

**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



# **Histological techniques: quality and impact on diagnosis**

## **1<sup>st</sup> TASTE Workshop Proceedings Deliverable 7.4**

**Falun, Sweden, 1<sup>st</sup> June 2012**

Department of Pathology and Clinical Cytology, Central Hospital Falun

## Preface

The following presentation contains the contributions presented at the First TASTE workshop, “TASTE Project: Histological techniques: quality and impact on diagnosis”, held on the 1<sup>st</sup> of June 2012 in Falun, Sweden. 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

This workshop is designed for all professionals involved in manufacturing and assessing histological, cytological, immunohistological and other preparations in routine diagnostic pathology and cytology. Pathologists, pathology residents, histo-technicians, cyto-technicians, medical students and student technicians are equally welcome to participate. As the first workshop in a series of six similar events, the meeting in Falun provided detailed information regarding the TASTE project; open the possibility of active participation of the attendees in creating, evaluating and standardizing a web-based good quality preparations image database with aim to form international standards for high technical quality of the preparations and maintaining good laboratory practice.

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. These are also the central questions the TASTE project will address. 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.

The international expert group of the TASTE project underlined the importance of wide implementation of the project already at its initial phase. Therefore, all the attendees of the workshops have been invited to contribute during the coming years with their

- own slides of top, average, or suboptimal quality,
- own experience in problem-solving and other technical issues,
- assessing the web-based images in scenarios standardized for students/residents, technicians and specialists,
- supporting the project by spreading information about its aims and by promoting its results.

The TASTE project is part of the European Commission's Lifelong learning project and as such aims to stimulate the interaction between established members of the pathology/cytology community and the future generation: the students and residents. At the same time, it may become a model how different categories of professionals at different levels of their education can successfully cooperate in problem-solving and in setting future technical standards.

**The mandate for standardization  
of pre-analytical processing of the tissues**

*Gianni Bussolati*

*University of Torino*

**Preparing pathology for personalized  
medicine:  
possibilities for improvement  
of the pre-analytical phase.**

Groenen PTA, Blokx WAM, Diepenbroek C, Burgers L,  
Visinoni F, Wesseling P & van Krieken JHJM.

Histopathology 59, 1-7, 2011

**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

## **Basis of histopathology**

- **Preservation**
  - Structure
  - Proteins
  - DNA / RNA

**“garbage in / garbage out”**

## **AIMS**

### **Preservation of**

- **Structure (morphological diagnosis)**
- **Proteins (Immuno-histochemistry)**
- **Nucleic Acids ( FISH + GEP )**

### **Standardization**

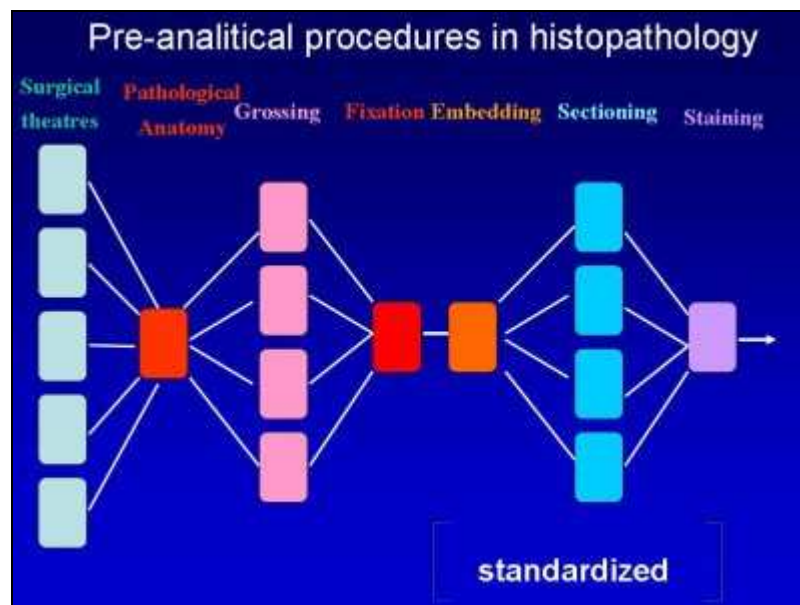
for present-day and future analyses

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



## Step 1

**"From the surgical theatre to the Pathology laboratory"**

## Step 2

**"From the grossing table to paraffin wax"**



## **Pre-Analytical Time Interval (PATI)**

**Interval 1: From the body (A) to the surgical table (B) (Temperature 37°C or more)**

**Depends on:**

- a) Type of operation**
- b) Modality of intervention**
- c) Ability of the surgeon**

## **Time between ligation of arteries and removal**

**Time negligible for:**

- Brain
- Breast
- (Liver)
- Lymph nodes
- Skin

## **Time between ligation of arteries and removal**

**Time from a minimum of ½ h to 1 h for:**

- Stomach
- Colon
- Lung
- Pancreas
- Thyroid



## Pre-Analytical Time Interval (PATI)

### Effects:

- Hypoxic conditions at 37° C for variable time
- Remarkable loss of RNA and antigen degradation if time longer than a few minutes.

### Additional caveats:

- Heath cutting
- Treatment with Lugol

*Histopathology* 2010; 56, 240-250. DOI: 10.1111/j.1365-2559.2009.01470.x

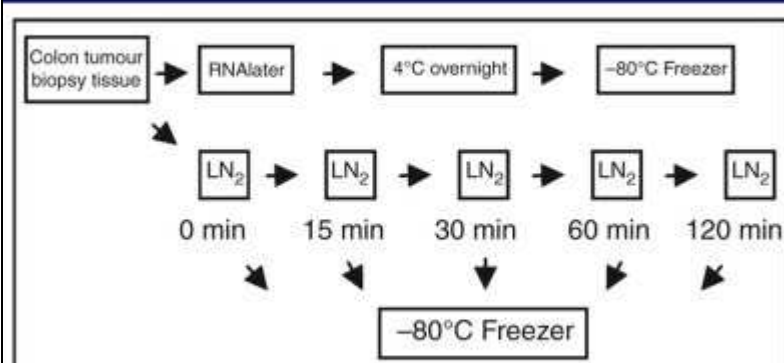
## Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,<sup>1</sup>

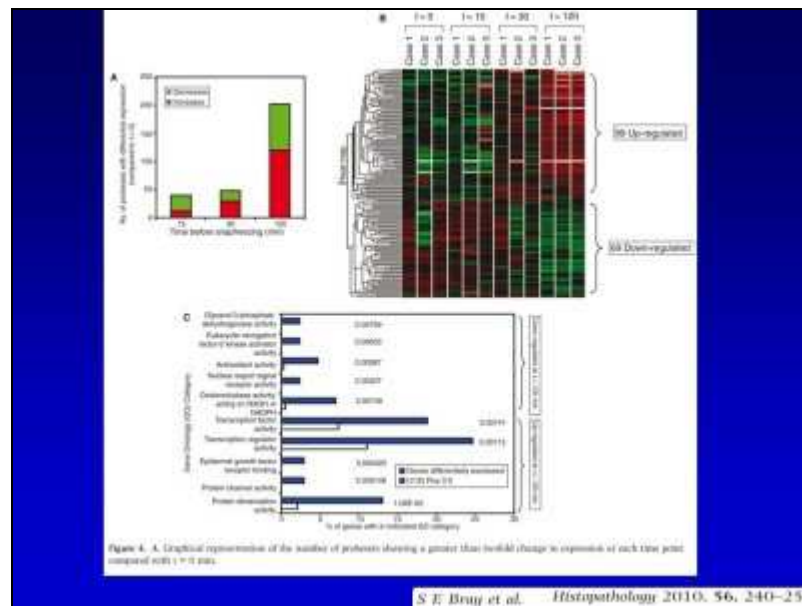
David A Levison,<sup>1</sup> Robert J C Steele & Neil M Kernohan<sup>1</sup>

Department of Surgery & Molecular Oncology, and <sup>1</sup>Department of Pathology & Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

S E Bray et al. *Histopathology* 2010; 56, 240-250



**Figure 1.** Protocol for sample collection in theatre.



*Histopathology* 2010; 56, 240–250. DOI: 10.1111/j.1365-2559.2009.01470.x

## Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,<sup>1</sup> David A Levison,<sup>1</sup> Robert J C Steele & Neil M Kernohan<sup>1</sup>  
 Department of Surgery & Molecular Oncology, and <sup>1</sup>Department of Pathology & Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

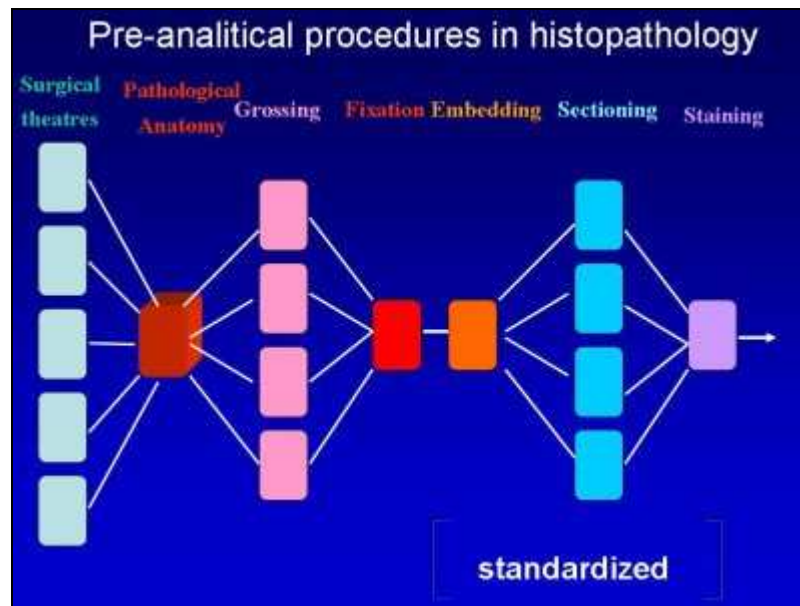
..... by 120 min there was a fourfold increase in the number of genes with a more than twofold change in the level of expression.

*Histopathology* 2010; 56, 240–250. DOI: 10.1111/j.1365-2559.2009.01470.x

## Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,<sup>1</sup> David A Levison,<sup>1</sup> Robert J C Steele & Neil M Kernohan<sup>1</sup>  
 Department of Surgery & Molecular Oncology, and <sup>1</sup>Department of Pathology & Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

**Conclusions:** Reliable interpretation of results of gene expression at the mRNA level requires standardized protocols for tissue procurement.



## Interval 2: From the surgical table (B) to the pathology lab (C)

### Alternatives :

#### a) Tissues left fresh

## Interval 2 -From (B) to (C):

### 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 between B and C: relatively short (it can vary)
- Time in C 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>

**Interval 2 -From (B) to (C):**

**Alternative: a) Tissues left fresh**

**Merits:**

- **No Fixation**  
(material available for banking)

**Interval 2 -From (B) to (C):**

**Alternative: a) Tissues left fresh**

**Dangers:**

- **Effect of delay on:**
  - **Structure**
  - **Proteins (antigens)**
  - **Nucleic acids**

**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**



## Interval 2: From the surgical table (B) to the pathology lab (C)

### Alternatives :

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

## Interval 2 -From (B) to (C):

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.

## Interval 2 -From (B) to (C):

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 <1cm. =  
Uniform fixation**

---

**Large specimen ( > 2 cm)**

- **Outside = Fixed**
- **Inside = Autolysis**

**Interval 2 -From (B) to (C):**

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”





**Molinette  
Hospital  
year 2007**

## **Interval 2: From the surgical table (B) to the pathology lab (C)**

### **Alternatives :**

- a) Tissues left fresh
- b) Tissues immersed in formalin
- c) Tissues under-vacuum + cooling**

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

#### **LETTER TO THE EDITOR**

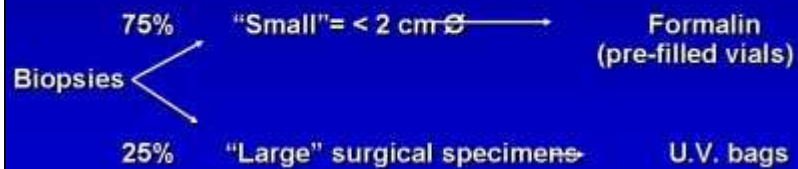
### **Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin**

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



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

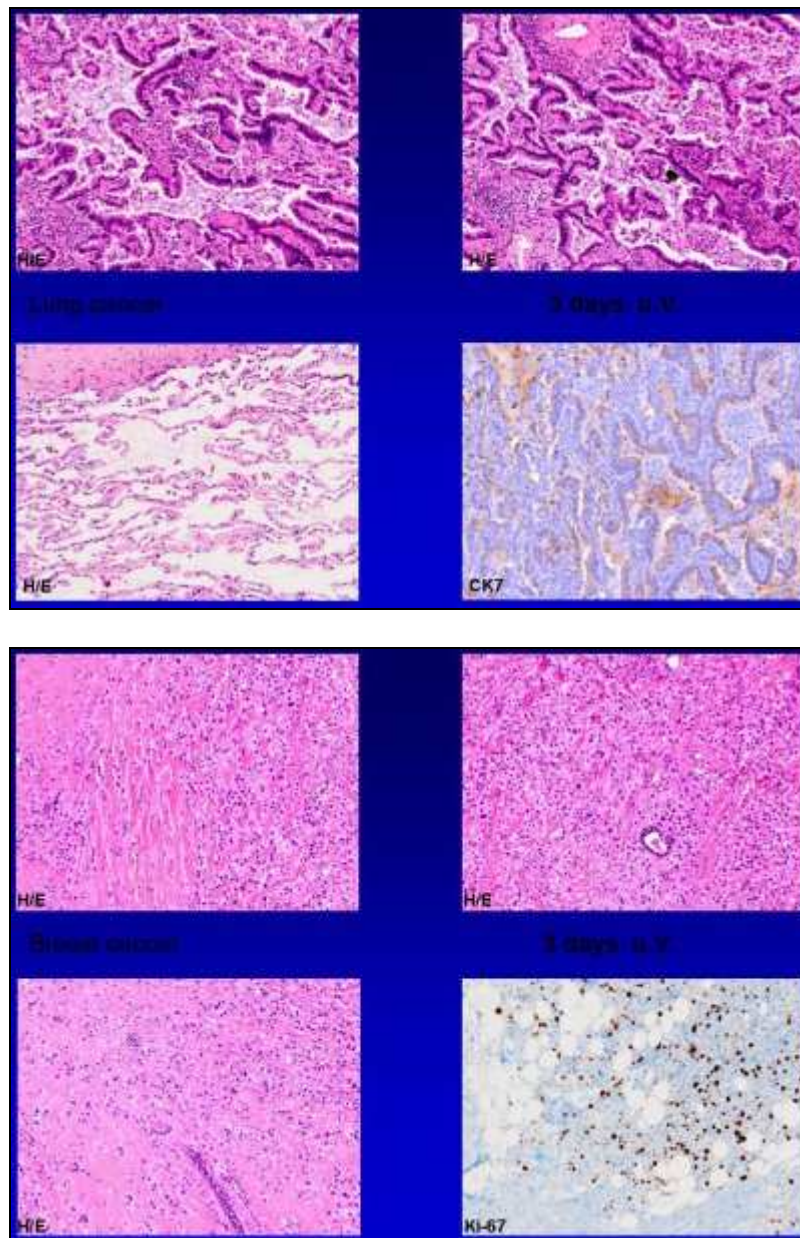
- 1162 Beds; >54.000 yearly admissions; > 40.000 histopathological exams. (2008)

