

La diagnostica anatomo-patologica: dalla morfologia ai test genetici

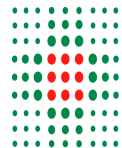
Prof. ssa Patrizia Querzoli
Anatomia Patologica
Azienda Ospedaliero-Universitaria S. Anna



**Dipartimento Attività Integrata
Diagnostica per Immagini e
Medicina di Laboratorio**

**IL TUMORE DELLA
MAMMELLA A FERRARA:
STATO DELL'ARTE E
INNOVAZIONE**

**Dipartimento di Morfologia,
Chirurgia e Medicina Sperimentale**



**SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA**
Azienda Ospedaliero - Universitaria di Ferrara

Sabato 8 Giugno 2013
Aula Magna Nuovo Arcispedale S. Anna
Cona, Ferrara



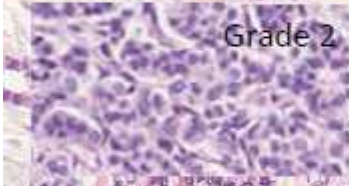
università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

Grouping of breast cancer

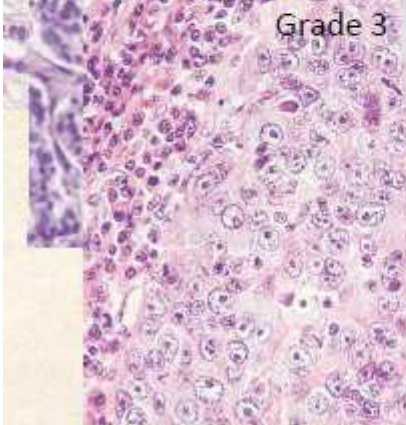
Histological grade



Grade 1

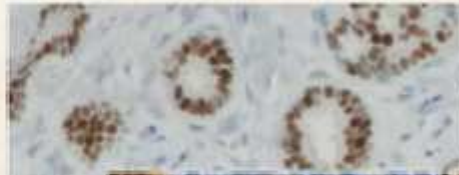


Grade 2

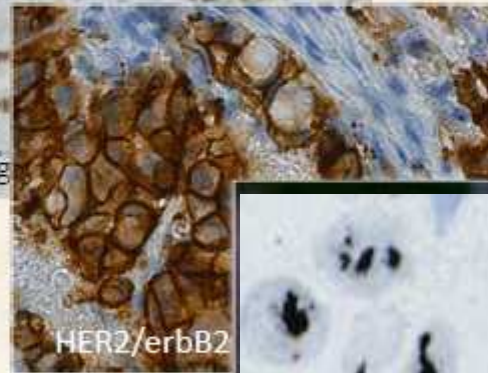


Grade 3

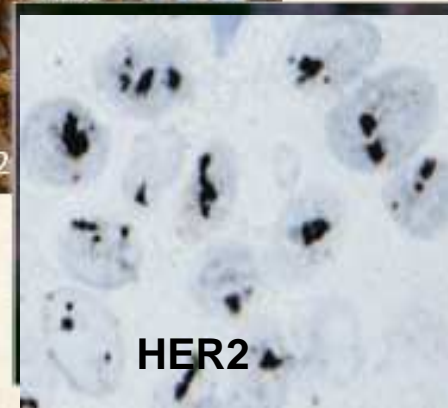
ER/PgR, HER2, Ki67



Estrog



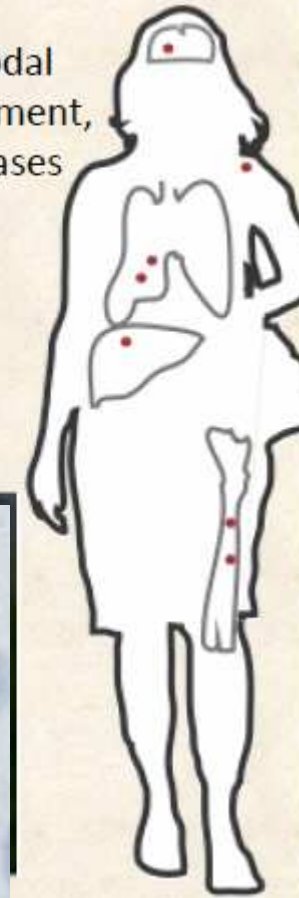
HER2/erbB2



HER2

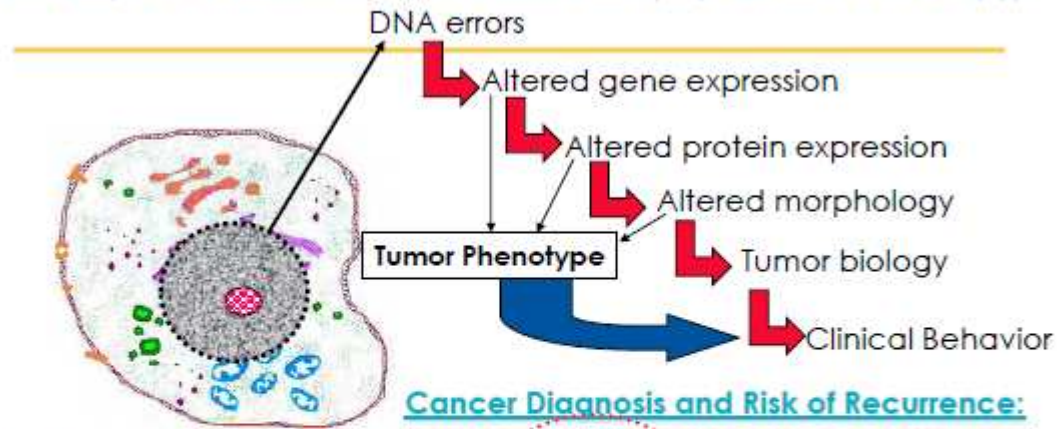
Size, nodal involvement, metastases
TNM

Age



ETEROGENEITA' CLINICA- ISTOPATOLOGICA- MOLECOLARE-RISPOSTA ALLE TERAPIE

Carcinogenesis, Tumor Biology and Clinical Behavior:
Clinically Useful Breast Cancer Classification (Implications for Therapy)

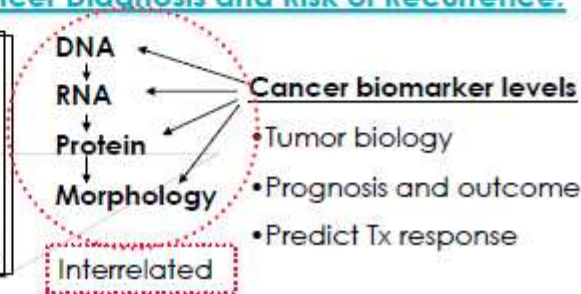


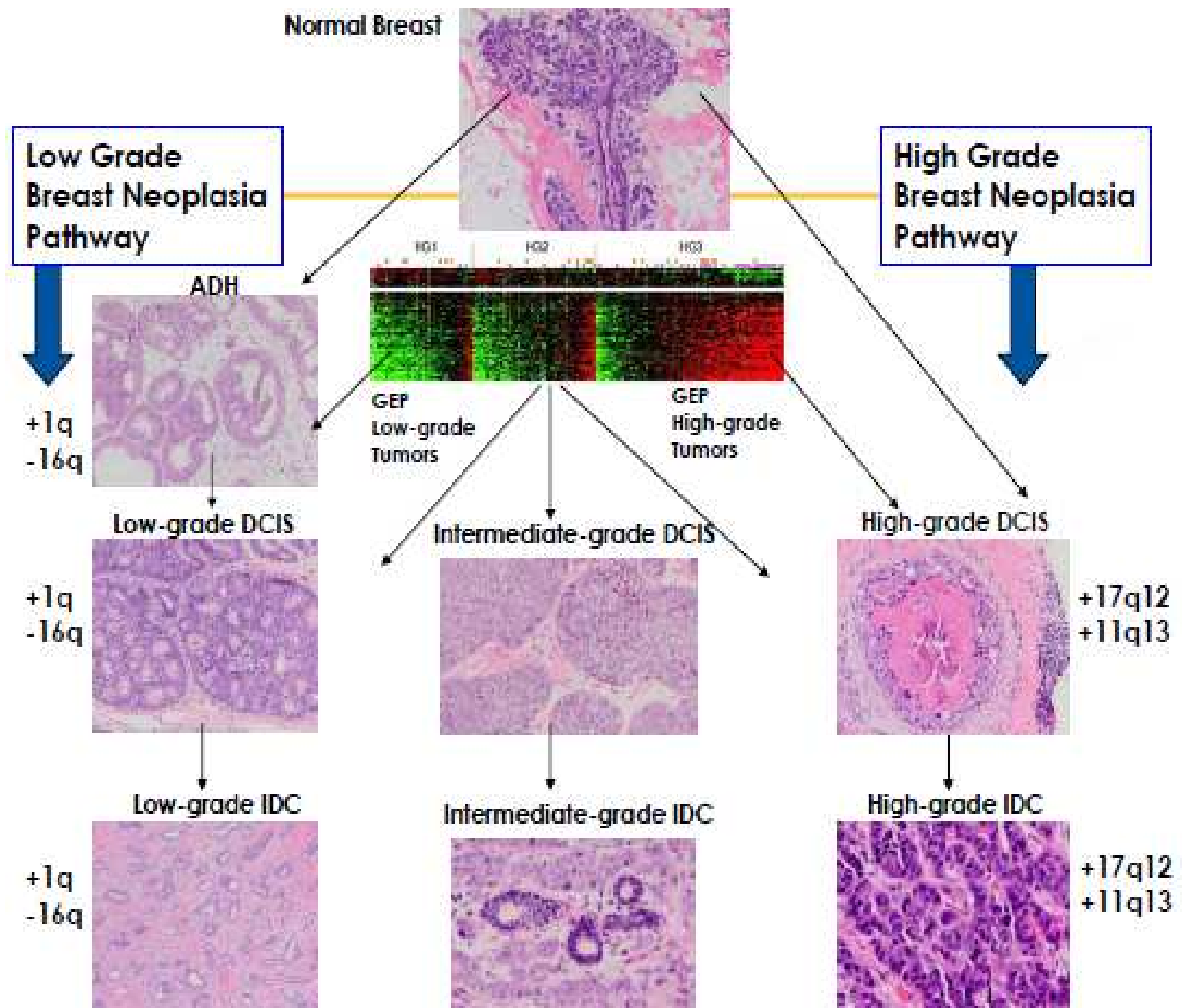
Key drivers:

