for performance...**

**60 IHC + 15 ISH**  
**slide capacity** enabling better patient case load management

**< 4 hour** turnaround time for **FISH slides**

**ID** logs all operator actions, **reduces errors and mitigates risks**

**...with distinct benefits**

- **Don't compromise time to delivery** by balancing fluctuations in daily/weekly test volumes
- Process patient case slides together and **save time** by reducing sorting steps
- Get the test results to the patient **quicker**
- **Confidence** that your lab will comply with **accreditation requirements**

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## Omnis Advisory Board

**Dako**  
A Better Path™



- **Prof. Hermann Herbst**, Vivantes, Berlin, Germany
- **Dr. Pedro Fernandez**, Hospital Clinic, Barcelona, Spain
- **Dr. Philippe Rochaix**, Institut Claudius Regaud, Toulouse, France
- **Mrs. Tracy Sanderson**, Northern General Hospital, Sheffield, UK
- **Mr. James Burchette**, Duke University, NC, US
- **Mr. Michael LaFriniere**, Cytology Services of Maryland, MD, US
- **Mrs. Cecelia A Dodson**, Indiana University Health, US
- **Prof. Ronald Stead**, Holburn & Gamma-Dynacare, Ontario, Canada
- **Mrs. Elizabeth Colley**, Holburn & Gamma-Dynacare, Ontario, Canada

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Relentless in our commitment  
to fighting cancer. Together.



Any questions?