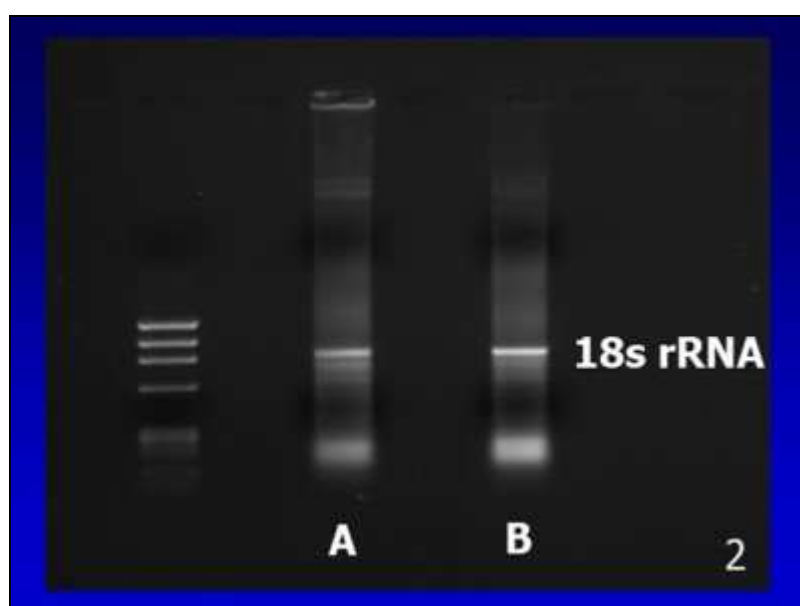
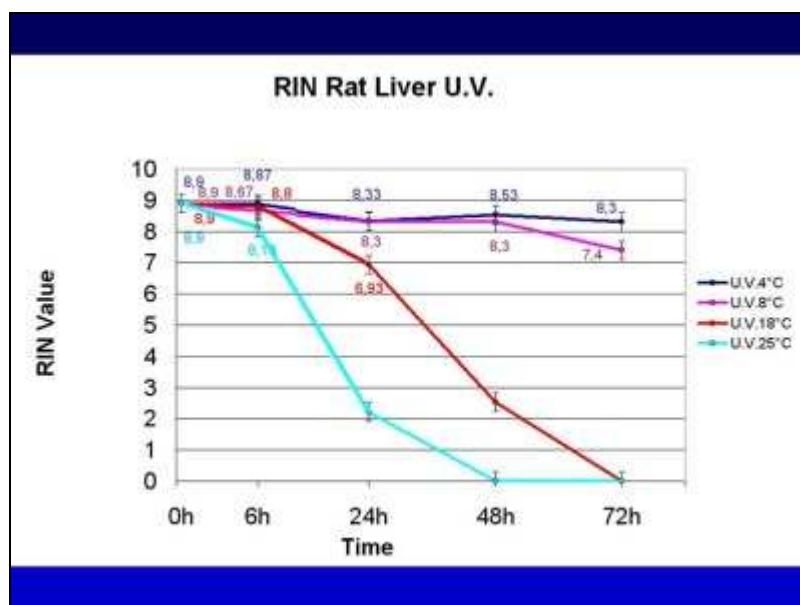
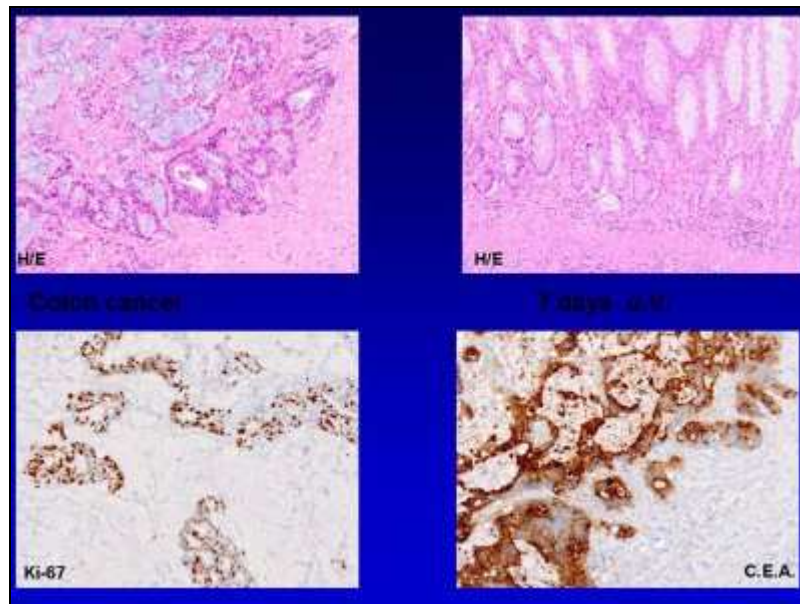




## Vacuum Sealing and Cooling as Methods to Preserve Surgical Specimens

Kristensen T, Engvad B, Nielsen O, Pless T, Walter S, Bak  
 Appl Immunohistochem Mol Morphol. 2011







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

Level of Satisfaction	Freq.		Percent	
	formalin	under-vacuum	formalin	under-vacuum
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	2.526087	0.4739596
COLOUR	1.173913	0.383223	2.913043	0.2848849
CONSISTENCY	2.021739	0.147442	2.23913	0.431266
# 4= endocrine / thyroid				
STRUCTURE	1.934783	0.2496374	2.526087	0.4739596
COLOUR	1.23913	0.431266	2.934783	0.2496374
CONSISTENCY	2	0.2108185	2.152174	0.3631584
# 5= liver / spleen				
STRUCTURE	1.904762	0.2971018	2.690476	0.4679011
COLOUR	1.190476	0.3974366	3	0
CONSISTENCY	1.97619	0.1543053	2.404762	0.4967958

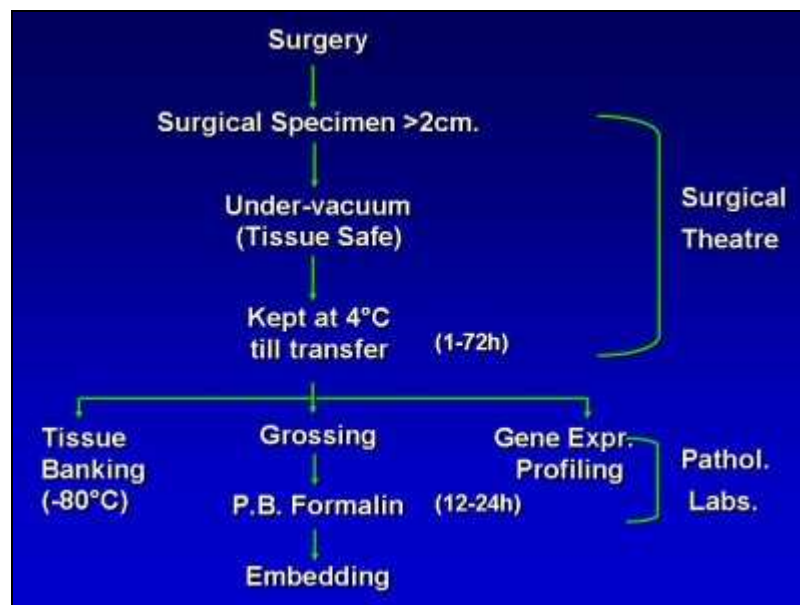
1= weak  
 2= satisfactory  
 3= good

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

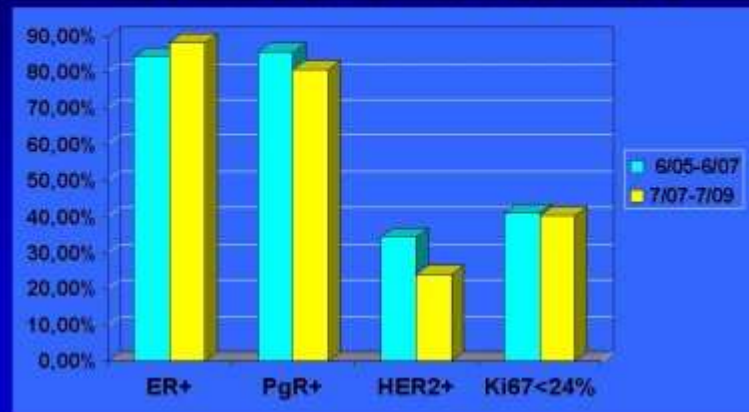
## Result of Survey among Staff Operators (October 2008 – April 2009)

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

Project: → F-I



ICC evaluation of therapeutic/prognostic parameters in a consecutive series of breast cancers  
 Years 2005-2007 vs 2007-2009, N=375



Breast cancers processed by USV and cooling (4°C)											
Slide ID	gpp	RIN	Slide ID	gpp	RIN	Slide ID	gpp	RIN	Slide ID	gpp	RIN
1201003	1h	9	8051003	4h	8,6	3401003	24h	8,7	8067003	7h	8,8
1305003	60h	7,8	8056003	24h	7,3	3673003	1h	6,6	8080003	24h	7,4
1394003	60h	7,9	8058003	24h	7,9	3701003	24h	7	9112003	24h	7,8
1399003	60h	7,4	8250003	2h	6,9	3764003	48h	8,6	9182003	4h	8,1
1271003	1h	7,8	8261003	5h	7,8	3889003	72h	7,8	9240003	48h	8
1408003	5h	7,5	8388003	72h	7,8	3888003	72h	8,2	9324003	24h	8,7
1422003	2h	8,7	8390003	72h	8	3883003	70h	7,4	9452003	5h	7,9
1267003	5h	8,2	8520003	24h	7,1	3903003	72h	7,6	9461003	7h	7,5
1898003	24h	8,7	8520003	24h	7,1	4034003	2h	7,6	9585003	24h	8,6
1782003	60h	7,6	8520003	24h	7,1	4073003	4h	9,1	9607003	4h	7,1
1844003	1h	8,2	8520003	24h	7,1	4075003	3h	7,9	1061003	5h	7,7
1886003	1h	8,2	8520003	24h	7,1	4122003	20h	7,3	1431003	5h	8
1804003	24h	7,3	8520003	24h	7,1	4221003	70h	7,4	3451003	2h	7,2
1903003	5h	7,1	8520003	24h	7,1	4231003	70h	8,2	4251003	5h	8,2
1992003	72h	8,1	8520003	24h	7,1	4275003	7h	9,4	5067003	8h	8,6
2022003	7h	7,2	8520003	24h	7,1	4298003	24h	6,6	6511003	5h	8,5
2043003	60h	8,1	8520003	24h	7,1	4471003	48h	8,6	6593003	3h	6,8
2201003	1h	8,8	8520003	24h	7,1	4553003	30h	8	6493003	23h	6,8
2238003	4h	8,4	8520003	24h	7,1	4594003	72h	8,2	10221003	72h	8,6
2277003	5h	8	8520003	24h	7,1	4617003	2h	8,9	12671003	5h	7,8
2574003	6h	7,9	8520003	24h	7,1	4668003	24h	8,6	13161003	5h	7,7
2621003	24h	7,6	8520003	24h	7,1	4831003	5h	8,3	14551003	24h	6,5
2622003	24h	6,8	8520003	24h	7,1	4937003	72h	8	16771003	4h	9
2712003	12h	7,4	8520003	24h	7,1	5008003	24h	8,2	17751003	7h	9,2
2605003	20h	7,8	8520003	24h	7,1	5118003	72h	8,2	19441003	7h	8
2703003	72h	6,4	8520003	24h	7,1	5237003	24h	7,3	19451003	4h	7,4
2828003	24h	7,2	8520003	24h	7,1	5525003	5h	7,8	20331003	5h	9,1
2873003	24h	7,8	8520003	24h	7,1	5598003	1h	7,9	20361003	24h	7,4
3218003	1h	7	8612003	72h	8,2	5861003	1h	7,1	20961003	24h	9,5
3340003	7h	8,2	8612003	72h	8,2	5764003	5h	6,2	20821003	5h	6,6
33480003	7h	7,7	8612003	72h	8,2	58221003	1h	7,4			

**129 consecutive cases of breast cancer, stored in UVS at 4°C for a time between 1 and 72 hours (means 23h), before grossing.**

**A specimen (punch biopsy) was taken, immersed in RNA later® and sent for Gene Expression Analysis (GEA).**

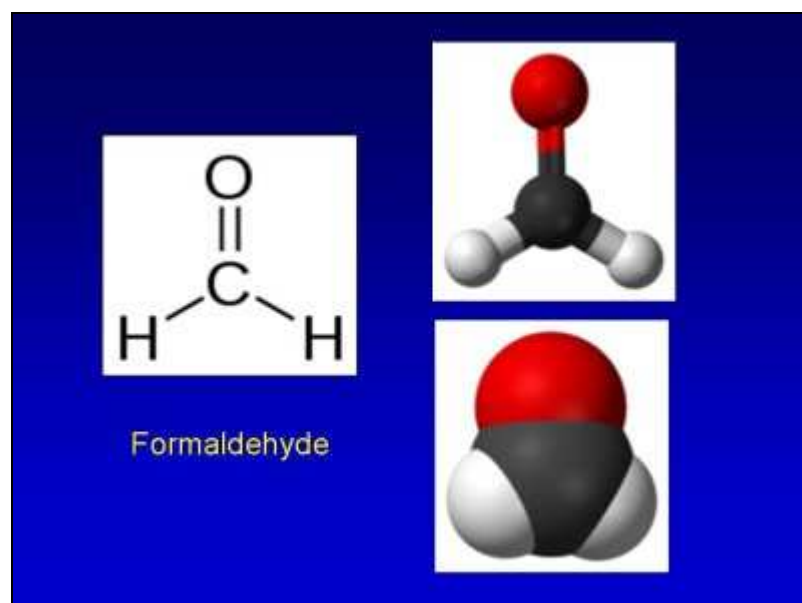
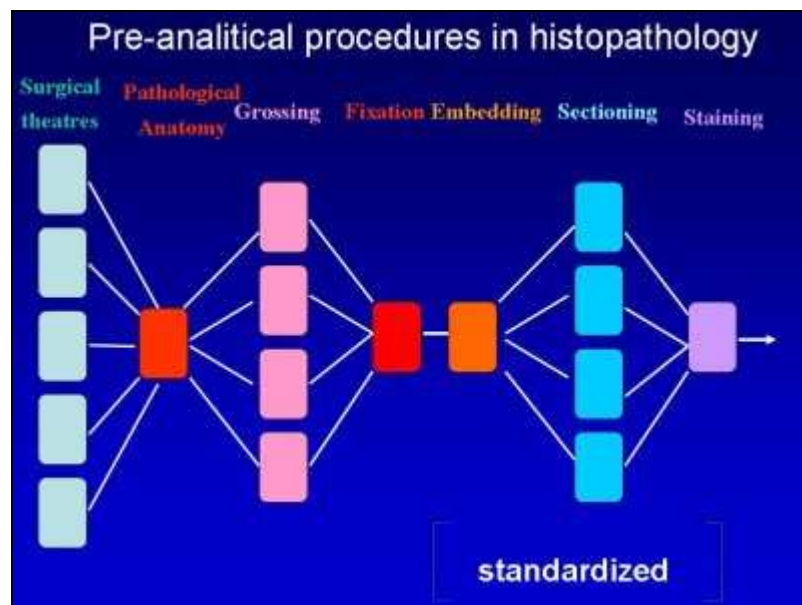
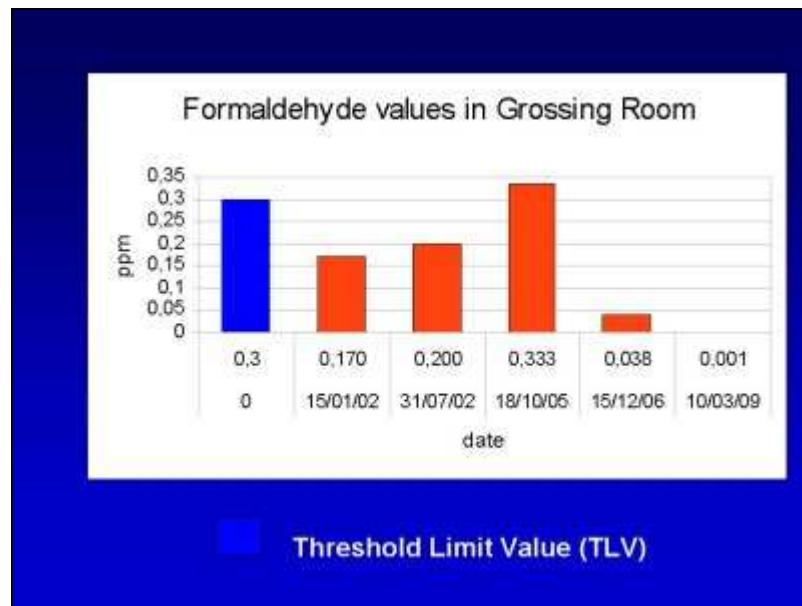
**Evaluation of RNA values proved that in all cases the material was fit for GEA analysis (RIN value mean 7.9)**