- Tumor biology
- Clinical behavior
- Therapeutic response

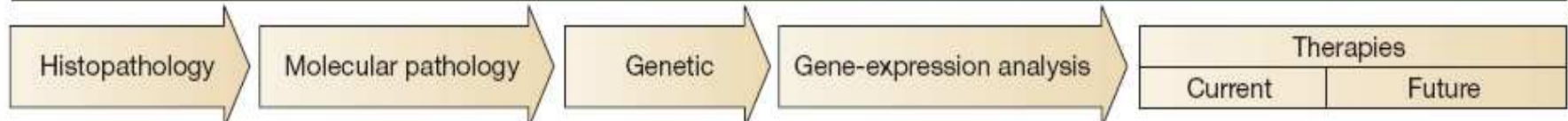
Testing

- FISH/CISH
- Microarray
- RTPCR
- IHC
- H&E Histology

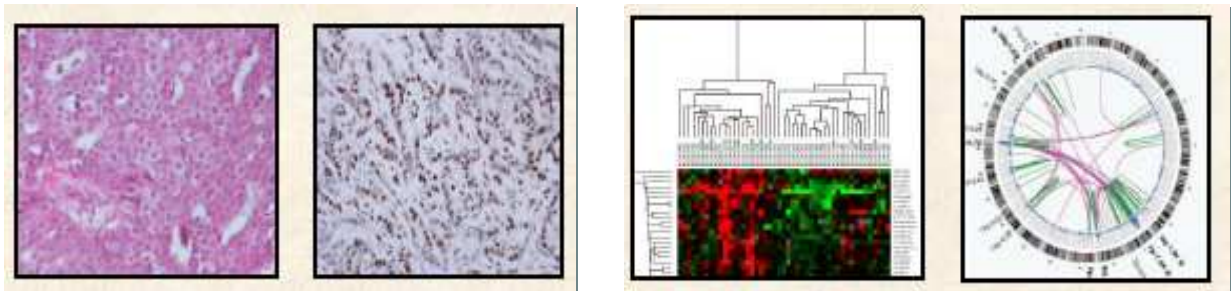




Year of publication



	Histopathology	Molecular pathology	Genetic	Gene-expression analysis	Therapies	
					Current	Future
Basal	Grade III Infiltrating ductal Medullary	ER ⁻ , PR ⁻ , ERBB2 ⁻ High Ki67 expression EGFR ⁺ , c-KIT ⁺ CK5 ⁺ and/or CK6 ⁺ , CK14 ⁺	Gain 6p21- p25	Poor prognosis ³⁰ Risk of metastasis ³² Recurrence score ³¹ Basal ^{17,21,23,24} Hypoxia ³⁵ , wound response ³⁴ Invasiveness ⁶² Bone metastasis ⁶¹	Chemotherapy Antiangiogenic therapy	PARP inhibitors Stem-cell inhibitors
Basoluminal	Grade III	ER ⁻ , PR ⁻ , ERBB2 ⁺ ER ^{-/+} , PR ⁺ , ERBB2 ^{-/+} ER ^{-/+} , PR ^{-/+} , ERBB2 ⁺ AR ⁺ , BCL2 ⁻	Gain 17q21	ERBB2 ^{17,21,23,24} Luminal B ^{17,21,23,24} Apocrine ²⁵	Chemotherapy Trastuzumab Lapatinib	Stem-cell inhibitors AR inhibitors
Luminal	Grade I Invasive lobular	ER ⁺ , PR ⁺ , ERBB2 ⁻ , GATA3 ⁺	Loss of 16q	Luminal A ^{17,21,23,24}	Tamoxifen Aromatase inhibitors	Stem-cell inhibitors



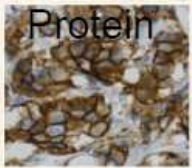

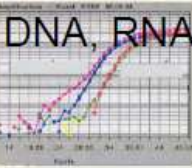

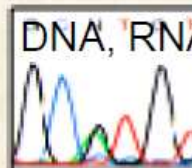
IHC	FISH	PCR	Microarrays	Sequencing
				
Protein	DNA, RNA	DNA, RNA	DNA, RNA, protein	DNA, RNA
Few proteins	Few genes	Single genes/subsets	Multiple	Single genes-whole genome
Visual interpretation, -simple	Visual interpretation, -simple	Automatized interpretation, -dependent on software	Automatized interpretation, -dependent on software	Automatized interpretation, -dependent on software
-correlation with type of cell, histology	-correlation with type of cell, histology	-No correlation with type of cell, histology	-No correlation with type of cell, histology	-No correlation with type of cell, histology
Variation between interpretators	Variation between interpretators			
Easy to integrate	Easy to integrate	Company based, difficult to integrate	Company based, difficult to integrate	From easy to difficult to integrate
Low cost	Low cost	Low cost/Costly	Costly	From low cost to very costly

Table 2. Methods of molecular pathology

- Microdissection (if necessary)
- FISH/CISH/break apart probes
- PCR/PCR variations in combination with ELISAs, and so on
- Microsatellite analysis
- LOH-analysis
- Comparative genomic hybridization
- DNA/RNA-array technology
- Direct sequencing Sanger, pyro, and so on
- Next-generation sequencing
- Protein arrays
- HPLC
- Protein analyses
- Methylation analyses

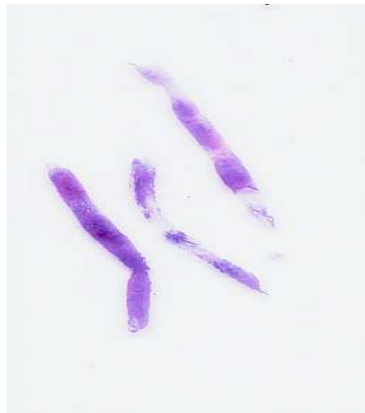
Abbreviations: CISH, chromogen *in situ* hybridization; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence *in situ* hybridization; LOH, loss of heterogeneity

RESPONSABILITA' DEL PATOLOGO NELLA PRATICA CLINICA

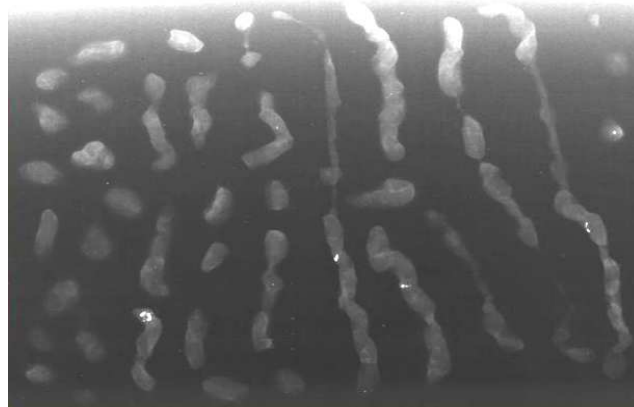
- **INTEGRAZIONE DELLE CLASSIFICAZIONI MORFOLOGICA, PREDITTIVA /MOLECOLARE**
- **IDENTIFICARE GLI ISTOTIPI SPECIALI :**
Tumori «Luminali»
Tumori «Tripli negativi»
- **ACCURATEZZA NELLA STADIAZIONE LINFONODALE : LNS**
- **ACCURATEZZA E RIPRODUCIBILITA' NELLA VALUTAZIONE DI ER, PR, MIB1 ED HER2**
- **BIOBANCHE DI CAMPIONI TUMORALI (-80° e FFPE) PER LA VALUTAZIONE DEI PROFILI DI ESPRESSIONE MOLECOLARE**