### Alternative c) Tissues preserved under vacuum

#### Merits:

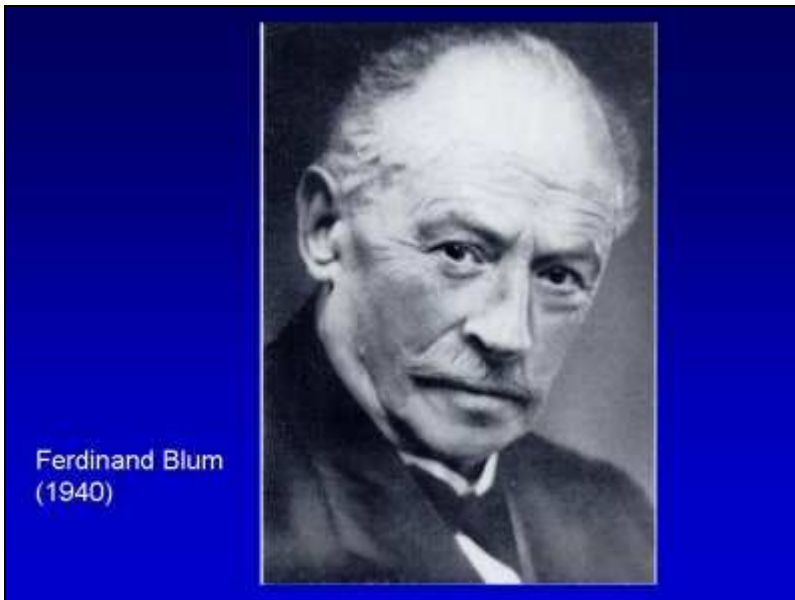
- No more formalin in surgical theatre (except for small specimens, where pre-filled tubes are employed)
- No spilling
- No fumes
- No drying of tissues
- Colours preserved
- Lack of insulating air around tissues allows fast cooling
- Tissues (bags) light and easy to carry
- Structure (RNA, Antigens)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





## 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)



## Formalin Fixation:

Advantages	Disadvantages
<ul style="list-style-type: none"> <li>• Cost</li> <li>• Safe for tissue</li> <li>• Fast penetration</li> <li>• Excellent morphology</li> <li>• ICC</li> </ul>	<ul style="list-style-type: none"> <li>• Unsafe for operators</li> <li>• ICC</li> <li>• Effect on Nucleic Acid</li> </ul>



**Edwin Klebs**  
 1834-1913



**Histology  
 (morphology)**



**Causes and seats of diseases**

## AIMS

### Preservation of

- Structure (morphological diagnosis)



## AIMS

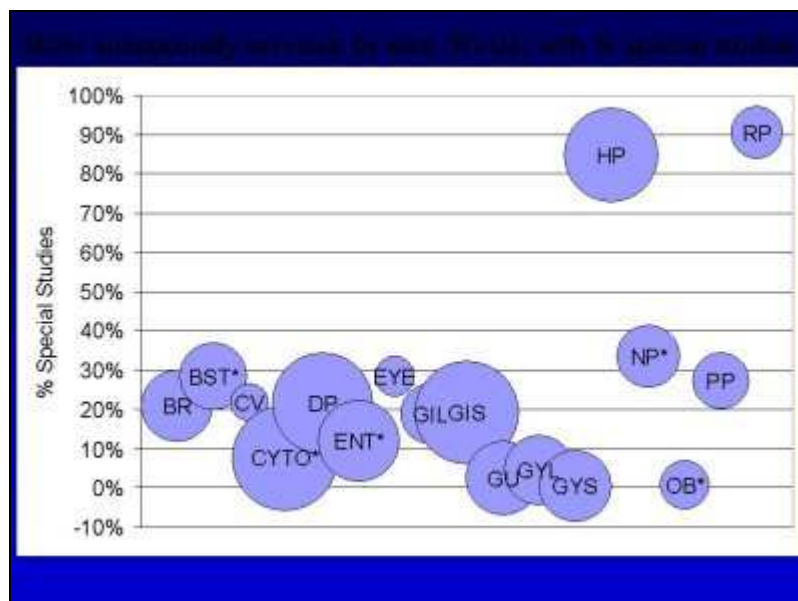
### Preservation of

- **Structure (morphological diagnosis)**
- **Proteins (Immuno-histochemistry)**

The Journal of Histochemistry and Cytochemistry  
 vol. 39, No. 6, pp. 741-748, 1991

**Antigen Retrieval in Formalin-fixed, Paraffin-embedded Tissues: An Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections**

SHAN-RONG SHI, MARC E. KEY,<sup>1</sup> and KRISHAN L. KALRA

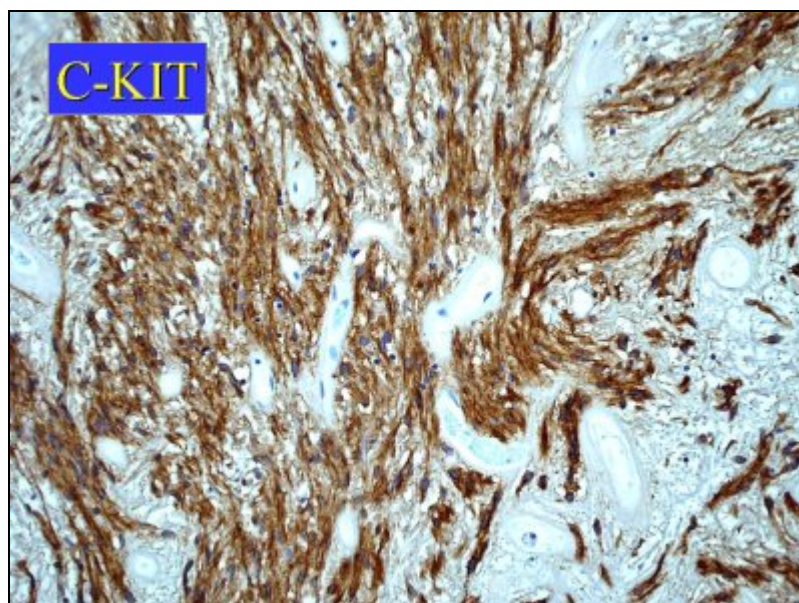






## The KIT revolution

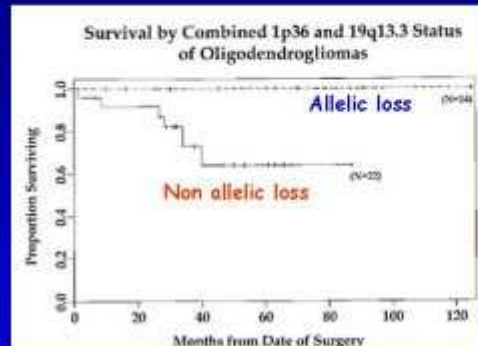
- KIT immunoreactivity defines a group of tumors showing differentiation towards interstitial cells of Cajal
- Activating *KIT* mutations
- Expression of KIT protein (CD117)
- Reliable phenotypic marker



# Alterations of Chromosome Arms 1p and 19q as Predictors of Survival in Oligodendrogliomas, Astrocytomas, and Mixed Oligoastrocytomas

By Justin S. Smith, Aris Perry, Thomas J. Barnell, Hyun K. Lee, Judith O'Fallon, Sandra M. Housh, David Kimmel, Allen Yates, Peter C. Burger, Bernd W. Schellhauer, and Robert D. Jenkins

J Clin Oncol. 2000 18(3):636-45.



## Pharmacopathology

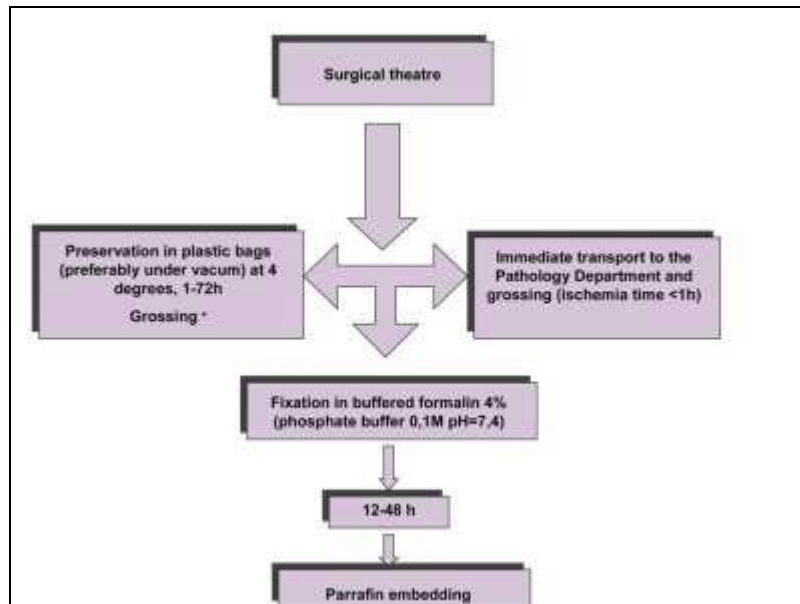
Receptors status (as evaluated by pathologists) of clinical - therapeutic interest in solid tumors

- ER/PgR
- HER<sub>2</sub>
- CD 117
- VEGFR
- SSTR<sub>1-5</sub>
- EGFR

## 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





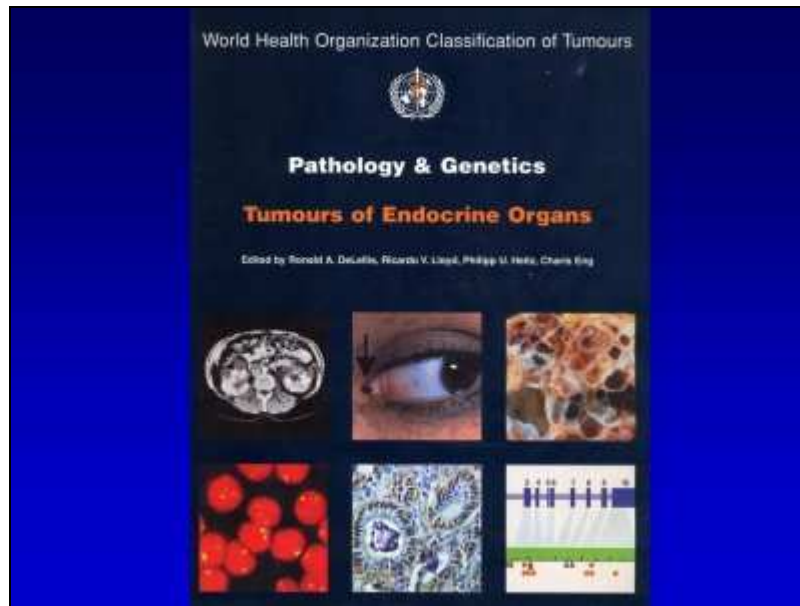
## Pharmacopathology

Receptors status (as evaluated by pathologists)  
 of clinical - therapeutic interest

### Problems involved

- Technique ( procedure, reagents, specificity, reproducibility)
- Evaluation ( which, where, what, when )
- Quantification ( operator; objectivity; visual vs. automatic )





Virchows Arch (2012) 460:129–130

#### INVITED COMMENTARY

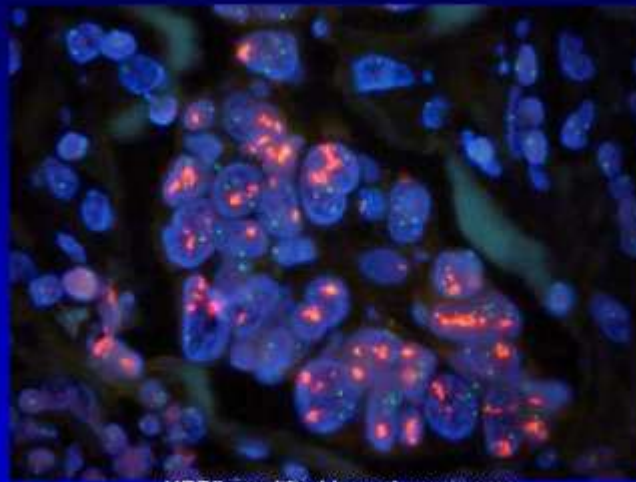
The times have changed:  
molecular pathology is here to  
stay

J. Han van Kreken & Gerald Hoefler

## AIMS

### Preservation of

- **Structure (morphological diagnosis)**
- **Proteins (Immuno-histochemistry)**
- **Nucleic Acids ( FISH + GEP )**



*HER2* amplified breast carcinoma  
Peripheral *HER2* gene signals

### RNA yield from FF-PET

- a) 10-15% of total
- a) Effect of strong protein-nucleotide bonds (?)
- b) Fragmented

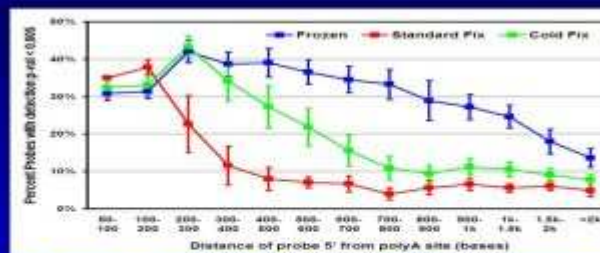
### Alternative tissue fixation

- RNA later
- HOPE
- Ethanol
- Methacarn
- M.W.

**PLoS One.** 2011;6(6):e21043. Epub Jun 15

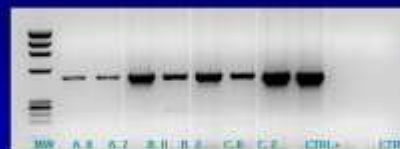
# A formalin fixation procedure preserving nucleic acid integrity.

Gianni Bussolati, Laura Annaratone, Enzo Medico, Giuseppe D'Armento and Anna Sapino.



Bussolati et al., Figure 3. Messenger RNAs from Cold-Fixed samples are detected by microarray probes hybridizing more than 500b upstream from the reverse transcription start site. Graph showing the fraction of probes with detectable signal (y-axis) for each bin of distance of target sequence from the mRNA poly(A) site from which RT is initiated (x-axis).

## Assessment of RNA conservation in Cold-Formalin Fixed Paraffin Embedded Tissue blocks after two years



RT-PCR for detection of CK-20 in colorectal cancer (500bp).

### Samples:

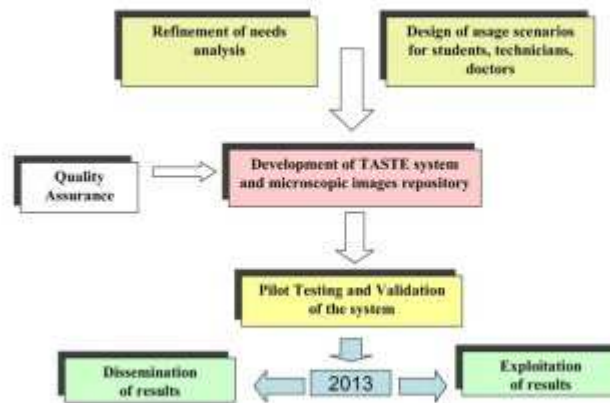
- A-0 RNA extraction year 2010
- A-2 RNA extraction year 2012
- B-0 RNA extraction year 2010
- B-2 RNA extraction year 2012
- C-0 RNA extraction year 2010
- C-2 RNA extraction year 2012

# MORPHOLOGY

IS

## Gene Expression profiling

**TASTE** - Telepathological Assessment of histopathological and cytological Techniques  
Project number : 519108 - LLP-2011-IT-KA3-KA3MP  
Grant Agreement number: 2011-4018/001-001 KA3 – ICT-Multilateral projects



Final Goal: Improvement of technical preparations of Histo- and Cyto-pathology at European level.





E. Ovcin  
First TASTE workshop  
Falun June 1, 2012

## The taste project

With the support of the Lifelong Learning Programme of the European Union



## Index

- The Partnership
- The Management Board
- Concerns
- TASTE Objective
- TASTE Target
- TASTE Goals
- Timeline
- The TASTE Project Workplan
- Where we are now & Next steps
- Info

## The Partnership



Consorzio per la Ricerca  
e l'Educazione Permanente  
Italy



Instituto de Patologia e Imunologia  
Molecular da Universidade de Porto  
Portugal



Institutul National de Cercetare Dezvoltare in Domeniul  
Patologiei si Stiintelor Biomedicale "Victor Babes"  
Romania



Università degli Studi di Torino  
Dipartimento di Scienze Biomediche ed Oncologia Clinica  
Italy



Institut Jules Bordet  
Belgium



Dalarna County Council Hospital  
Laboratory Medicine Dalarna Department of  
Pathology and Clinical Cytology  
Sweden

## The Management Board

- **COREP:**
  - Gianni Bussolati - Scientific coordinator
  - Emanuela Ovcin - Project Manager
- **UNITO:** Anna Sapino
- **IPATIMUP:** Fernando Schmitt
- **IJB:** Francesco Feoli
- **IVB:** Maria Comanescu
- **FALUPAT:** Tibor Tot

## Concerns

**Histological and cytological preparations are the basis for pathological diagnosis** performed in daily practice throughout Europe in a number of several millions per year and correctness and reproducibility of such diagnosis are heavily dependent on the technical quality. Yet, **quality is variable in different places and countries**, related to school level, technicians' dedication, standard of apparatuses and reagents. What is even more important, **variation in technical quality** of the preparations **prevents their open circulation at European level.**

## TASTE Objective

- The TASTE project tackles the abovementioned problems by building-up an ICT environment -**TASTE System**- whereby users from different countries will submit via Web, using Tele-pathology procedures featuring "virtual slides", the microscopic images of their own preparations to a panel of internationally recognized experts who will give comments and suggestions.



## TASTE Target

### ■ Primary Target

- **Technicians** working on histological and cytological preparations (about 15.000 in EU)
- **Doctors** specialists in Anatomic Pathology (about 12.000 in EU)
- **Students** becoming technicians and residents training in pathology

## TASTE Target

### ■ Secondary Target

- European Society of Pathology
- Associations of professionals
- Universities
- Hospitals
- National Health systems

## TASTE Goals

1. The identification of **TASTE usage scenario** for each kind of user (doctors, technicians and students), both for training and VET (Vocational Education and Training) purposes then to join a **community** where comparing preparations/ diagnosis assessed by experts. These scenarios will be defined tailored to the characteristics of the end users and assessed with real users.

## TASTE Goals

2. The realization of a **TASTE system** which will combine a fully sustainable system of e-learning and lifelong learning solutions with a microscopic images and slides repository (a sort of "virtual microscope") collected and continuously updated in different languages;

## TASTE Goals

- 3) The set up of a **TASTE virtual community** and network where students and experts in the field of histology, cytology and anatomy-pathology could compare preparations and diagnosis with a large number of already developed cases and in continuous update.

## Timeline

- Period: Nov 2011-Oct 2014, **3 years project**
- **October 2012:**
  - First TASTE System Demo
  - Needs Analysis
  - Desing of Taste Users Scenario
  - Set up TASTE community
- **June 2013:** 1° version of TASTE repository of images and slides
- **October 2014:** Final version of the TASTE system and database.

## TASTE project workplan (+ leader)

- WP1: Project management (COREP)
- WP2: Refinement of Needs analysis (IPATIMUP)
- WP3: Design of TASTE users scenarios (IVB)
- WP4: Development of TASTE system and microscopic images repository (UNITO)
- WP5: Pilot Testing and Validation (COREP)
- WP6: Quality Assurance (COREP)
- WP7: Dissemination (IJB-ULB)
- WP8: Exploitation (UNITO)

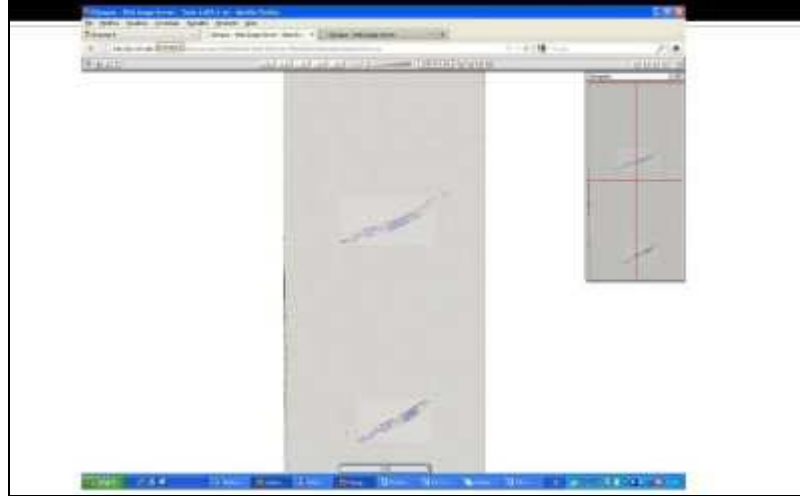
## Where we are now & next steps

- First demo of the system
- Developing scenario
- Preparing investigation with real users (want to join?)
- Setting up images and slides repository

## Where we are now? -SYSTEM



## Where we are now? DATABASE



## Info

- Thanks for your attention!
- For further info: **[www.tasteproject.eu](http://www.tasteproject.eu)**
- To join our network:
- **[info@tasteproject.eu](mailto:info@tasteproject.eu)**



## The TASTE system: design of the users scenarios

Maria Comanescu

With the support of the Lifelong Learning Programme of the European Union



- Designing the most appropriate TASTE users scenarios to be adopted in the innovative TASTE system, considering several aspects:
  - contents,
  - learning infrastructure/ICT-enabled learning tools
  - methodology.
- It focuses on the definition of specific scenarios for the different target groups, that will access a common framework of applications, but will have at their disposal different functionalities according to their real needs
- Preliminary users scenarios will be designed and proposed to relevant target groups.



- However, only final users after a trial/simulation can suggest improvements or define smart changes.
- For these reasons, prototype of scenarios will be presented to representative groups of potential final users, in order to obtain information on their needs as well as on functionalities they perceive to be useful or required to meet their purposes.
- On the basis of feedbacks received, scenarios will be refined and designed in their final version





- Short description – create a website pathways that will help students/doctors realising which are the criteria that make a histological/cytological preparation of low/high quality and ultimately improving the quality of slides, through examples - PROBLEM SOLVING THROUGH CASE STUDIES.



- 4 categories of access for each one of the defined categories:
  - students-technicians,
  - students-doctors/residents,
  - workers-technicians/biologist,
  - workers-doctors.
- From each category, the participant will be directed to a quiz.



- Digital Slidebox “Mottura” is a digital slide and multimedia management system allowing users to create their web-based digital resources.
- It can be used to exploit the power of digital slides in education, training and External Quality Assurance applications.
- The project aims at combining this system with the Course Management System “Moodle” (Modular Object-Oriented Dynamic Learning Environment).



- Thanks to the combined system, from the one hand a dedicated web based repository will host images and slides, from the other hand, according to TASTE users scenarios the system will be developed taking advantage from various functionalities offered by Moodle (chat, forum, other utilities) and will be tailored to the learning/training/networking objectives of different target users.



- First selection will refer to the 2 big areas:
- **CYTOLOGY – cervical, mammary, urinary, FNAB**
- **HISTOLOGY- punch biopsy, surgical specimens.**
- The correct pathways to follow will be decided by the experts and the participants will be guided to follow this correct pathways.



- 1) Quiz with a numerical answer to be included.  
Example:  
– On Mottura presents 3 cases of different preparations, in Moodle enter the N° of the optimal preparation.
- 2) Quiz with a numeric answer such as "matching".  
Example:  
– On Mottura presents 3 cases of different preparations. Moodle presents 3 possible alternatives. Enter the number corresponding with the case to your judgment until you have completed the 3 alternatives.
- 3) Quiz with electronic response of "matching".  
Example:  
– On Mottura present 3 cases of different preparations. Moodle presents 3 possible alternatives. Combine the matches by clicking on the case + alternative.



- 4) Long texts.  
 Example:
  - Case X corresponds to a description, with one or more words to be inserted.
- 5) Selection of alternatives  
 Example:
  - Presentation of two preparations. Select which presents the alteration X.
- 6) Choosing between different areas of the same preparation.  
 Example:
  - In a preparation areas are selected and marked with a circle and a number. The quiz is required to identify the number corresponding to the alteration.
- 



- 7) Comparison between preparations made with the same staining on serial sections (or several smears) of the same case  
 Example:
  - Identify in a multiple choice quiz application, which artifact is/is not present on one of the two preparations.
  - Choose between two preparations of the same case, which is preferred.
- 8) Comparison between the preparations made in different stainings (histological, histochemical, cytologic or immuno-histochemistry) of serial sections (or several smears) of the same case  
 Example:
  - Identify in a multiple choice quiz, which number corresponds to a certain color preparation.
  - Matching quiz

*Thank you!*

## Neuro-endocrine tumours: Update on the classification and future prospects

**Gianni Bussolati.**  
University of Turin, Italy and  
COREP

### DIFFUSE NEURO-ENDOCRINE SYSTEM

#### PROPERTIES

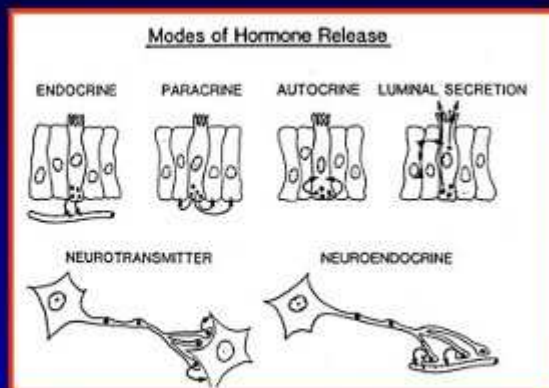
##### Function

##### Mechanisms

endocrine

paracrine

autocrine



Products: hormones & amines

Argyrophilia / Argentaffinity

### DIFFUSE NEURO-ENDOCRINE SYSTEM

Common Features

• Structural

• Differentiation Functional (Markers) →

• Embriologic (Neuro-crest)

• Pathology →

Hyperplasia  
↓  
Neoplasia  
Indolent ——— Aggressive

? Drug Sensitivity ?

**“Granules” + Vesicles at EM**

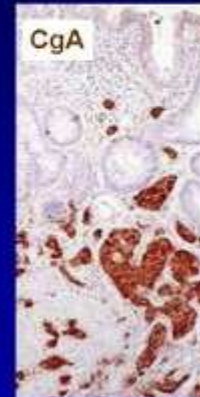




## Immunohistochemical markers in NENs: *definition of NE phenotype*

### Pan-endocrine markers

- ✓ Cytosolic (NSE, PGP 9.5)
- ✓ Related to secretory granules (**chromogranin A**)
- ✓ Related to synaptic vesicles (**synaptophysin**, VMAT)
- ✓ Intermediate filaments (NF, CK HMW)
- ✓ Adhesion molecules (N-CAM)



*Modern Pathology* advance online publication 2 March 2012

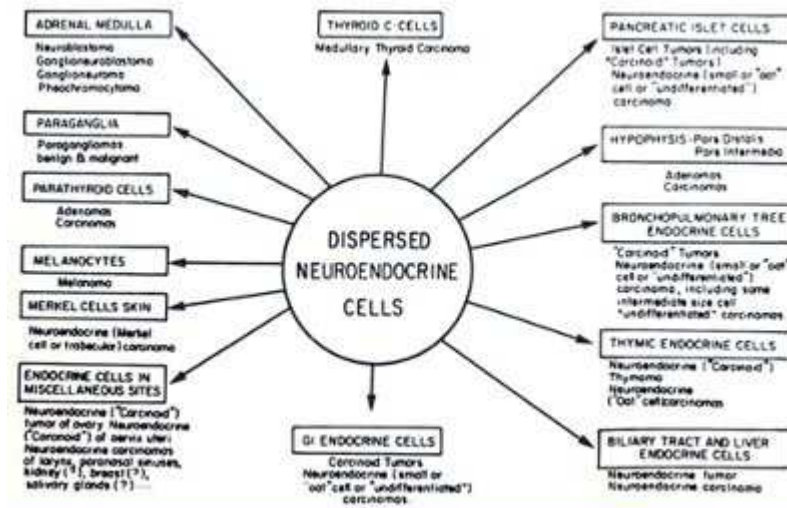
Value of Islet 1 and PAX8 in identifying metastatic neuroendocrine tumors of pancreatic origin  
 Jamie Koo, Richard B Mertens, James M Mirocha, Harlin L Wang and Deepthi Dhall

Site	n	Islet 1 (%)	PAX8 (%)	TTF1 (%)	CDX2 (%)
Pancreas	33	27 (82)	29 (88)	0	0
Pulmonary	31	2 (6)	7 (23)	16 (52)	0
Ileum	23	0	0	0	20 (87)
Rectum	14	12 (86)	11 (79)	0	0
Stomach	9	0	2 (22)	0	0

### Definition of NE phenotype

- ✓ Compromise between sensitivity and specificity
- ✓ Do not rely on a single marker to establish or disprove the diagnosis of NEN
- ✓ Immunohistochemical findings must be interpreted in the context of the microscopy (and, if necessary, the clinical and biochemical picture)





V. Gould and R. De Lellis, 2003

NE Tumours

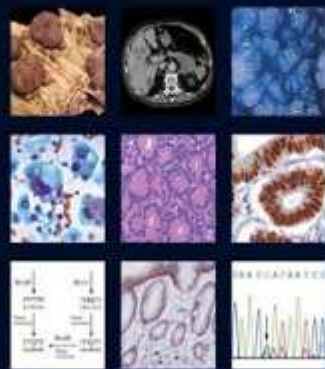
GEP

Lung

Breast

### WHO Classification of Tumours of the Digestive System

Edited by Fred T. Bosman, Filipe Carneiro, Rajen H. Hruban, Neil D. Theise



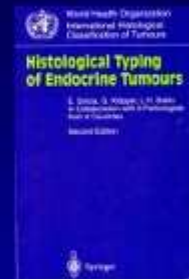
## WHO 2000 classification of GEP endocrine tumors

**Combined clinico-pathological parameters...**

location, diameter, angioinvasion, presence of metastases

**... and functional data (clinico-pathological correlates)**

type of hormonal secretion and clinical syndrome eventually present



## WHO 2000 classification of GEP endocrine tumors

**Well-differentiated endocrine tumor**  
 - benign/uncertain behavior



**Well-differentiated endocrine carcinoma**  
 - Low grade malignant



**Poorly differentiated endocrine carcinoma**  
 - High grade malignant



**Mixed Exocrine-Endocrine carcinoma / MEEC**

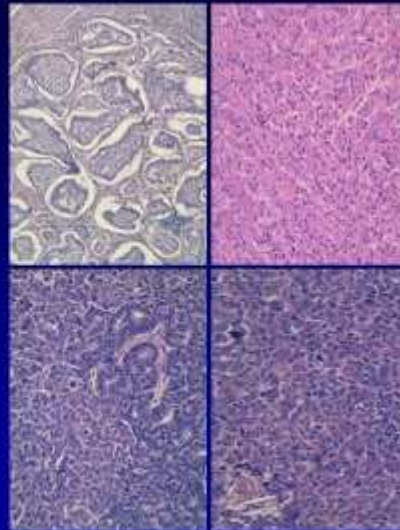
## Neuroendocrine Neoplasms WHO Classification 2010 of the Digestive System

- **Working principles**
  - “Neuroendocrine” defines the peptide hormone-producing tumours and share neural-endocrine markers
  - “Neuroendocrine neoplasm” includes well- and poorly differentiated tumours
- **Premise: All neuroendocrine neoplasms (NENs) have a malignant potential**

Bosman FT, et al. WHO Classification of Tumours of the Digestive System. Lyon, France: IARC Press; 2010.