PERCORSO DELLA PAZIENTE CON CARCINOMA MAMMARIO

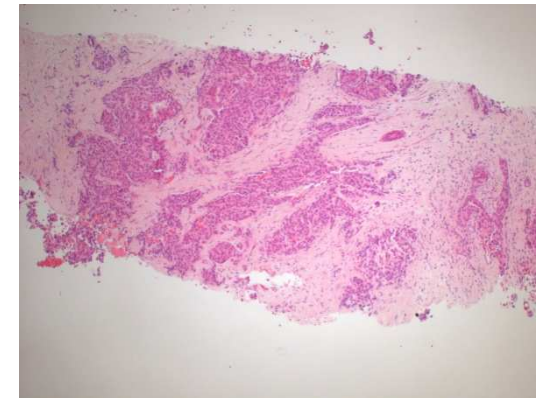
✓ DIAGNOSI PRE-OPERATORIA



CORE BIOPSY

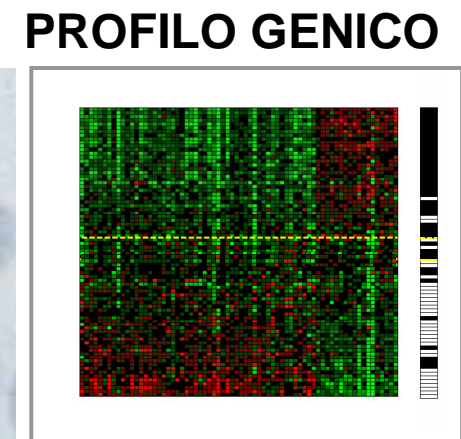
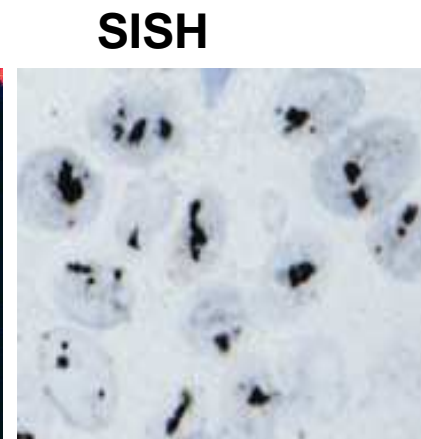
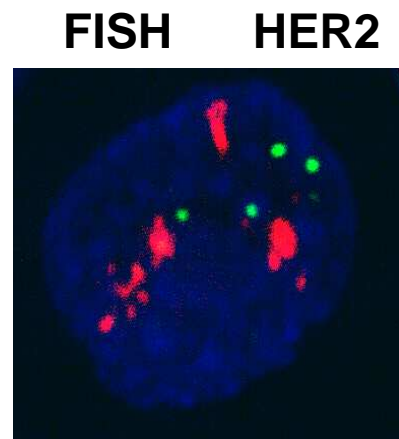
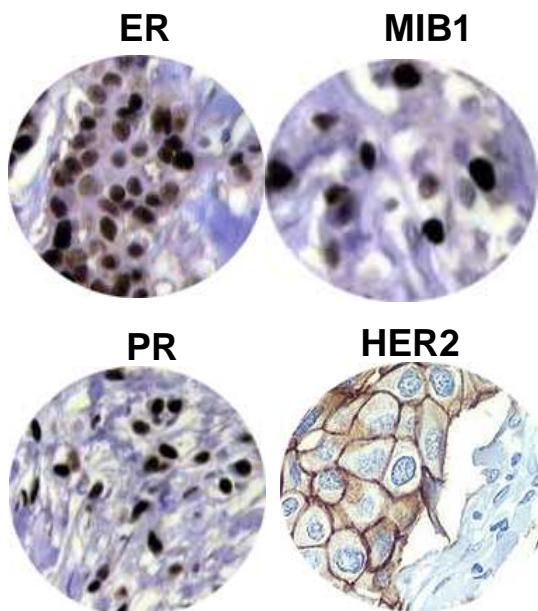


VAB (ECO/Mx/RM)



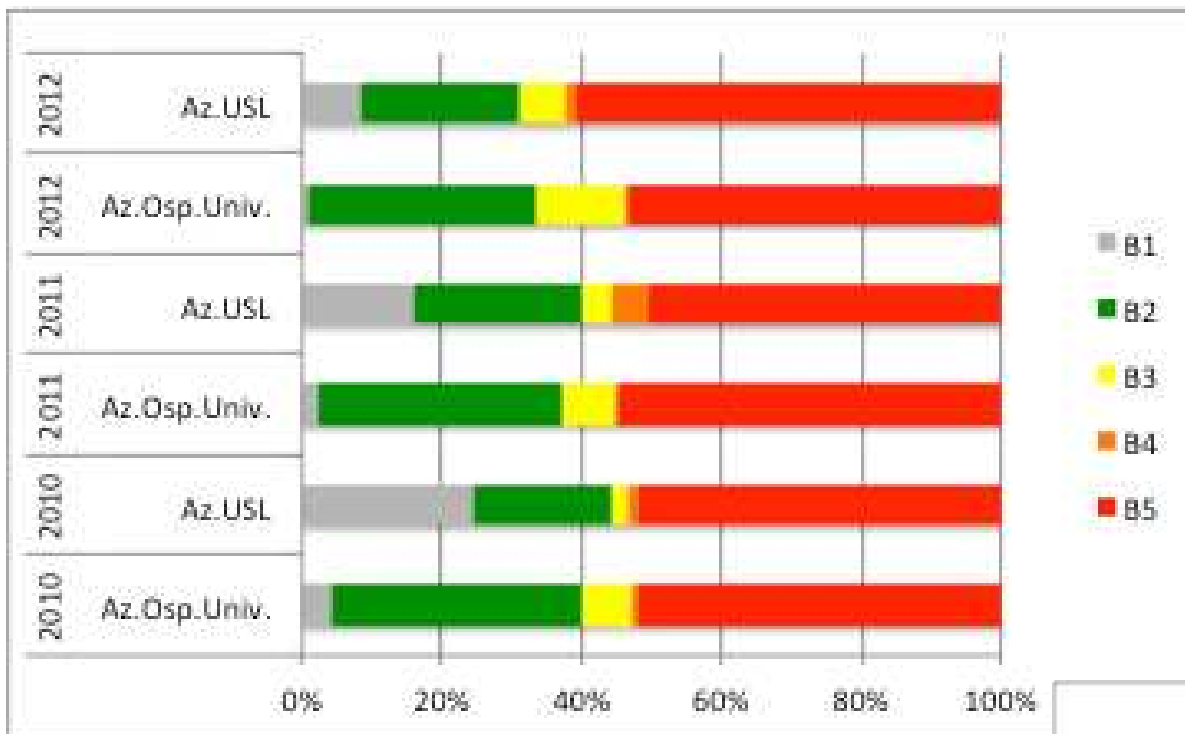
EE

✓ CARATTERIZZAZIONE PROGNOSTICO-PREDITTIVA



DIAGNOSI PREOPERATORIA

BIOPSIA PERCUTANEA (CORE BIOPSY/VAB- US-Mx-MR)



B1 Tessuto normale/non diagnostico

B2 Lesioni benigne

B3 Lesioni a potenziale di malignità incerto

B4 Lesioni sospette di carcinoma

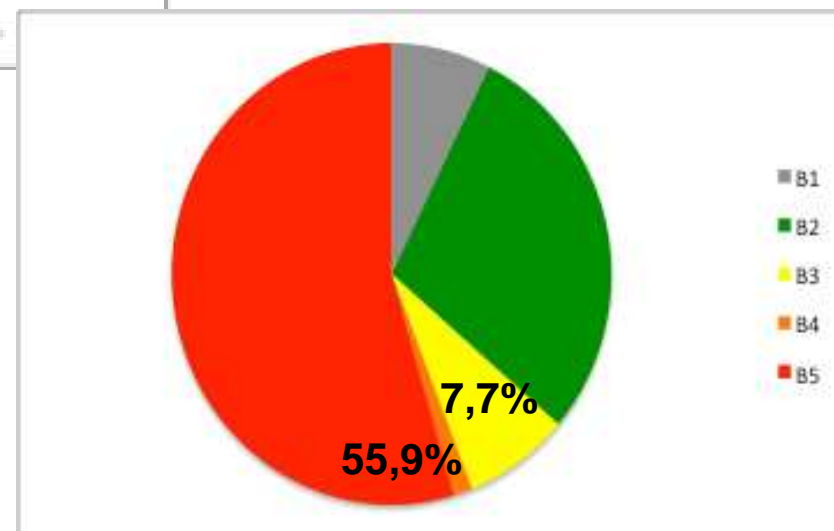
B5 Lesioni maligne (carcinomi in situ/car. invasivi)

2010-2013 (15/05) 2518 CASI

-1613 (64,5%) Az Osp-Univ e 905 (36,5%) Az USL

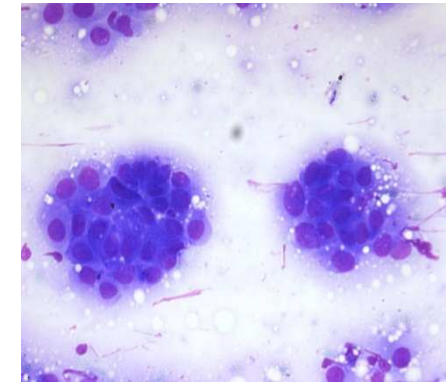
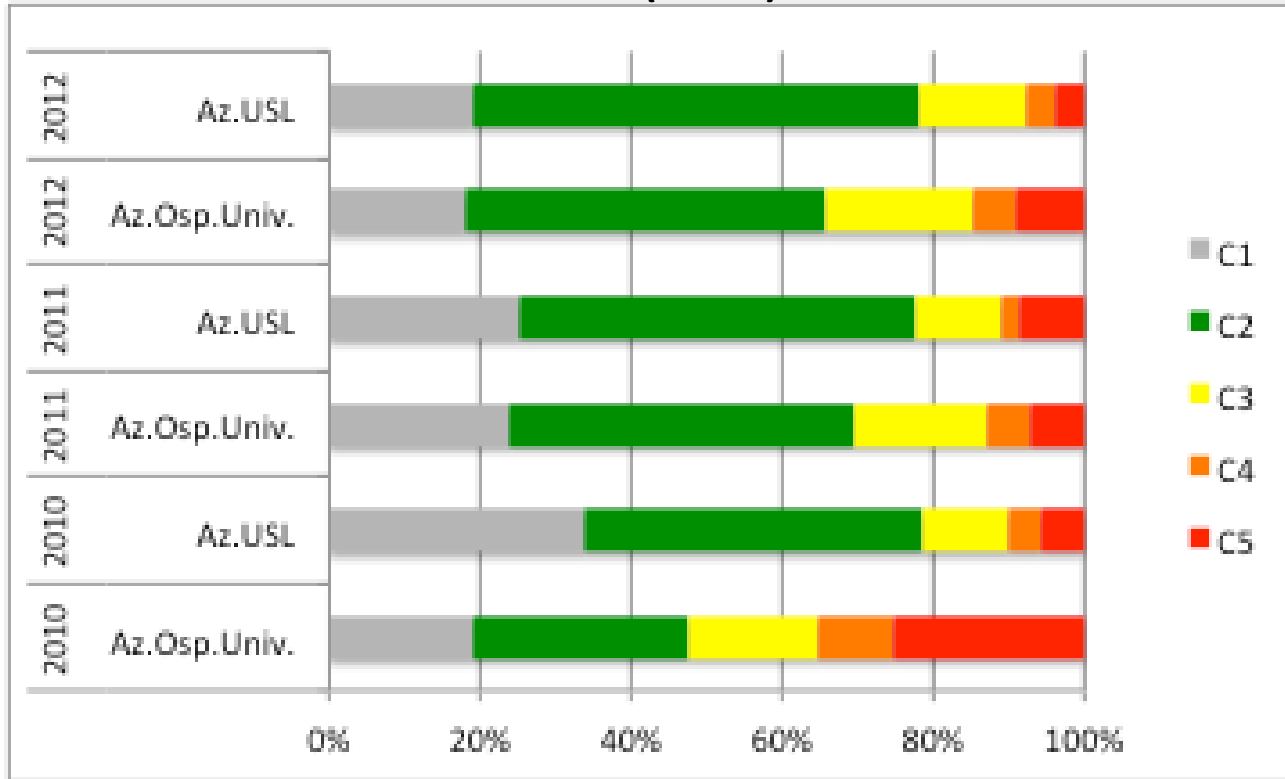
-B5 1374 e B4 35 =1409 (55,9%)

-B3 195 (7,74%)



DIAGNOSI PREOPERATORIA

CITOLOGIA AGOASPIRATIVA (FNAC)



C1: Inadeguato

C2: Benigno

C3: Atipie in lesioni probabilmente benigne

C4: Sospetto di malignità

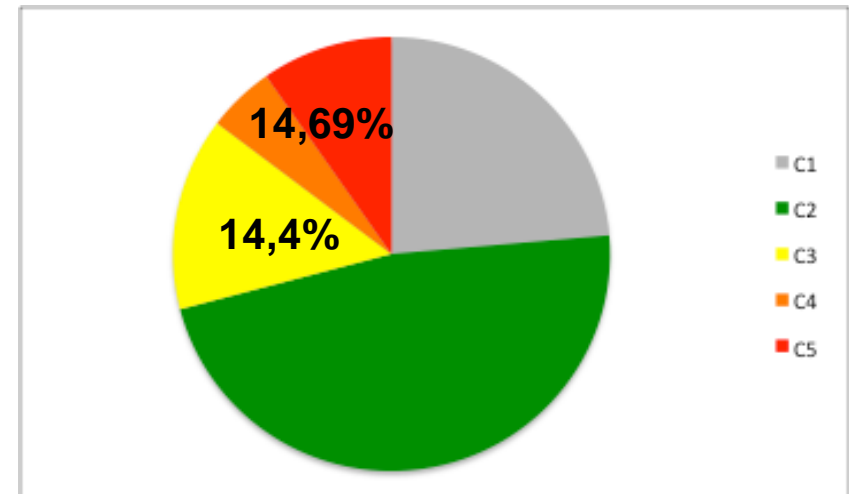
C5: Maligno

2010-2013 (15/05) 1041 CASI

-364 (35%) Az Osp-Univ e 677 (65%) Az USL

-C5 101 e C4 52 =153 (14,69%)

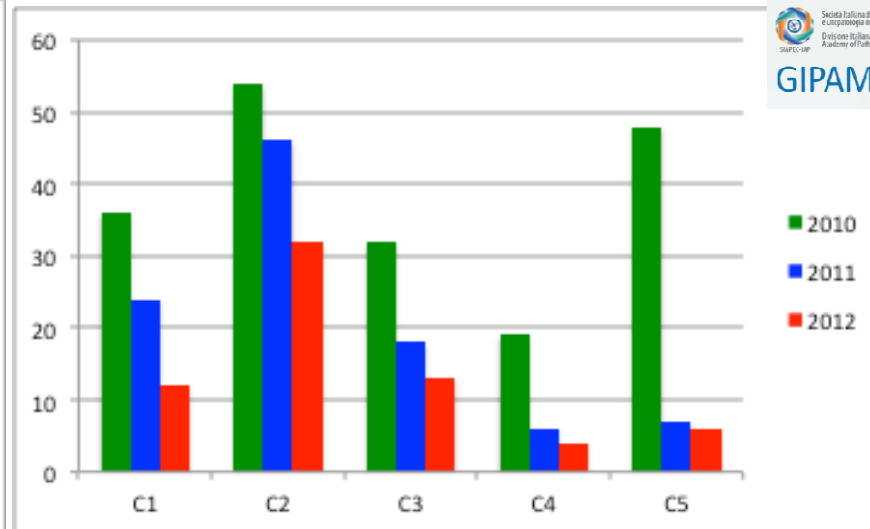
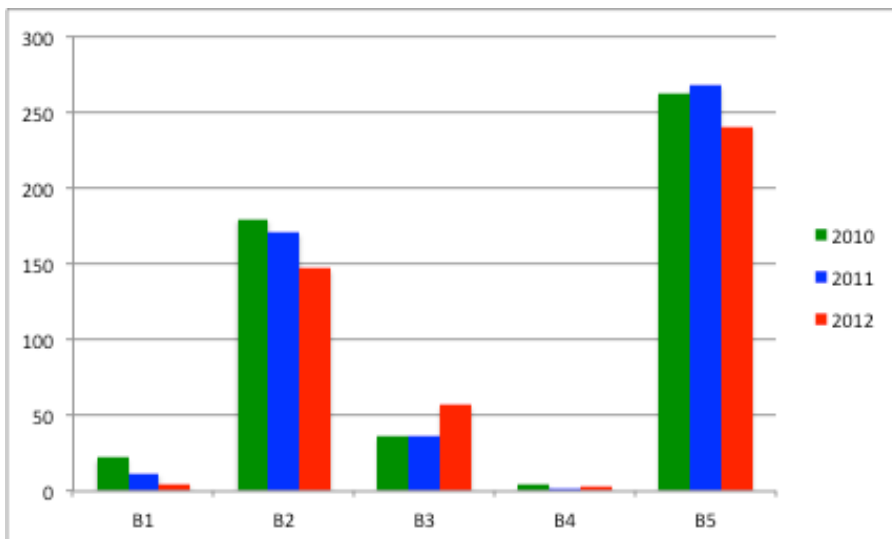
-C3 150 (14,4%)



BIOPSIA PERCUTANEA

2010 – 2012 AZ OSP – UNIV

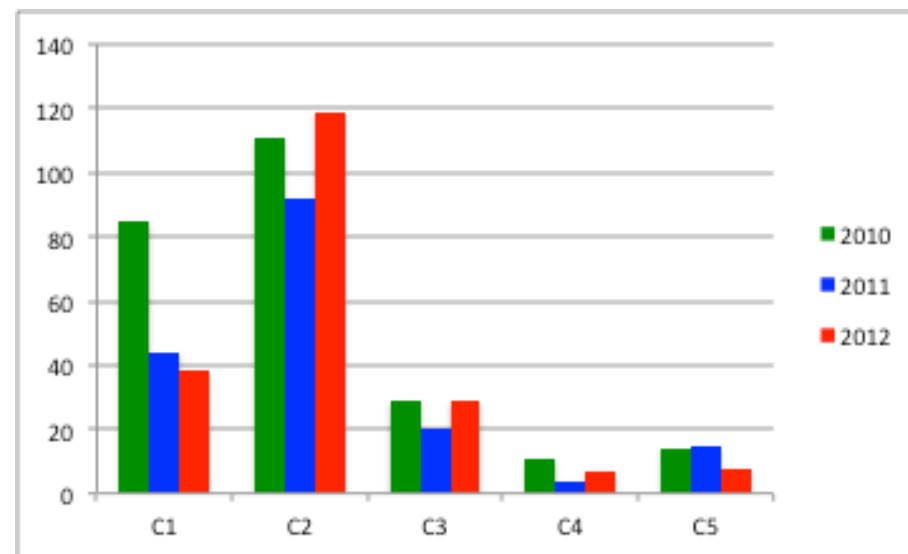
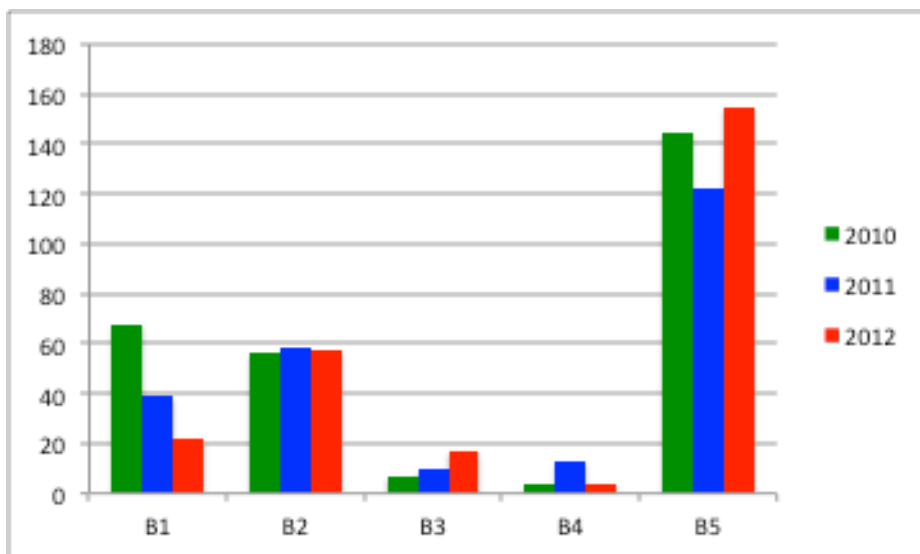
CITOLOGIA



BIOPSIA PERCUTANEA

2010 – 2012 AZ. USL

CITOLOGIA



CASI 785 731 808 294 (15/05/2013)

CASI 439 276 268 58 (15/05/2013)

CARATTERIZZAZIONE BIOPATOLOGICA –PIANIFICAZIONE TERAPEUTICA

TUMORE

Referto diagnostico

Check list

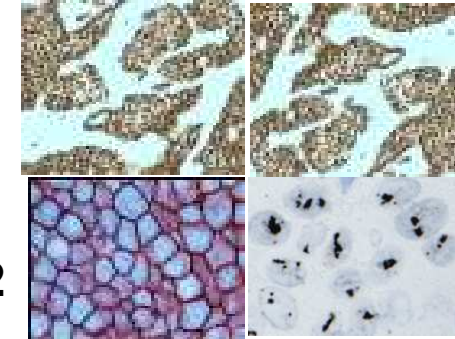
Referto Biotecnologico/BT

Età
Istotipo
pT,G,pN,IVP

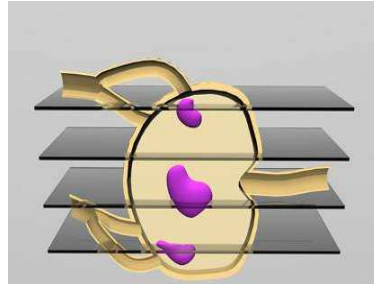
ER,PR,MIB
HER2



ER
PR
MIB1
HER2



LINFONODO SENTINELLA dal 1998 >3400 (anche in anestesia locale)