## Morphological patterns in NENs

- ✓ Insular (nodular solid nests with peripheral invading cords)
- ✓ Trabecular (anastomosing trabeculae or ribbons)
- ✓ Glandular (tubules, acini or rosettes)
- ✓ Poorly differentiated with no well-organized growth pattern

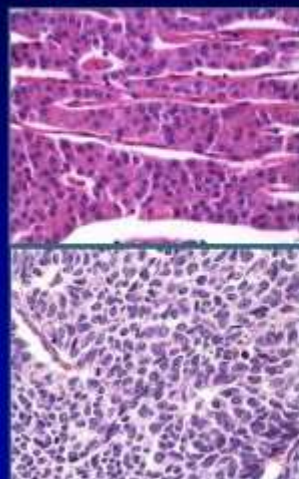


NEN vs. non-NEN = Morphology + Markers

## Neuroendocrine Neoplasms WHO Classification 2010 of the Digestive System

WHO 2000	WHO 2010
Well-differentiated endocrine tumour (WDET)	Neuroendocrine tumour
Well-differentiated endocrine carcinoma (WDEC)	
Poorly differentiated endocrine carcinoma/small-cell carcinoma (PDEC)	Neuroendocrine carcinoma

## Neuroendocrine Neoplasms WHO Classification 2010 of the Digestive System



Neuroendocrine  
tumor/NET (Carcinoid)

NET vs. NEC = Structure + Grade

Neuroendocrine  
carcinoma / NEC



## Grading of GEP-NENs According to ENETS/WHO/AJCC

Grade	G1	G2	G3
Ki67 index	≤2	3–20	>20
MI	<2	2-20	>20



1. Rindi G, et al. *Virchows Archiv*. 2006;449:395-401. 2. Rindi G, et al. *Virchows Archiv*. 2007;451:757-762.

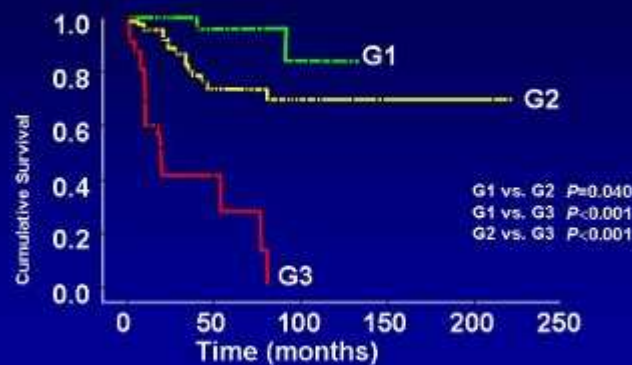
## Ki67 Counting

- **Options to quantify Ki67**
  - Systematically counting a defined number of tumors cells (500-2000) and calculating the positive percentage
  - Using a computerized digital image analysis system to measure the positive percentage
  - A general “eyeballed” estimate of the percentage of positive cells
- The result should be reported as a **single percentage** reflecting the average of the regions counted, rather than a range of value

## Mitotic index evaluation

- ✓ A total of **50 fields** should be counted
- ✓ The mitotic rate should be expressed based on **the number in 10 high power fields (2 mm<sup>2</sup>)**

## Grading of GEP-NENs according to ENETS/WHO/AJCC



Pape UF et al. *Cancer*. 2008;113:256-265.

## TNM Classification of GEP-NENs

- ✓ SITE-specific
- ✓ Based on depth of invasion and size

### ENETS: 2006/2007

Rindi, Klöppel, Ahlman, Wiedenmann. TNM staging of foregut, midgut and hindgut (neuro) endocrine tumours: A consensus proposal including a grading system. *Virchows Archiv*. 2006;449:395-401, and 2007;451:757-762.

### UICC/AJCC: 2009

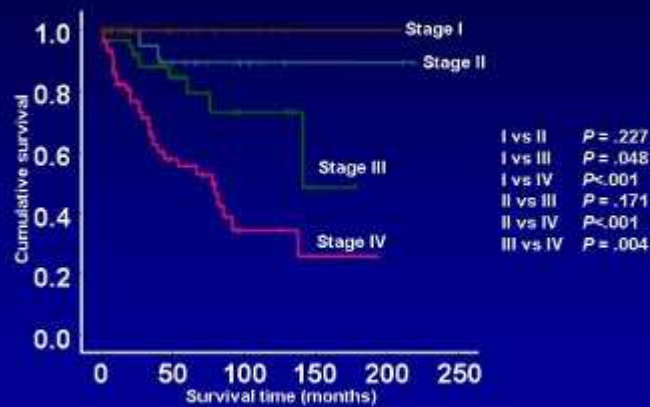
Sobin, Gospodarowicz, Wittekind. *TNM Classification of Malignant Tumours*. Wiley-Blackwell. 7th Edition; 2009.

## Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

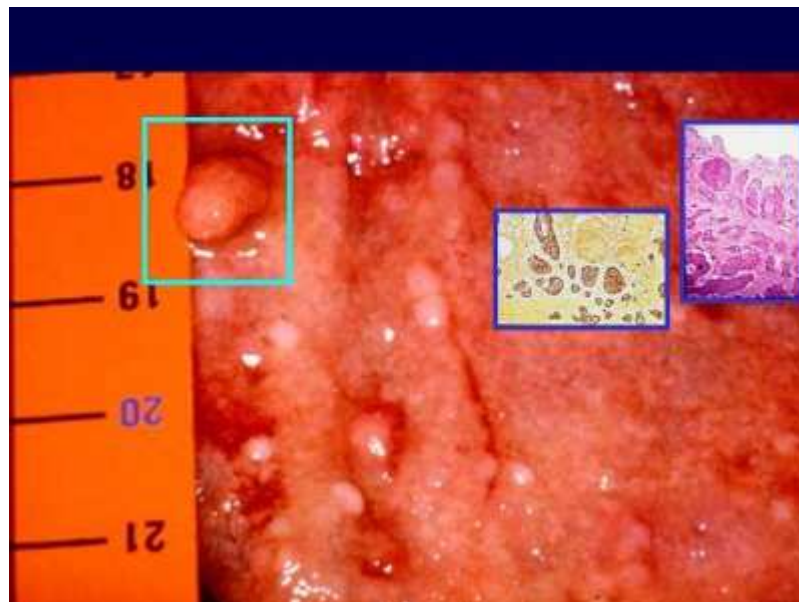
- ✓ Similar TNM classifications:
  - Stomach
  - Duodenum
  - Jejunum/ileum
  - Colon/rectum



## Staging of upper digestive NENs according to ENETS/WHO/AJCC

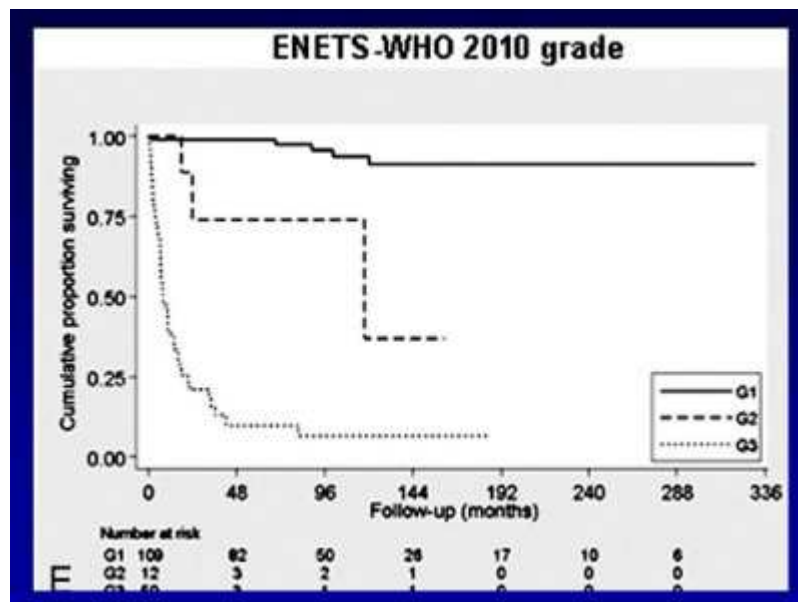
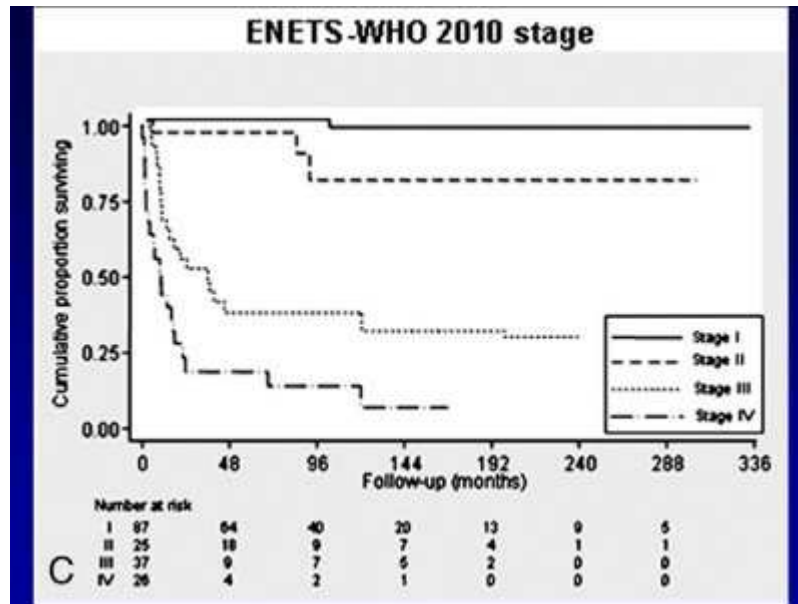


Pape UF et al. *Cancer*. 2008;113:256-265.



## Histologic characterization and improved prognostic evaluation of 209 gastric neuroendocrine neoplasms

La Rosa et al *Human Pathology* (2011) 42, 1373–1384



**The European NeuroEndocrine Tumor Society–World Health Organization 2010 staging system proved effective. Introduction of novel T (T1a and T1b or deep submucosal) and N categories (N1, <3 nodes metastases; N2, ≥3) allowed a simplified, equally informative 3-stage TNM system.**

La Rosa et al. 2011

## Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

### ✓ Similar TNM classifications:

- Stomach
- Duodenum
- Jejunum/ileum
- Colon/rectum

### ✓ Different TNM classification

- Appendix
- Pancreas

J Clin Oncol. 2011 Aug 1;29(22):3044-9.

**Prognostic validity of a novel American Joint Committee on Cancer Staging Classification for pancreatic neuroendocrine tumors.**

Strosberg JR, Cheema A, Weber J, Han G, Coppola D, Kvols LK.

**Both the novel AJCC classification and the ENETS classification were highly prognostic for survival.**

Strosberg et al. 2011

## T Category Criteria for Appendiceal NENs is Different Between ENETS and UICC/AJCC

	ENETS TNM	UICC/AJCC TNM
T1	≤1 cm; invasion of muscularis propria	T1a: ≤1cm T1b: >1– 2 cm
T2	≤2 cm; and <3 mm invasion of subserosa/ mesoappendix	>2–4 cm; or invasion of cecum
T3	>2 cm; or >3 mm invasion of subserosa/ mesoappendix	>4 cm; or invasion of ileum
T4	invasion of peritoneum/ other organs	invasion of peritoneum/ other organs

### NET of the Appendix



**TUMOR STAGING BUT NOT GRADING IS ASSOCIATED TO ADVERSE CLINICAL OUTCOME IN NEUROENDOCRINE TUMORS OF THE APPENDIX: A RETROSPECTIVE CLINICAL PATHOLOGICAL ANALYSIS OF 138 CASES.**

Volante et al. 2011 (sent for publication)



pT stage and positive resection margins are the best predictors of adverse outcome in the small proportion of clinically aggressive cases here analyzed.

Volante et al. 2011

...only four patients died of the disease. Adverse outcome was significantly associated with extra-mural extension (including mesoappendix), well-differentiated carcinoma diagnosis (2000 WHO classification), pT3-4 stage, older age and presence of positive resection margins, but not with tumor size, nor mitotic or proliferative indexes, and consequently not with the 2010 WHO categories.

Volante et al. 2011

## Pathology report of NENs

- Define **location** and **tumor type** based on WHO classification
- Define **tumor grade** (including Ki-67 proliferative index)
- Describe the presence of **additional histologic features** (multicentric disease, non-ischemic tumour necrosis, vascular or perineural invasion)
- Assess the **TNM stage**
- Define the **resection margins**
- Define the **hormonal production**, if any

*Upon request, assess prognostic or predictive factors useful for target therapy (e.g. somatostatin receptors, mTor pathway molecules, other target enzymes, ...)*

See also: Klimstra D, et al. *Am J Surg Pathol.* 2010;34:300-313.





Expression of Somatostatin receptor types 1-5 in 81 cases of gastrointestinal and pancreatic endocrine tumors. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis.

Papotti M, Bongiovanni M, Volante M, Allia E, Landolfi S, Helboe L, Schindler M, Cole SL, Bussolati G.

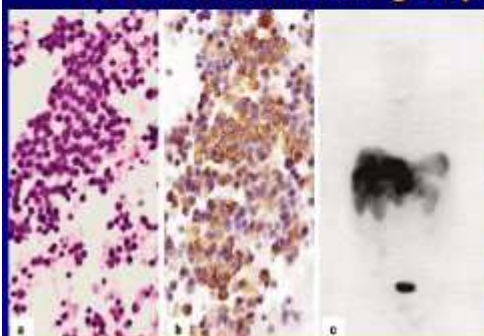
Virchows Arch. 2002 May;440(5):461-75

**Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy**

Modern Pathology (2007) 20, 1172-1182

Marco Volante<sup>1</sup>, Maria Pia Brizzi<sup>1</sup>, Antoniazio Faggiaro<sup>2</sup>, Stefano La Rosa<sup>1</sup>, Ida Rapa<sup>1</sup>, Anna Ferrero<sup>1</sup>, Gelsomina Mansueti<sup>1</sup>, Laisella Righi<sup>1</sup>, Silvana Garancini<sup>1</sup>, Carlo Capella<sup>1</sup>, Gaetano De Rosa<sup>1</sup>, Luigi Dogliotti<sup>1</sup>, Annamaria Colao<sup>3</sup> and Mauro Papotti<sup>1</sup>

**107 cases... including 41 pre-operative samples**



**Correlation with**

**Scintigraphy: 77%**  
(107 cases)

**Tx response: 75%**  
(28 patients)

## Integration of SSTR IHC and Somatostatin Analog-based Imaging (SRS):

### SSTR IHC

- ✓ Cost effective
- ✓ Detection of protein
- ✓ Identification of SSTR subtype
- ✓ Identification of cell type expressing SSTR
- ✓ Applicable retrospectively

### SRS

- ✓ Identification of "functional" receptors
- ✓ Detection of SSTR expression in the whole tumor mass

## Current therapy modalities in NETs

- ✓ Biotherapy (somatostatin analogs and interferon)
- ✓ Peptide receptor radionuclide therapy
- ✓ Cytotoxic treatment
- ✓ Targeted therapies

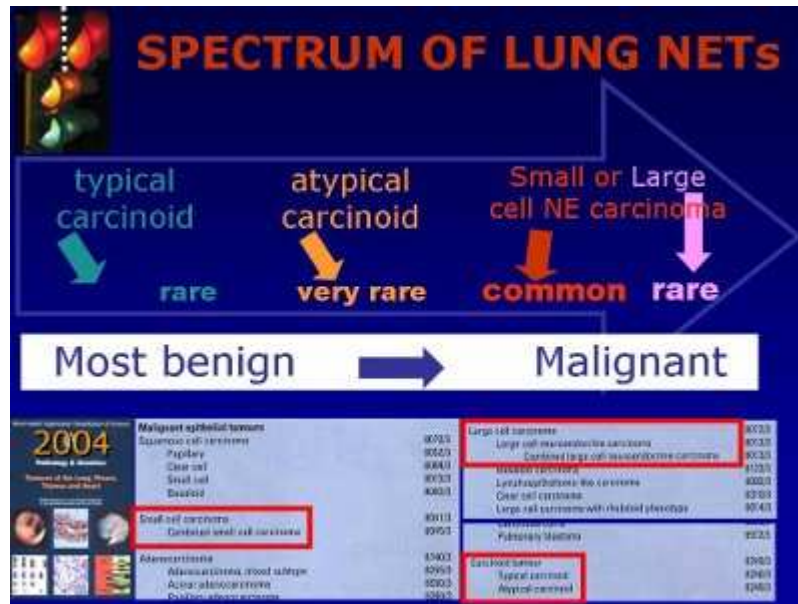
### Now in **GEP-NETs** only:

- NEN vs NET
- grade & TNM
- ➡ 2 groups
- ➡ 3 grades
- ➡ 4 stages



WHO Classification of Tumours of the Digestive System





**TC**

**AC**

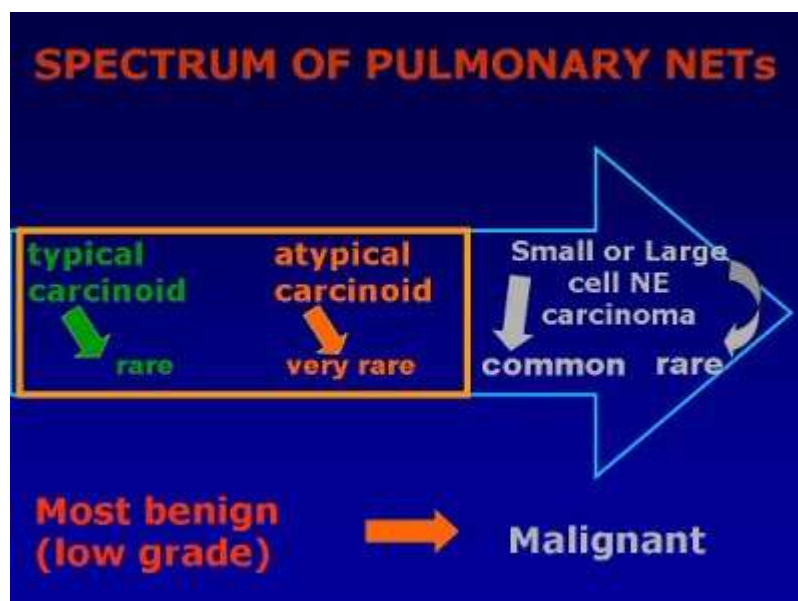
**LCNEC**

**SCLC**

- Different & separate subtypes in the WHO
- Different epidemiology and pathology
- Different therapy & clinical behavior
- Different Authors.....

among others

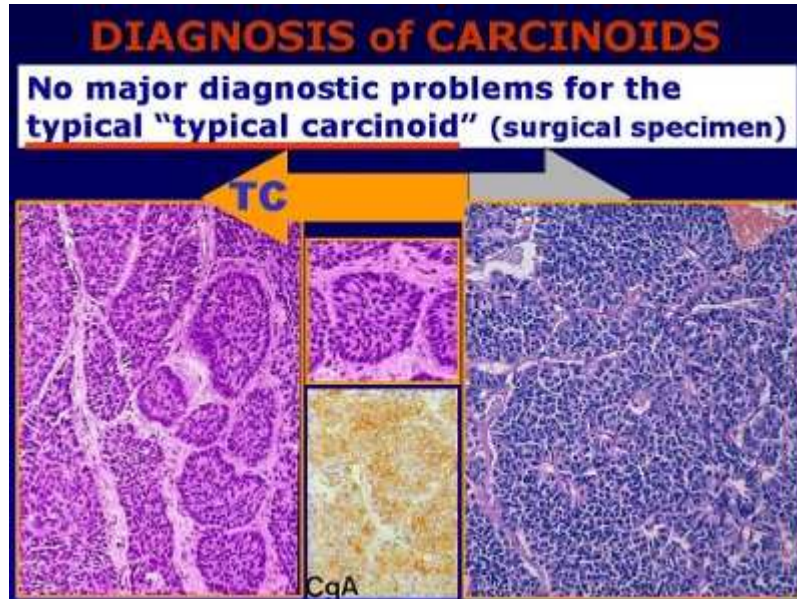
**SCLC**





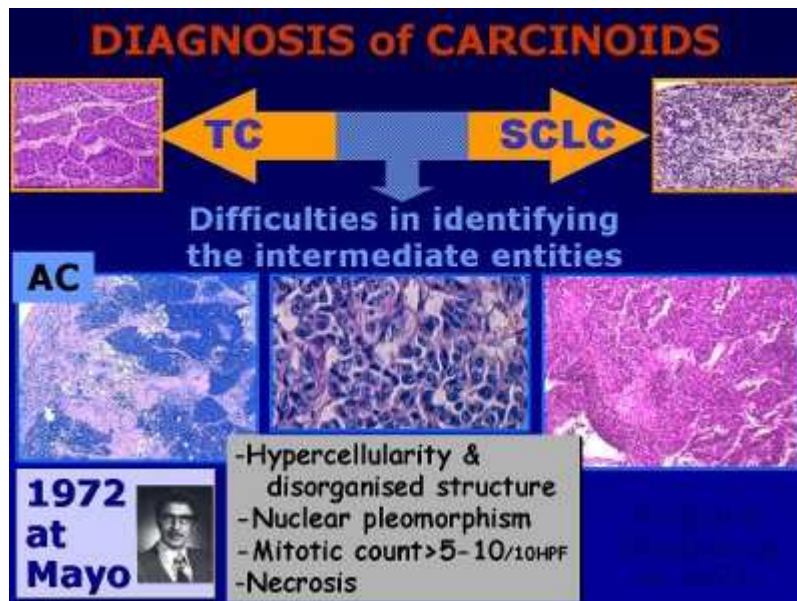
### DIAGNOSIS of CARCINOIDS

**No major diagnostic problems for the typical "typical carcinoid" (surgical specimen)**



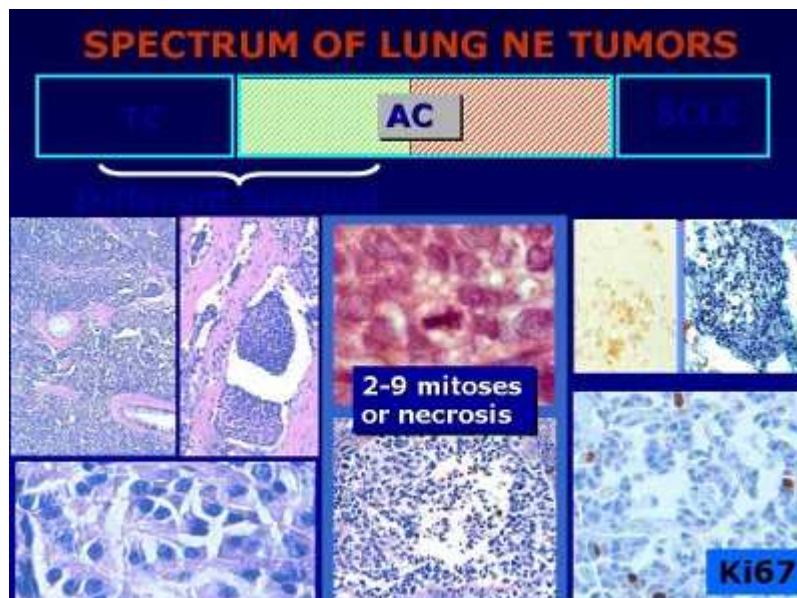
The diagram illustrates the diagnosis of a typical carcinoid (TC). It features a central orange arrow labeled 'TC' pointing to the right. To the left of the arrow is a large histological image of a typical carcinoid showing nests of uniform cells. To the right of the arrow is a large histological image of a small cell lung carcinoma (SCLC) showing a dense population of small, blue cells. Below the TC arrow, there are two smaller images: a histological image of a typical carcinoid and an immunohistochemistry (IHC) image labeled 'CgA' showing brown staining, indicating chromogranin A positivity.

### DIAGNOSIS of CARCINOIDS



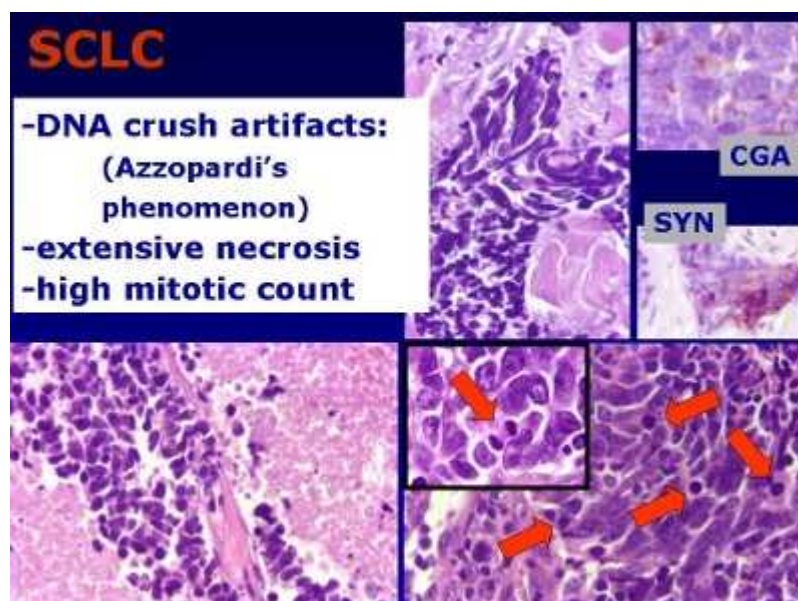
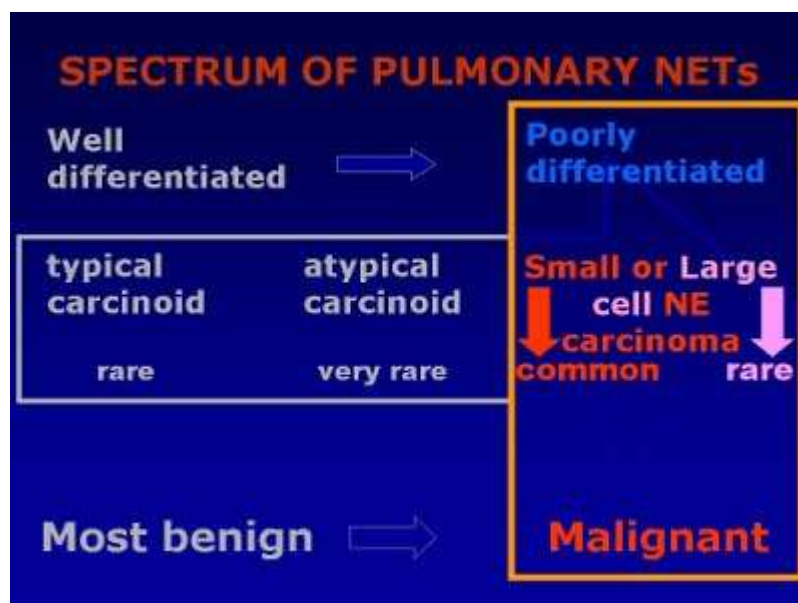
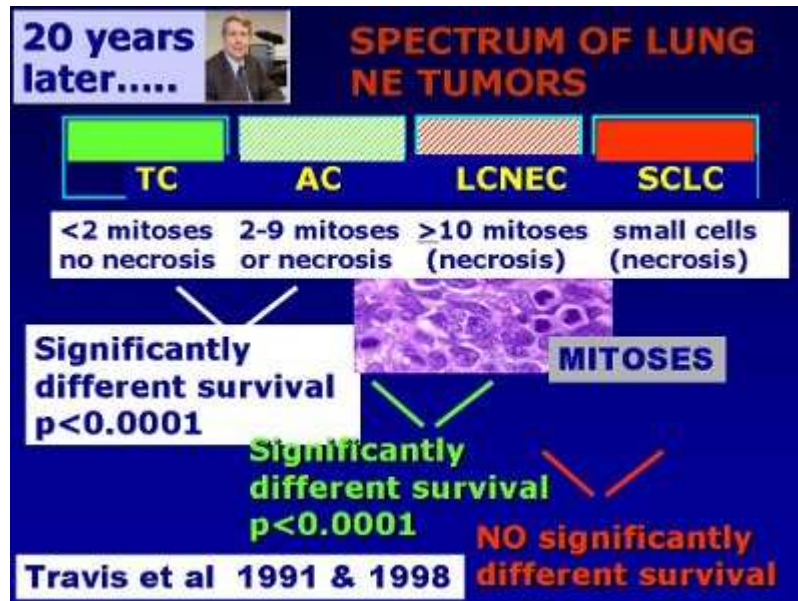
The diagram illustrates the difficulties in identifying intermediate entities between typical carcinoid (TC) and small cell lung carcinoma (SCLC). It features a central blue arrow pointing from left to right, with 'TC' on the left and 'SCLC' on the right. Below the arrow, the text 'Difficulties in identifying the intermediate entities' is displayed. To the left of the arrow is a small histological image of a typical carcinoid. To the right of the arrow is a small histological image of a small cell lung carcinoma. Below the central text, there are three histological images: one labeled 'AC' (atypical carcinoid) showing nests of cells with some nuclear atypia, and two others showing intermediate forms. A text box lists the following features: '-Hypercellularity & disorganised structure', '-Nuclear pleomorphism', '-Mitotic count >5-10/10HPF', and '-Necrosis'. To the left of this text box is a small portrait of a man and the text '1972 at Mayo'.


### SPECTRUM OF LUNG NE TUMORS



The diagram illustrates the spectrum of lung neuroendocrine tumors. It features a horizontal bar with four colored segments: a blue segment labeled 'TC' (typical carcinoid), a green segment labeled 'AC' (atypical carcinoid), an orange segment, and a blue segment labeled 'SCLC' (small cell lung carcinoma). Below the bar, there are several histological images. One image shows a cluster of cells with a label '2-9 mitoses or necrosis'. Another image shows a cluster of cells with a label 'Ki67'.







Small cell carcinoma Combined small cell carcinoma	 <b>Pathology &amp; Genetics</b> Tumours of the Lung, Pleura, Thymus and Heart	8041/3 8045/3
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**Classical oat cell type:**

- solid growth of small cells
- 3 lymphocytes in size
- dark nucleus
- Finely granular chromatin

**No major diagnostic problems**

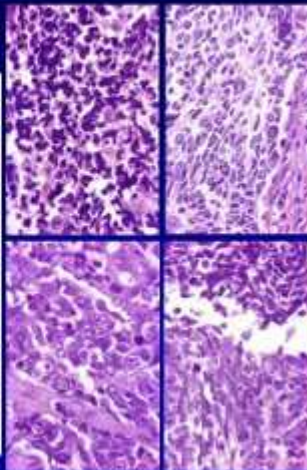


Small cell carcinoma Combined small cell carcinoma	 <b>Pathology &amp; Genetics</b> Tumours of the Lung, Pleura, Thymus and Heart	8041/3 8045/3
---	--	------------------

**HISTOLOGICAL TYPES**

**WHO 1981: A- oat cell, B- intermediate cells (round or spindled) C- combined with a NSCLC histotype**

**IASLC 1988 : A- oat cell, B- mixed small & large cell, C- combined small & non-small cell lung carcinoma**

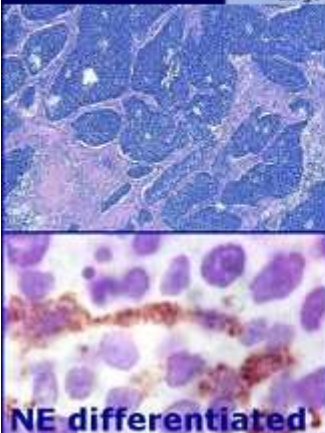


<b>LCNEC: HISTORY</b>	Large cell carcinoma	8012/3
	Large cell neuroendocrine carcinoma	8013/3
	Combined large cell neuroendocrine carcinoma	8013/3
	Basaloid carcinoma	8123/3
	Lymphoepithelioma-like carcinoma	
	Clear cell carcinoma	
	Large cell carcinoma with rhabdoid ph	

**NE differentiated**

**Travis WD, et al. Neuroendocrine tumors of the lung with proposed criteria for large-cell NE carcinoma.**  
 AJSP 1991;15:529-53

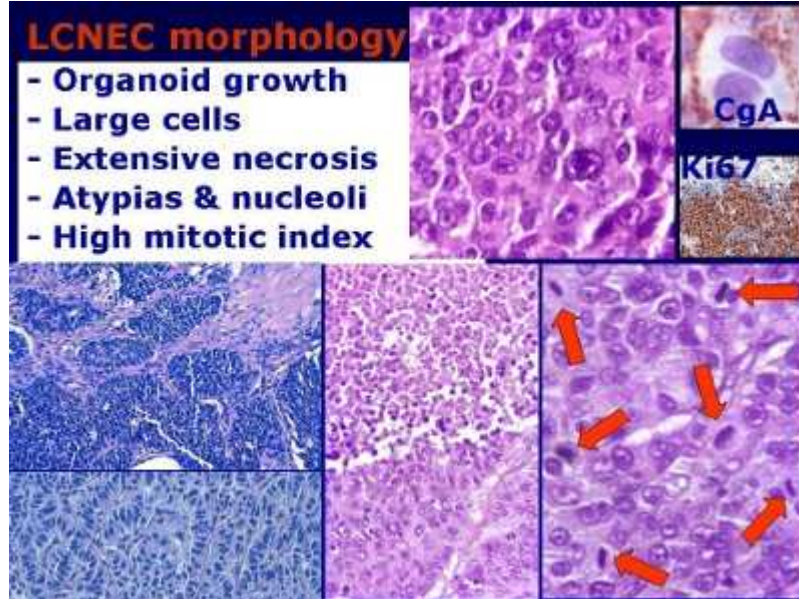
**Travis WD, et al. Survival analysis of 200 pulmonary NE tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid.**  
 AJSP 1998;22:934-44