EE
PCT

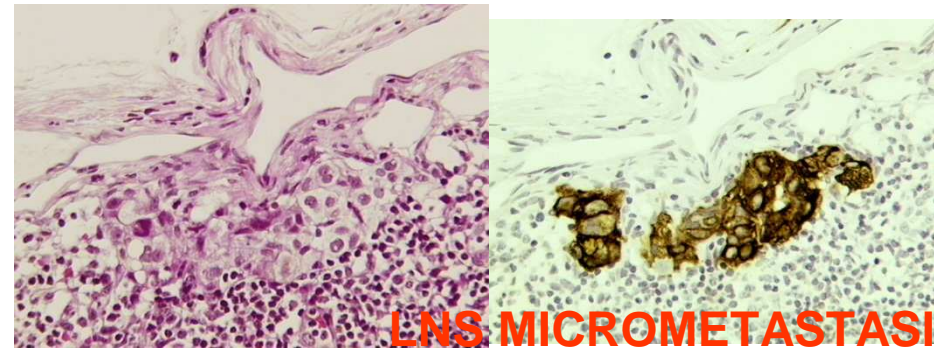
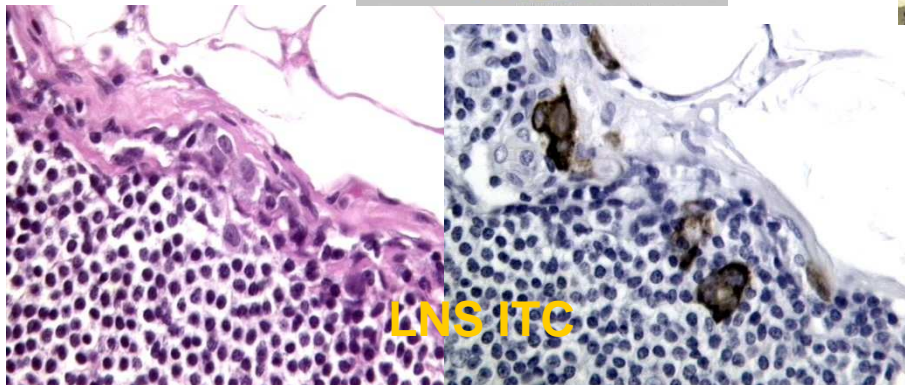


pN0(i-)(sn) (63,3%)

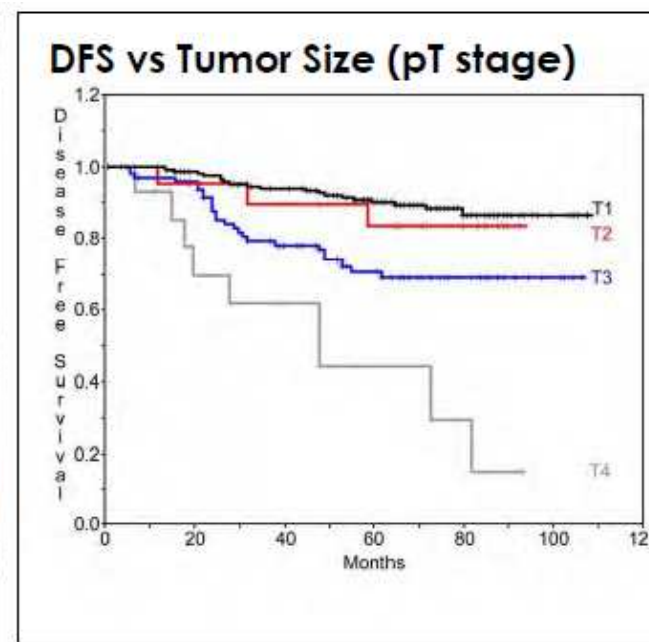
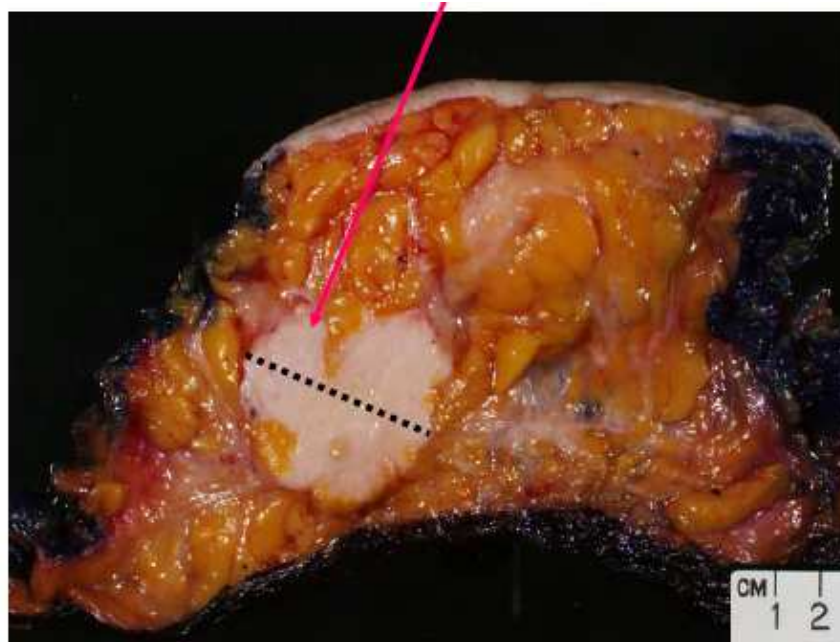
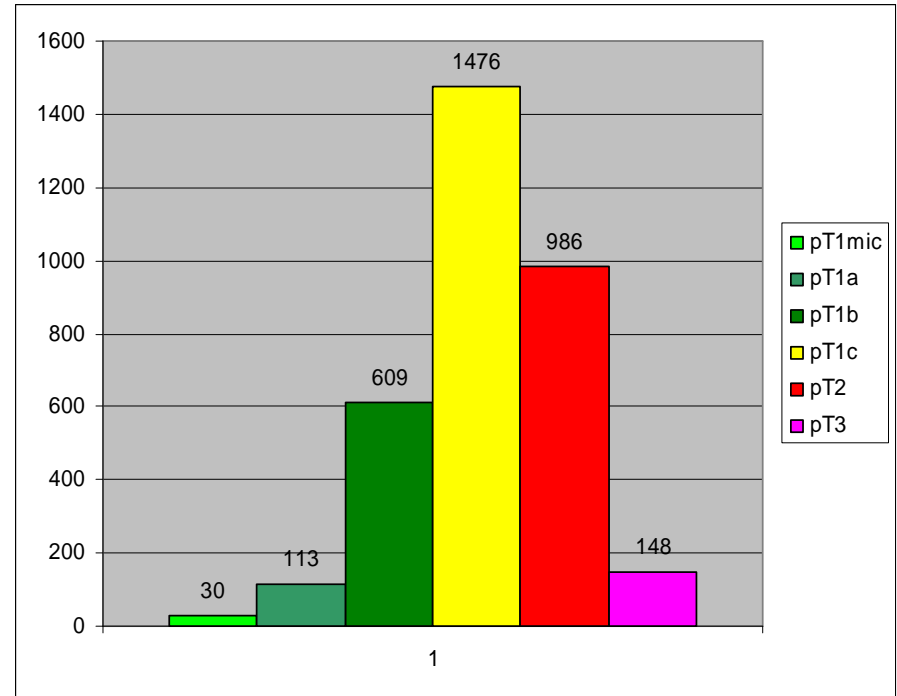
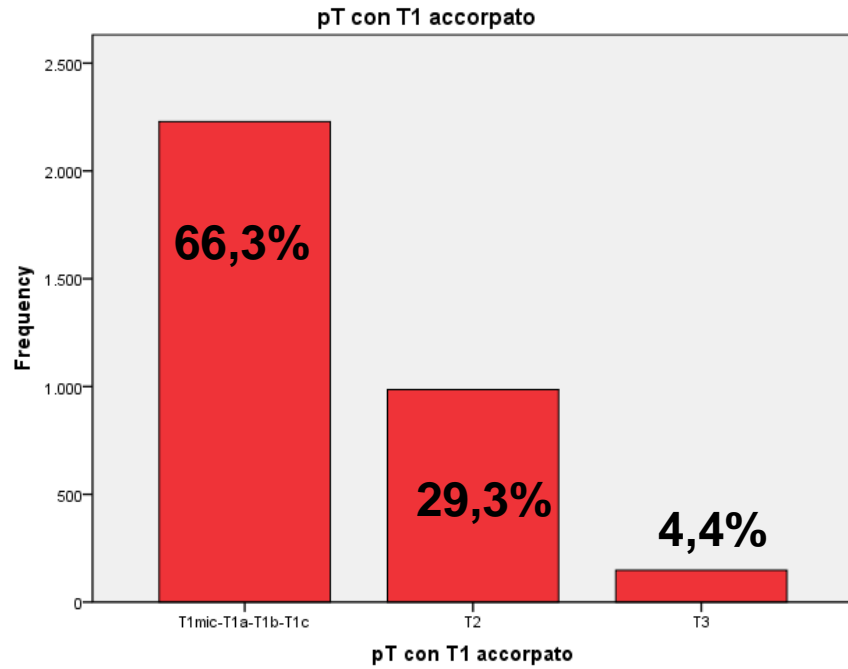
pN0i(i+)(sn) (7,1%)

pN1mi(sn) (8,9%)

pN sn+ (20,7%)