**LCNEC morphology**

- Organoid growth
- Large cells
- Extensive necrosis
- Atypias & nucleoli
- High mitotic index




**DISTINGUISHING SCLC FROM LCNEC: does it matter?**

**Significant indicators for differential dx:**  
 organoid growth,  
 tumor cell size,  
 N/C ratio,  
 nuclear molding,  
 rosette formation,  
 prominent nucleoli



Sun, Pathol Int 2009

**However, large degree of overlap exists:**  
 eg: cell size  
 chromatin texture  
 immunoprofile



Hiroshima, Modern Pathol 2006

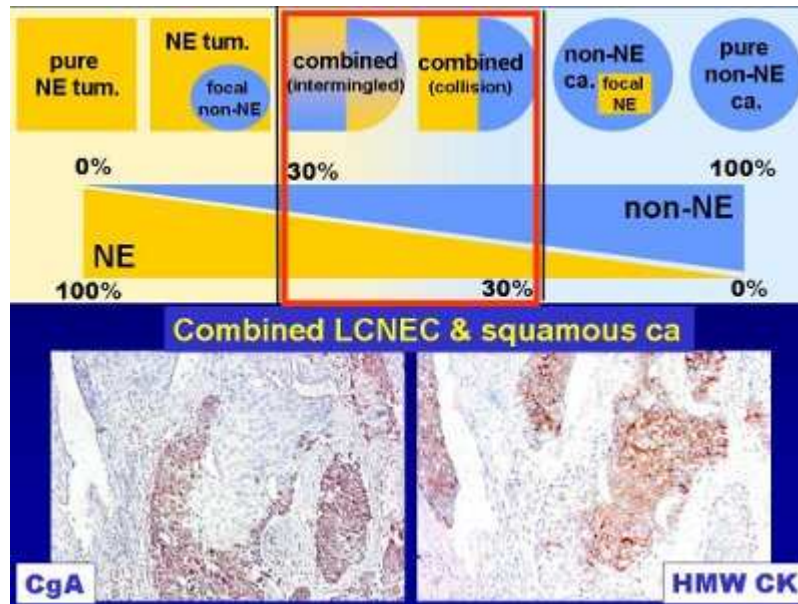
**In addition:**

- Similar behavior & survival.
- Similar therapy of LCNEC and SCLC



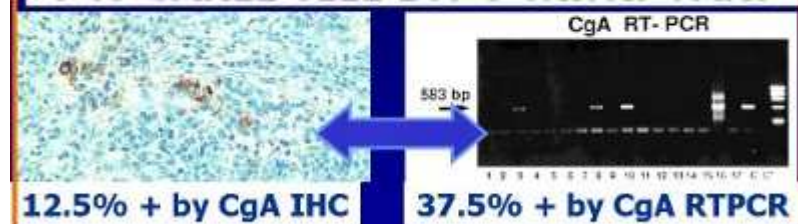
Asamura et al JCO 24, 70-76, 2006

**DISTINGUISHING SCLC FROM LCNEC: does it matter?**  
**NO !!!!!!!!!!!!!!!**



**Unfortunately,** the identification of a NE component is largely depending on the selected method & marker

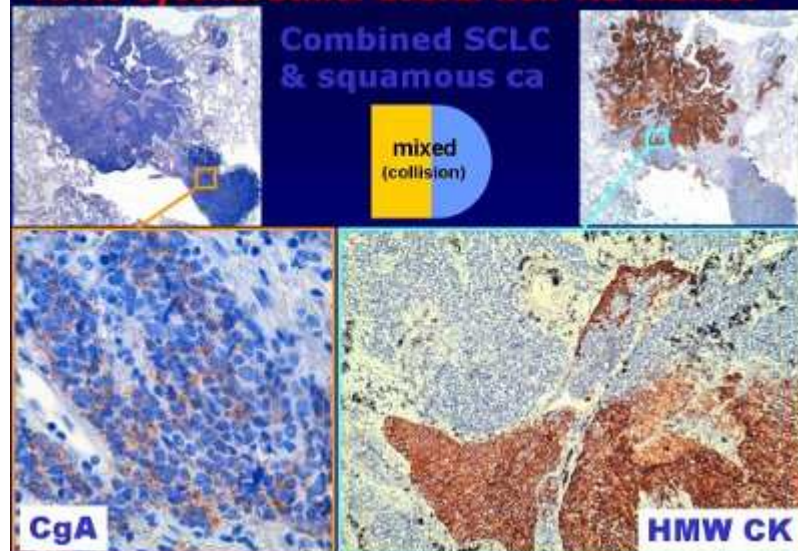
### CHROMOGRANIN A GENE EXPRESSION IN NON-SMALL CELL LUNG CARCINOMAS



GIANCARLO ABBONA, MAURO PAPOTTI, LAURA VIRELLI, LUGLIA MACRÌ, ANNA STELLA AND GIANNI BUSOLATI\*

JOURNAL OF PATHOLOGY  
*J. Pathol.* 186: 151–156 (1998)

### HMW cytokeratins: useful non-NE marker





## COMBINED LUNG CARCINOMAS

SCLC + Squamous ca

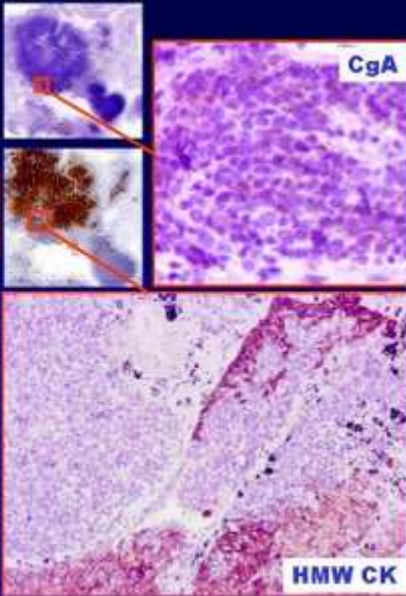
*The individual elements constituting CSCLCs are closely related in most cases despite their distinct morphologic appearances. The prevalent expression of synaptophysin and CD56 and loss of 22q13 suggest that these tumors are biologically closer to SCLC.*

**7 cases studied**

**Combined Small Cell Lung Carcinomas**  
 Genotypic and Immunophenotypic Analysis of the Separate Morphologic Components

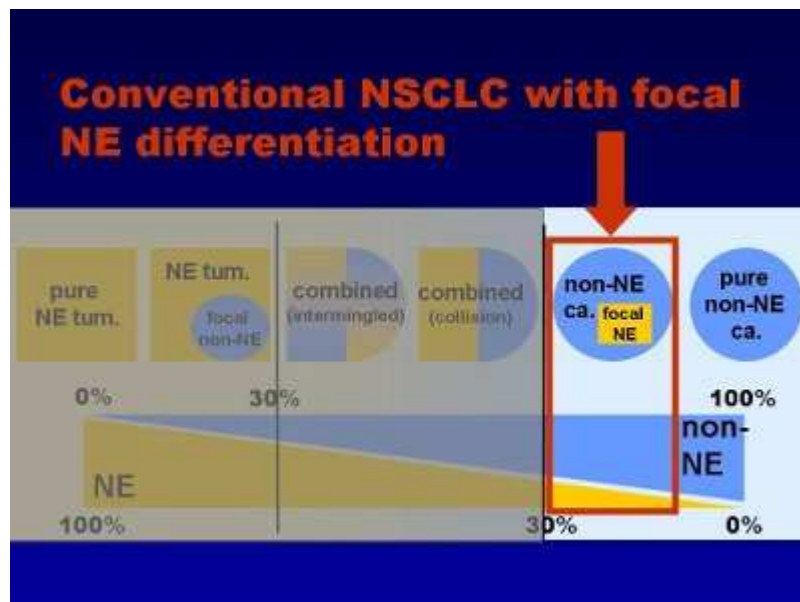
David L. Wispas, MD,\* Nisha Vaidyanath,\* Yuxi Song, MD, PhD, PhD,\* and David N. Karp, MD

**Am J Clin Pathol 131, 376, 2009**



CgA

HMW CK




## NE DIFFERENTIATION in NSCLC

**PATHOLOGIST:**  
 "Present in up to 25% of NSCLC, depending on the method used to assess NE phenotype"

**ONCOLOGIST:**  
 "Define the clinical significance, if any !!"

**CHROMOGRANIN A GENE EXPRESSION IN NON-SMALL CELL LUNG CARCINOMAS**

CgA RT-PCR



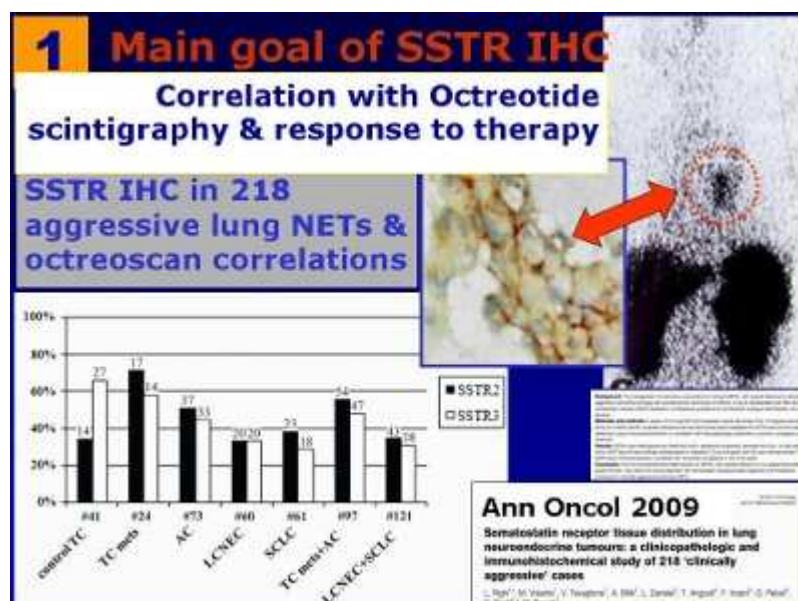
583 bp

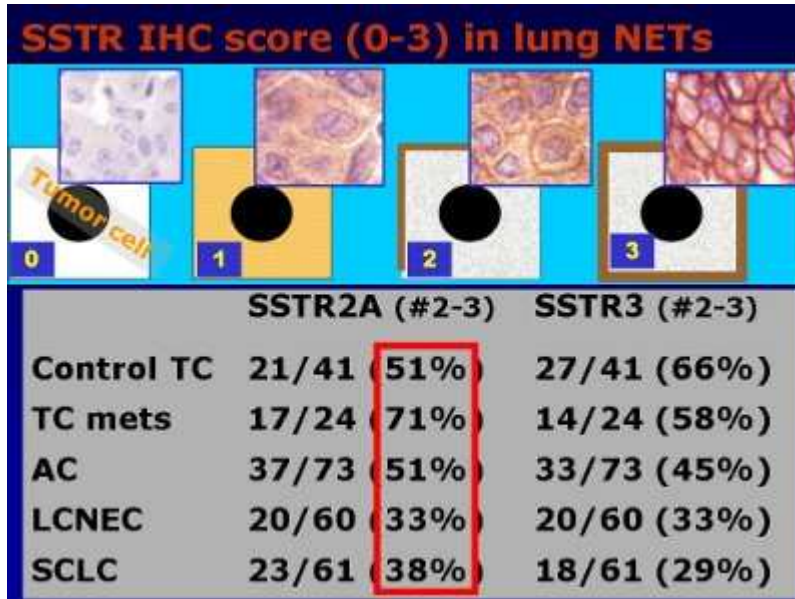
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

**Y/N ? NSCLC**

**YES** STOMACH & PROSTATE CANCERS

**NO** BREAST and COLORECTAL CANCERS





**2**

A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas *British Journal of Cancer* (2006) 95, 1148

## TARGETING mTor

-Ongoing clinical trials testing the efficacy of mTOR inhibitors in metastatic (well differentiated) NE tumors.

-Little is known on mTOR activation patterns in the spectrum of lung NETs.

**Any predictive value in patients treated with mTOR-targeted therapies??**

IHC expression of phosphorylated-mTOR and of its major targets (p70-S6K and 4EBP-1) in a large surgical series of lung NETs

NE Tumours

GEP

Lung

Breast



1963	Feyter	Morphology
1977	Cubilla	Grimelius staining
1980	Capella	Electron microscopy
1984	Jundt	Small cell variant
1987	Bussolati	Chromogranin A (Chr A)
1989	Papotti	Synaptophysin/somatostatin rec.
1990	Capella	Immuno-EM for chromogranin
1990	Pagani	NB, WB analysis for Chrs
1991	Scopsi	Argyr/chr B male breast.
1994	Maluf	Endocrine differentiation of breast carcin
1995	Maluf	Solid papillary carcinoma (DCIS)
1996	Tsang	E-DCIS
1996	Birsak	Sex steroid receptor

## DEFINITION 1

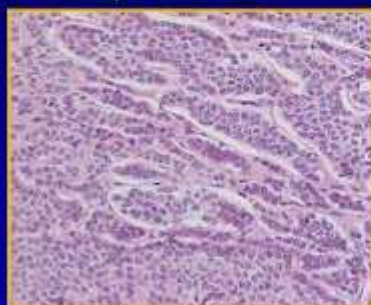
*WHO Classification of Tumours Series. Lyon, France: IARC Press; 2003*

Primary neuroendocrine breast carcinomas (NEBC) are a special group of breast carcinomas, which exhibits morphological features similar to those of endocrine tumors of gastro-intestinal tract and lung  
**AND**  
 express neuroendocrine markers in more than 50% of cells

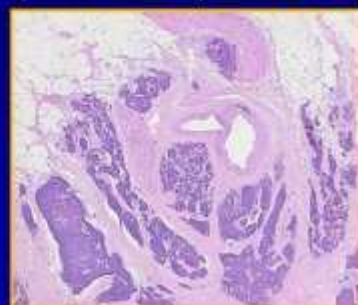
## CLASSIFICATION 1

*WHO Classification of Tumours Series. Lyon, France: IARC Press; 2003*

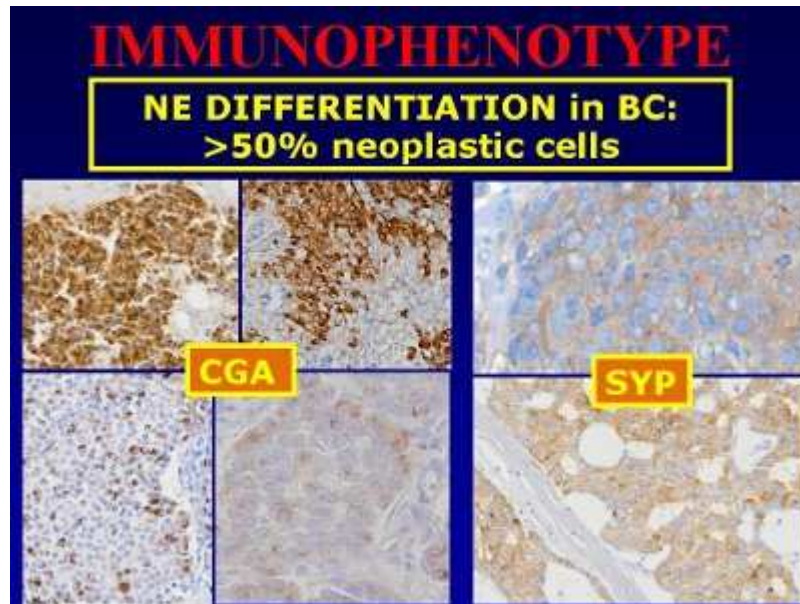
Solid variant: round nests, spir, mito, nec: **MORE COMMON**