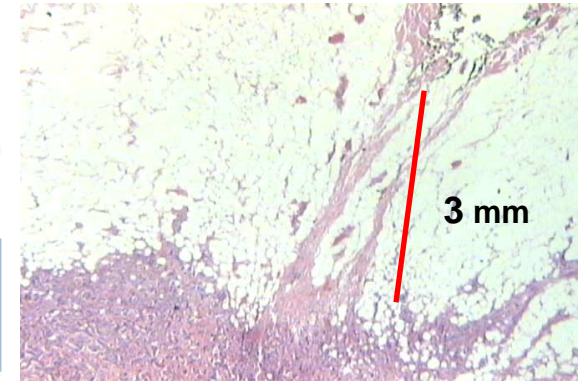
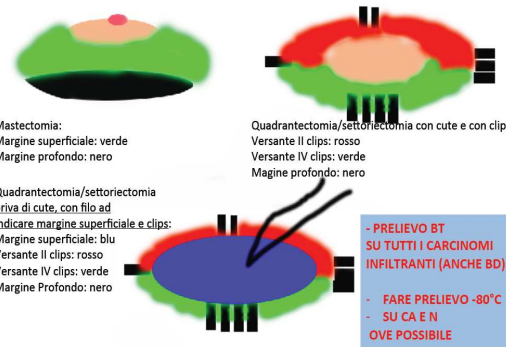
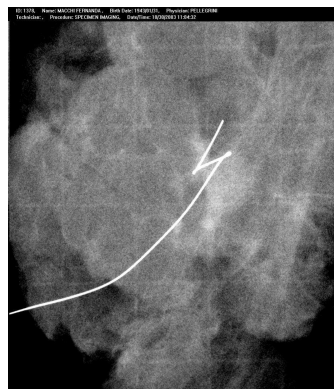
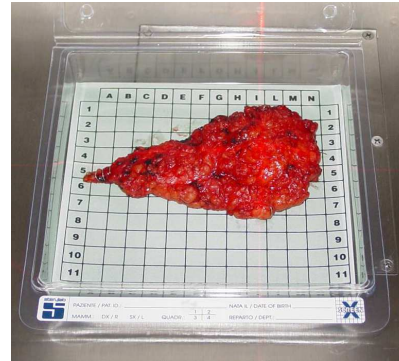
DIMENSIONI



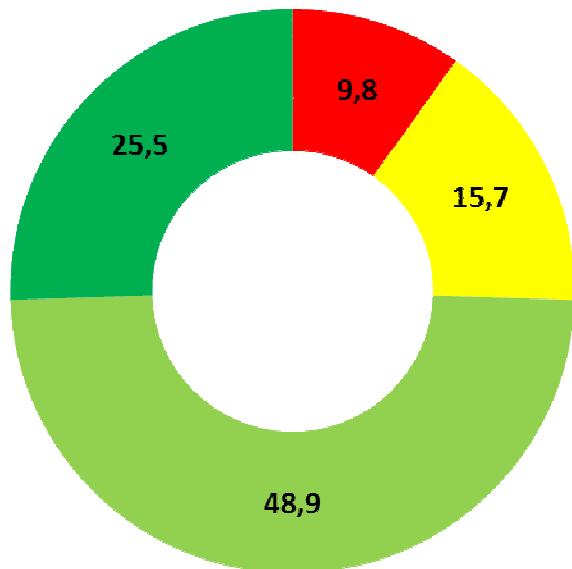
MARGINI

corretto invio del campione:

- ✓ integro
- ✓ radiografia del pezzo operatorio
- ✓ punti di reperi in sedi prefissate



Casistica consecutiva incidente 2009 – 2010: 470 casi

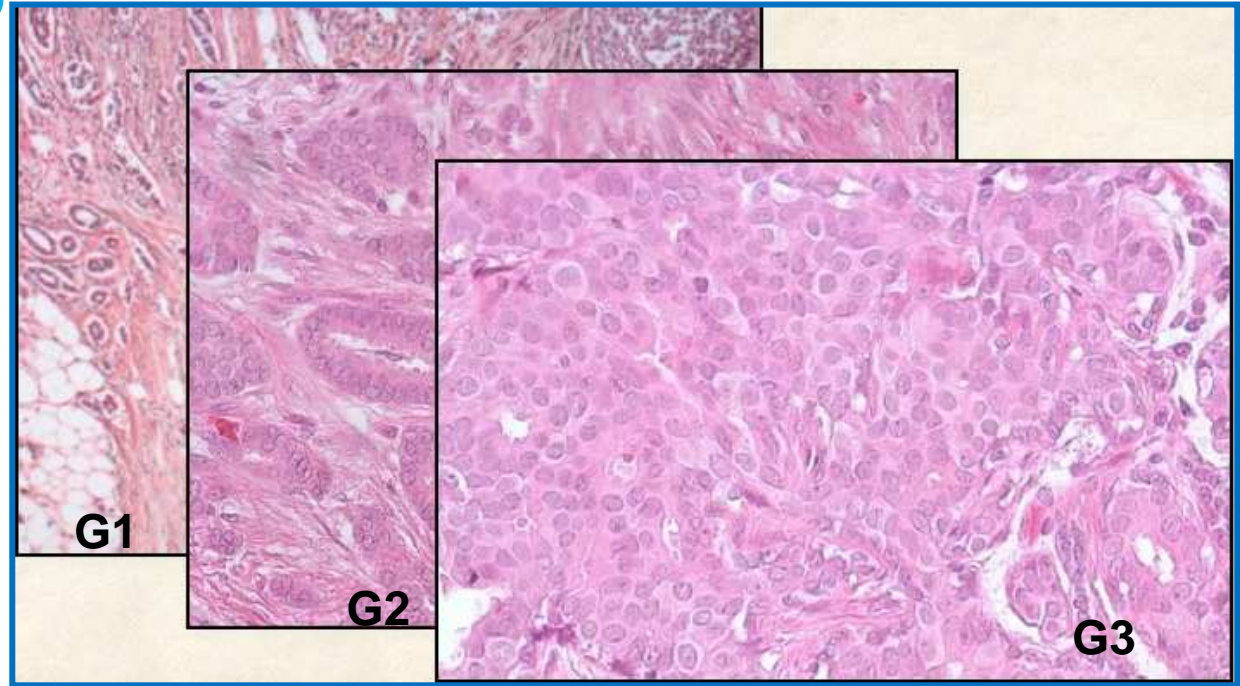


- Presente sul margine
- Fino a 2 mm
- Da 2,1 a 10 mm
- > 10 mm

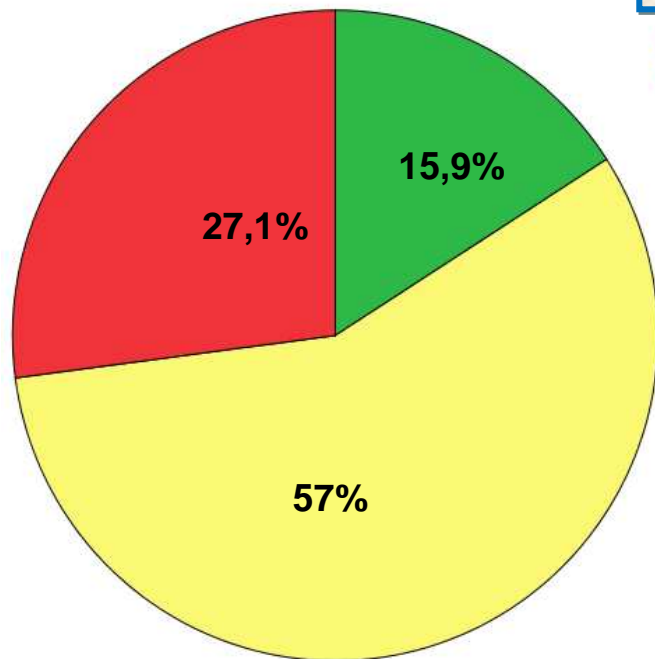
Margini di resezione chirurgici (numero ed estensione) sono uno dei principali fattori predittivi di recidiva locale

Valutazione microscopica (AIRO) Margini indenni: > 2mm

GRADO ISTOPATOLOGICO



G1
G2
G3



AN PAT FE

Histological types according to prognosis

GOOD

Tubular
Cribriform
Mucinous
Adenoid cystic

Juvenile secretory
Tubulolobular
Metaplastic low-grade

INTERMEDIATE

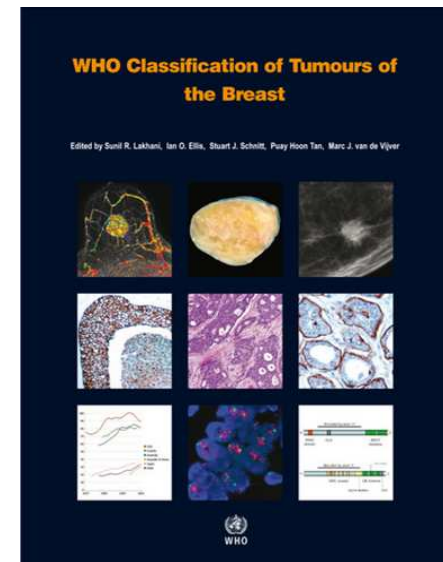
NOS/NST
Lobular
Medullary
Ductal & Lobular

Apocrine

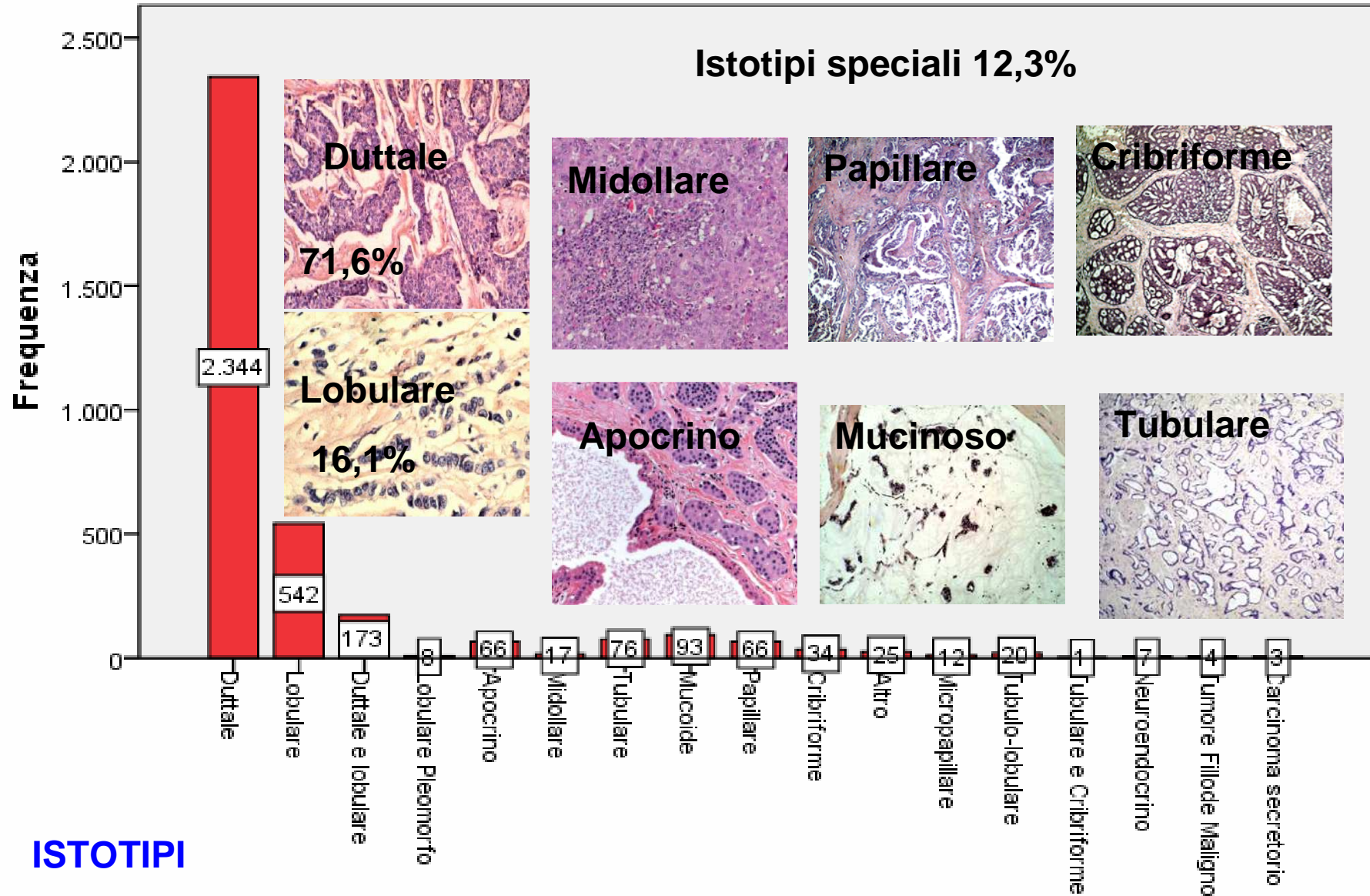
POOR

Inflammatory
Micropapillary
Pleom. lobular
Metaplastic high grade

ISTOTIPI



Diagnosi Carcinoma Infiltrante



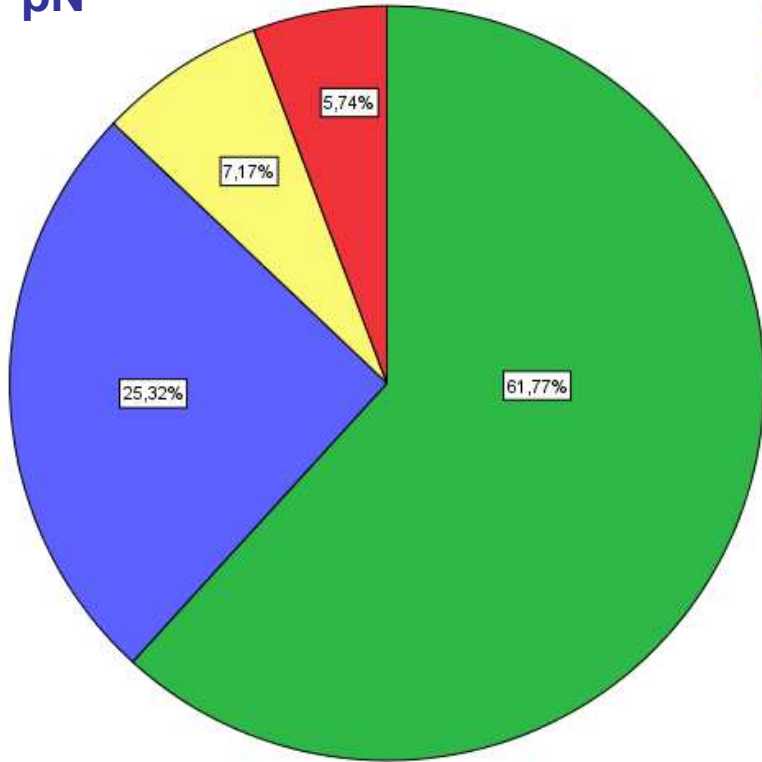
ISTOTIPI

Diagnosi Carcinoma Infiltrante

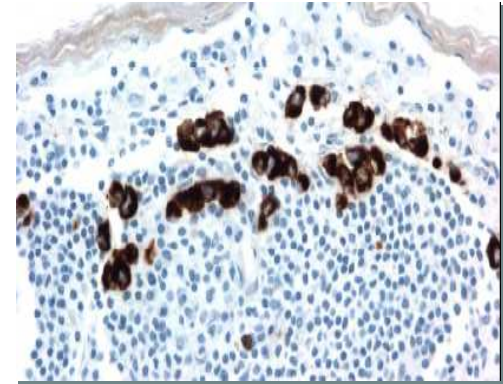
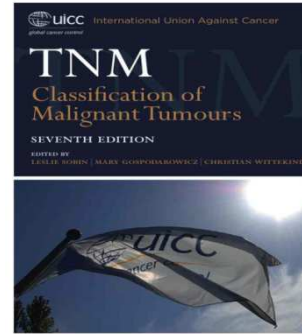
AN PAT FE

pN

Stato linfonodale



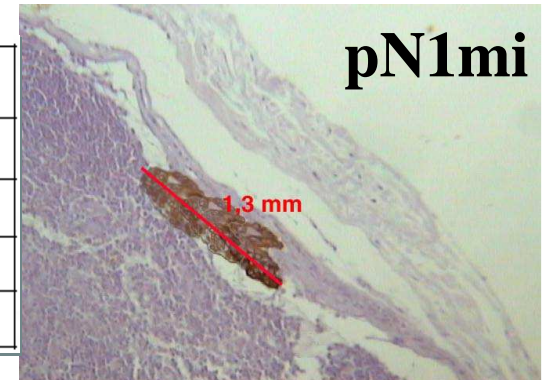
- pN0
- pN1a
- pN2a
- pN3a



pN0(i+)

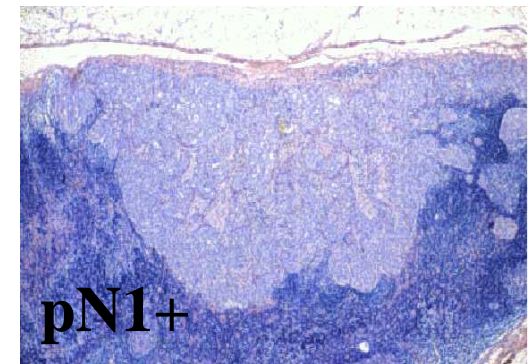
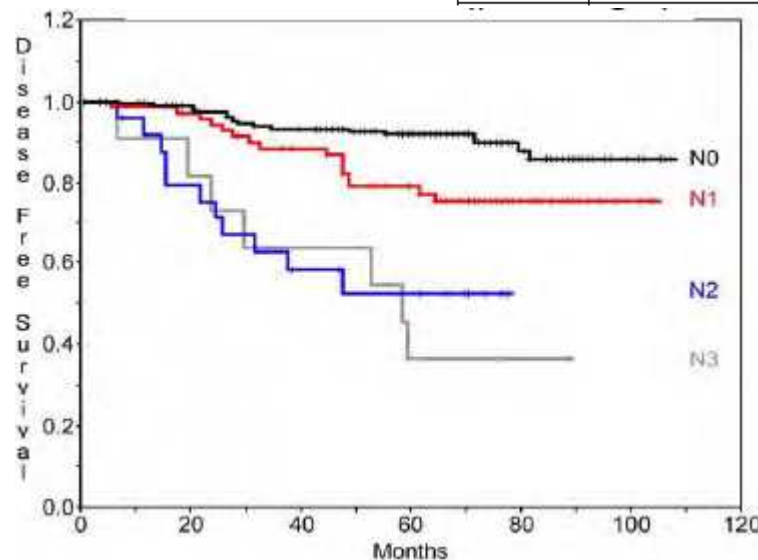
ITC fino a 0,2 mm o < 200 cellule tumorali

Nodal stage	Nodal involvement	5 year survival
N0	Negative lymph nodes	82.8%
N1	1-3 positive nodes	73%
N2	4-9 positive nodes	45.7%
N3	10 or more positive nodes	28.4%



pN1mi

> di 0,2 mm e/o > 200 cellule fino a 2mm



pN1+

AN PAT FE

CARATTERIZZAZIONE BIOPATOLOGICA –PIANIFICAZIONE TERAPEUTICA

TUMORE

Referto diagnostico

Check list

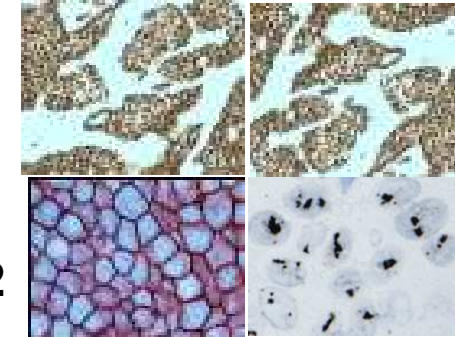
Referto Biotecnologico/BT

Età
Istotipo
pT,G,pN,IVP

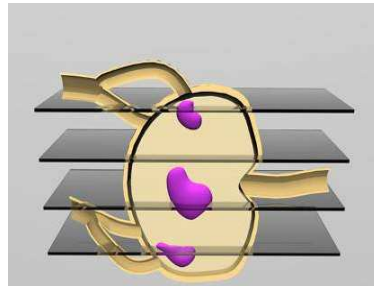
ER,PR,MIB
HER2



ER
PR
MIB1
HER2



LINFONODO SENTINELLA dal 1998 >3400 (anche in anestesia locale)