Small cell / Large cell variant: necr, cytoplasm, h, nuclei, mito: **VERY RARE**







## DEFINITION 2

*Tavassoli F. Eusebi V: Tumors of mammary gland –  
 AFIP Atlas of Tumor Pathology, Series 4, 2009*

Endocrine tumors are neoplasms

histologically characterized by a solid, trabecular or glandular arrangement of cells with may also form pseudorosettes or tubulo-acinar structures

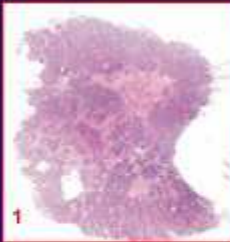
## CLASSIFICATION 2

*Tavassoli F. Eusebi V: Tumors of mammary gland –  
 AFIP Atlas of Tumor Pathology, Series 4, 2009*


1. Well differentiated endocrine tumor (VERY RARE)
2. Well differentiated endocrine carcinoma (MORE COMMON) (Spindle cell/ Solid Papillary)
3. Poorly differentiated endocrine carcinoma - Small cell carcinom (VERY RARE)

# GROWTH PATTERNS

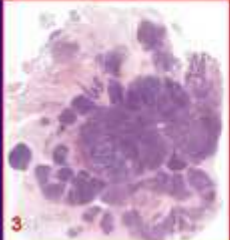
*Sapino et al 2000*




1



2



3



4

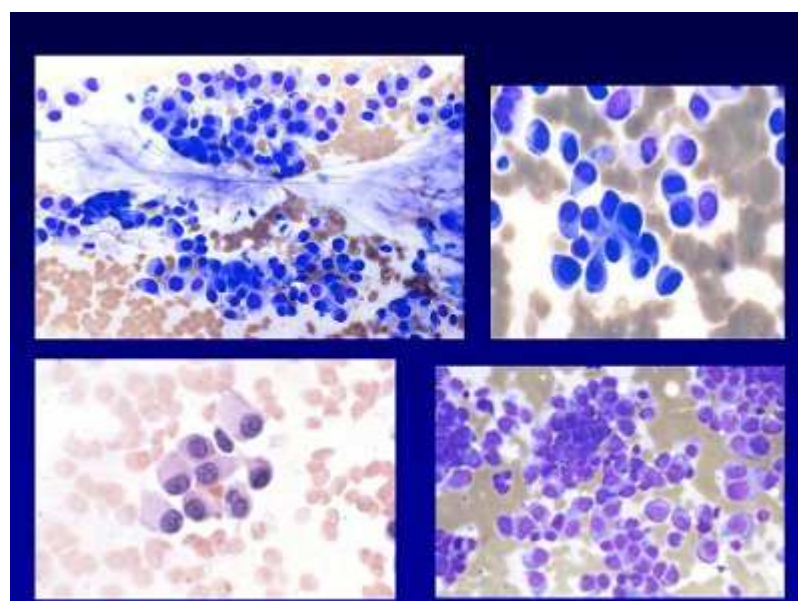
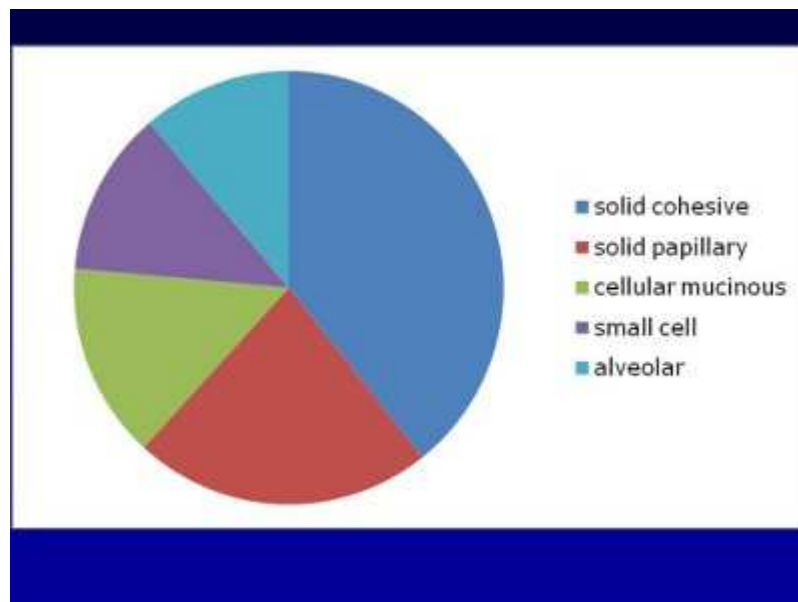
**1. Solid-cohesive**  
infiltrating appearance,  
dense central core

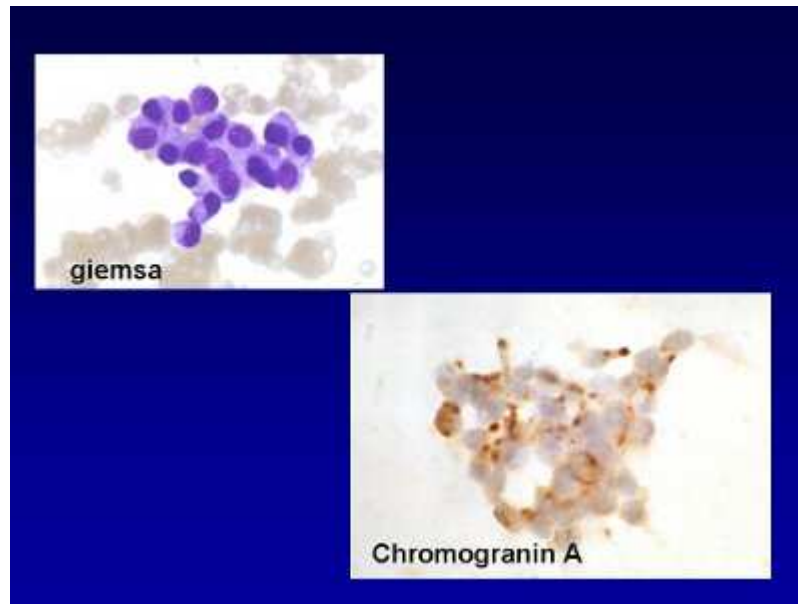
**2. Alveolar**  
Infiltrative growth,  
scanty dense stroma

**3. Small cell**  
Expansive growth

**4. Solid-papillary**  
Expansive growth

**5. Mucinous**





**Molecular subtype of NEBC**

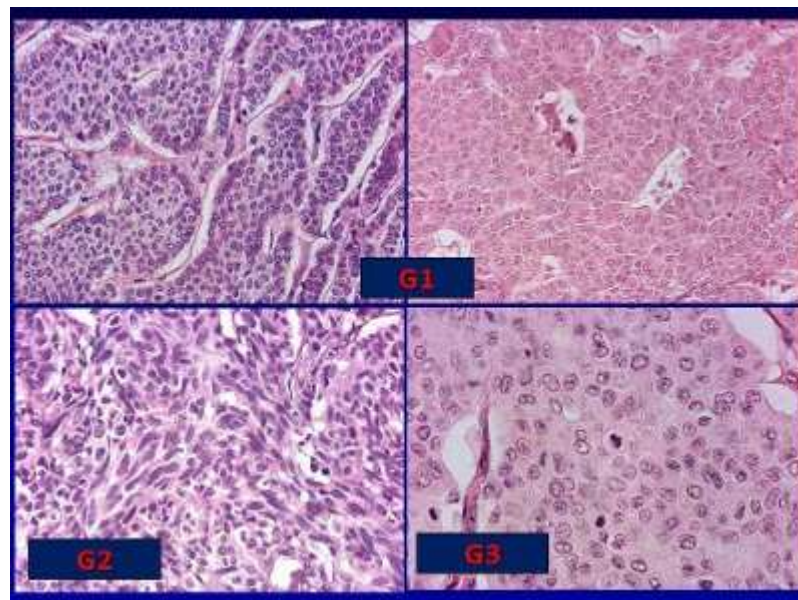
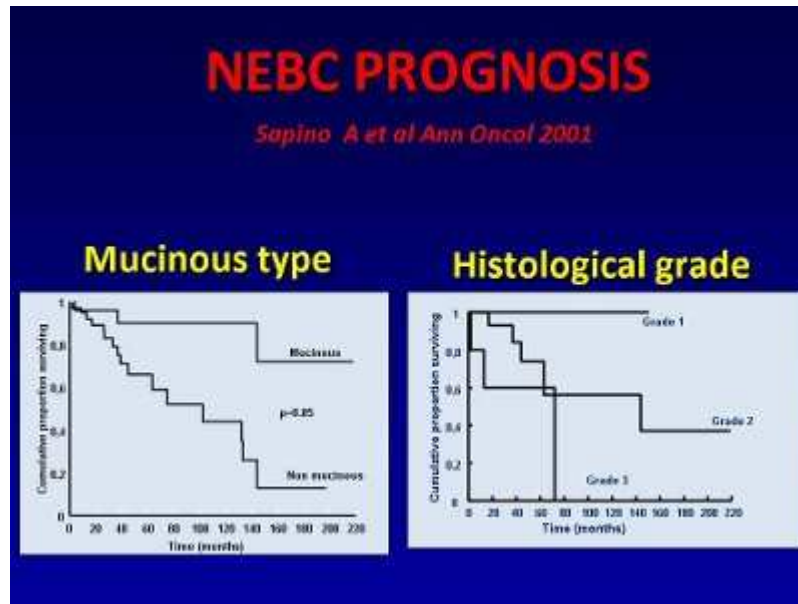
**Luminal A**  
*Lopez-Bonet 2008*  
*Weigelt 2009*

**PR+** **ER+** **HER2-** **EGFR-** **CK5/14-** **HMWCK-**

**SSTR2A+** **ki67**

CLINICAL FEATURES	
PATIENT'S AGE	ELDERLY (mean 67y)
CLINICAL PRESENTATION	ROUNDISH NODULE
CLINICAL BEHAVIOUR	GENERALLY GOOD
TUMOR COMPONENTS	MIXED NE and non-NE
PREVALENT GROWTH PATTERN	SOLID COHESIVE
CELL TYPE	Plasmacitoid , signet ring, clear, small cell
DIAGNOSTIC MARKERS	CGA, SYP
RECEPTORS	ER+, PR+, HER2-, AR+, SSTR+
PROLIFERATIVE STATUS	VARIABLE, GRADE
MOLECULAR SUBGROUP	LUMINAL A
GENE EXPRESSION	Connective





## NEBC PROGNOSIS 2011

### 74 CASI

#### Invasive Neuroendocrine Carcinoma of the Breast

A Distinctive Subtype of Aggressive Mammary Carcinoma

Bing Wei MD<sup>1</sup>, Zhen Tian MD, PhD<sup>2</sup>, Bing Wei MD, PhD<sup>3</sup>, Feng Tang MD, PhD<sup>4</sup>, Wei Wei MS<sup>5</sup>, Michael Z. Gilcrease MD, PhD<sup>6</sup>, Lei Huo MD, PhD<sup>6</sup>, Constance T. Albarracín MD, PhD<sup>6</sup>, Susan A. Abraham MD, PhD<sup>6</sup>

**Prognostic significance of tumor grading and staging in mammary carcinomas with neuroendocrine differentiation**

Human Pathology (2011) 42, 1169–1177

Histopathology 2011, 58, 106–115, DOI: 10.1111/j.1365-2559.2011.01880.x

#### Invasive mammary carcinoma with neuroendocrine differentiation: histological features and diagnostic challenges

Feng Tang,<sup>1,4</sup> Bing Wei,<sup>2,3</sup> Zhen Tian,<sup>2</sup> Michael Z Gilcrease,<sup>4</sup> Lei Huo,<sup>4</sup> Constance T Albarracín,<sup>4</sup> Erika Resekova,<sup>4</sup> Hong Zhang,<sup>4</sup> Aysegül Sahin,<sup>4</sup> Jieqing Chen,<sup>4</sup> Hong Bu,<sup>2</sup> Susan Abraham<sup>4</sup> & Yun Wu<sup>4</sup>



## DIFFERENTIAL DIAGNOSIS

*WHO Classification of Tumours Series, Lyon, France: IARC Press; 2003*

- Metastatic NEC (carcinoid / small cell carcinoma)
- Mammary lobular carcinoma
- 10-18% Breast Cancer with NE (<50%) differentiation
- Breast cancer "usual type"

ER, PGR, GCDFP15

E-CAD

## TAKE HOME MESSAGE

### When to suspect a NEBC?

- Old Patient F/M
- NE patterns (carcinoid/Small cell)
- Growth: solid/solid-papillary/mucinous
- Low grade IDC
- (tubules 3; pleomorphism 1; mitoses 1/2)

CGA/SYP

### WHO 2010 applicability in the "preoperative" setting?

- ✓ Definition of a NE neoplasm possible (suspect + markers)
- ✓ Definition of NET vs NEC usually possible (cell size, atypia, necrosis, growth pattern)
- ✓ Grading applicable, although with some limitations



## open issues...

- ✓ Applicability of new WHO classification to be tested
- ✓ Grading to be implemented (G2, location)
- ✓ Optimal staging system to be validated
- ✓ Prognostic/predictive markers to be validated
- ✓ Quantitative and reproducible evaluation of immunohistochemical markers
- Gene expression profiling

Custom-made Arrays Containing Genes of Your Choice



➔ Gene expression profiling by RT-qPCR on Formalin-fixed Paraffin embedded tissues.

Case 238131

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERG 1	SOX 2	GATA 3	ESR 4	CHD8 5	CDK6 6	UHRF 7	PTK 8	PDGF 9	PCSK 10	SH 11	GPC 12
B	PRK 13	CCA 14	ESR 15	ESR 16	ESR 17	ESR 18	ESR 19	ESR 20	ESR 21	ESR 22	ESR 23	ESR 24
C	ESR 25	ESR 26	ESR 27	ESR 28	ESR 29	ESR 30	ESR 31	ESR 32	ESR 33	ESR 34	ESR 35	ESR 36
D	ESR 37	ESR 38	ESR 39	ESR 40	ESR 41	ESR 42	ESR 43	ESR 44	ESR 45	ESR 46	ESR 47	ESR 48
E	ESR 49	ESR 50	ESR 51	ESR 52	ESR 53	ESR 54	ESR 55	ESR 56	ESR 57	ESR 58	ESR 59	ESR 60
F	ESR 61	ESR 62	ESR 63	ESR 64	ESR 65	ESR 66	ESR 67	ESR 68	ESR 69	ESR 70	ESR 71	ESR 72
G	ESR 73	ESR 74	ESR 75	ESR 76	ESR 77	ESR 78	ESR 79	ESR 80	ESR 81	ESR 82	ESR 83	ESR 84
H	ESR 85	ESR 86	ESR 87	ESR 88	ESR 89	ESR 90	ESR 91	ESR 92	ESR 93	ESR 94	ESR 95	ESR 96

Case 228131

	1	2	3	4	5	6	7	8	9	10	11	12
A	IRS 1	DCO 2	GAST 3	DET 4	CHGA 5	CD56 6	CD56 7	PTH 8	POU 9	FOXC2 10	SHI 11	GPC 12
B	PRG 13	CCA 14	ESBE 15	TSAB 16	CD45 17	POGF 18	MSMT 19	MSDB 20	CD333 21	CD34 22	HHHE 23	IRS 24
C	BRG1 25	CD45 26	PRG 27	CD3 28	CD3 29	CD3 30	CD3 31	CD3 32	CD3 33	CD3 34	CD3 35	CD3 36
D	CD45 37	CD3 38	CD3 39	CD3 40	CD3 41	CD3 42	CD3 43	CD3 44	CD3 45	CD3 46	CD3 47	CD3 48
E	CD3 49	CD3 50	CD3 51	CD3 52	CD3 53	CD3 54	CD3 55	CD3 56	CD3 57	CD3 58	CD3 59	CD3 60
F	CD3 61	CD3 62	CD3 63	CD3 64	CD3 65	CD3 66	CD3 67	CD3 68	CD3 69	CD3 70	CD3 71	CD3 72
G	CD3 73	CD3 74	CD3 75	CD3 76	CD3 77	CD3 78	CD3 79	CD3 80	CD3 81	CD3 82	CD3 83	CD3 84
H	CD3 85	CD3 86	CD3 87	CD3 88	CD3 89	CD3 90	CD3 91	CD3 92	CD3 93	CD3 94	CD3 95	CD3 96