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PCT

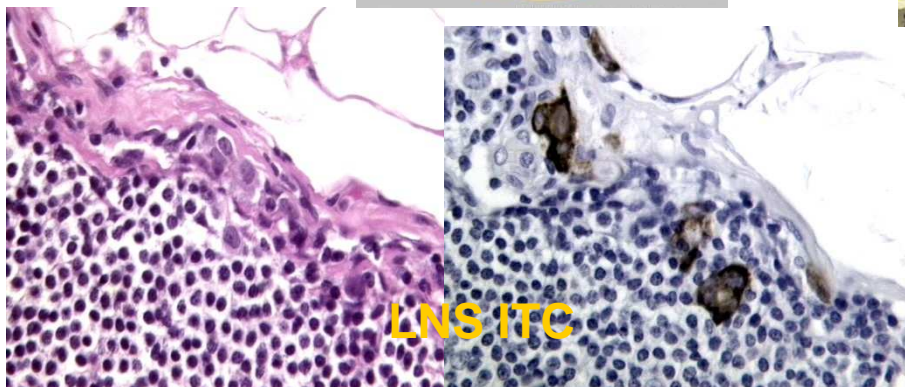


pN0(i-)(sn) (63,3%)

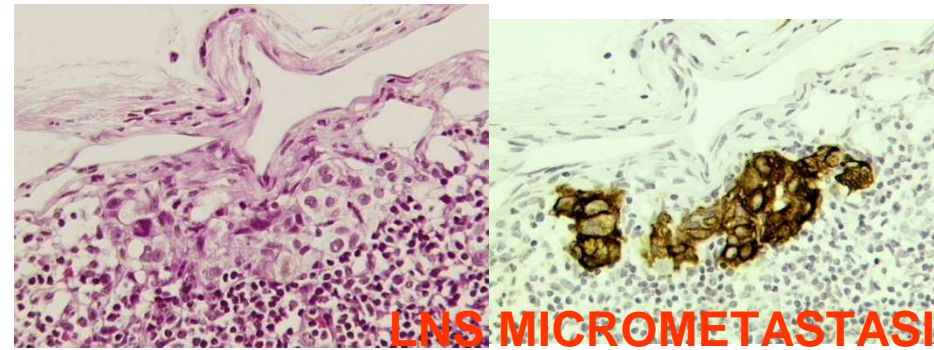
pN0i(i+)(sn) (7,1%)

pN1mi(sn) (8,9%)

pN sn+ (20,7%)



LNS ITC



LNS MICROMETASTASI

Sentinel Node and Bone Marrow Micrometastases and Nanometastases

Elia Biganzoli · Massimo Pedriali · Patrizia Querzoli ·
Italo Nenci · Stefano Iacobelli · Mauro Piantelli ·
Saverio Alberti

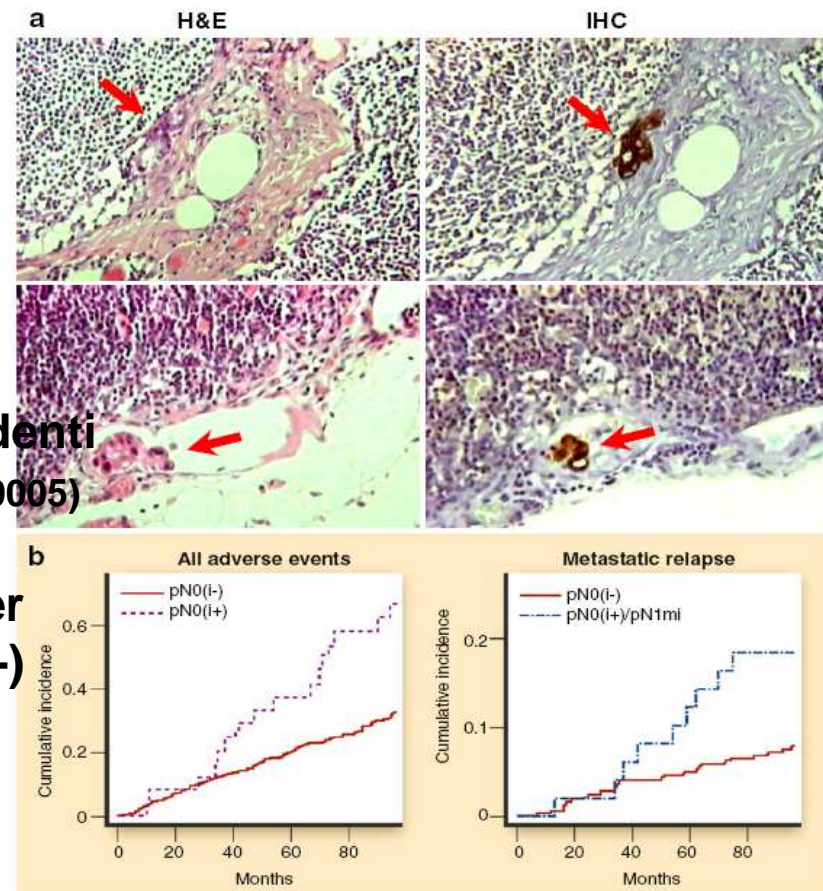
98

Author's personal copy

Curr Breast Cancer Rep (2010) 2:96–106

- Quantificare l'impatto di itc/mm
- storia naturale BC
- staging
- planning terapeutico

Fig. 1 a Examples of lymph node occult metastatic cells as analyzed by hematoxylin and eosin (H&E) staining or immunohistochemistry (IHC). The locations of tumor deposits in pairs of consecutive slides (upper panel, case 1; bottom panel, case 2) are indicated by *arrows*. b Left panel: cumulative incidence curves for the time to any adverse event for pN0(i-) and pN0(i+) cases; right panel: non-parametric estimates of crude cumulative incidence for metastatic relapse of pN0(i-) and pN0(i+)/pN1mi patients as a function of time [65]



- Itc e mm indicatori prognostici indipendenti per tutti gli eventi avversi RR di 2.2 (p=0.0005)
- Nessuna differenza RR itc e mm
- Curve di Incidenza Cumulativa (CCI) per itc e mm iniziano a separarsi dalle pN0(i-) dopo 3 aa

Dipartimento di Diagnostica per Immagini e Medicina di Laboratorio

ANATOMIA E ISTOLOGIA PATOLOGICA

SCHEMA DIAGNOSTICA

Esame Numero: **CL2011-000608**

C
L

Data di nascita: 01 06 1940

Sesso: F

Ente: **Azienda Ospedaliero-Universitaria Ferrara**

Ospedale: ARCISPEDALE S. ANNA - FERRARA

Reparto: CHIR.GEN.DEG. II PIANO ALA A

Ambulatorio:

Data: 29 08 2011

Richiedente: FRANCESCO PELLEGRINI

Caso di Riferimento

B2011-005751

.CARCINOMA DELLA MAMMELLA

CARCINOMA duttale infiltrante N.A.S

GRADO ISTOLOGICO: **III**

CARCINOMA IN SITU (coesistente):

Intraduttale, grado nucleare alto, tipo solido e solido con necrosi

STATO LINFONODALE:

Linfonodi ascellari - N° totale 13

Metastasi in N° 1

INFILTRAZIONE CUTANEA: **Assente**

INVASIONE VASCOLARE: **Presente**

MARGINI DI EXERESI: **Indenni, distanza minima mm 10**

DIMENSIONI DELLA NEOPLASIA: **Carcinoma infiltrante mm 25 x 15**

STADIO PATOLOGICO (TNM, VII ed.): **pT2 - pN1a**

NOTTINGHAM PROGNOSTIC INDEX: **Alto rischio, score 5,5**

SEDE: **Mammella sinistra - Quadrante superiore-esterno**

Patrizia Querzoli

Prof.ssa Patrizia QUERZOLI

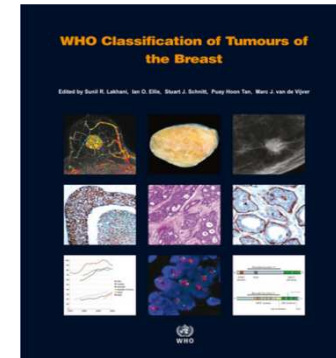
(29 08 2011)

Referto firmato digitalmente ai sensi delle norme vigenti

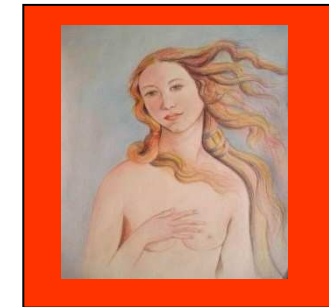
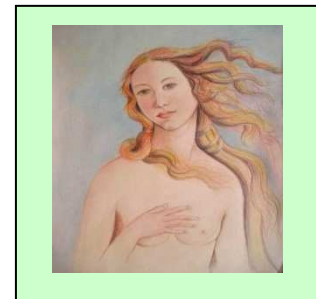
Via Fossato di Mortara, 64 - Tel. 0532 236350-0532 236812-0532 455501

Per informazioni sul significato di questa diagnosi si può accedere alla pagina www.ospfe.it/diagnosi-istocitopatologiche

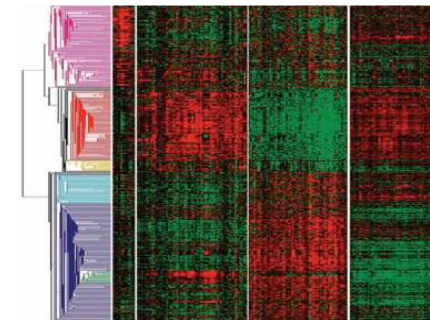
- **CLASSIFICAZIONE ISTOPATOLOGICA WHO**
19 istotipi principali



- **CLASSIFICAZIONE PREDITTIVA ST. GALLEN**
Endocrino responsiva
Non Endocrino responsiva

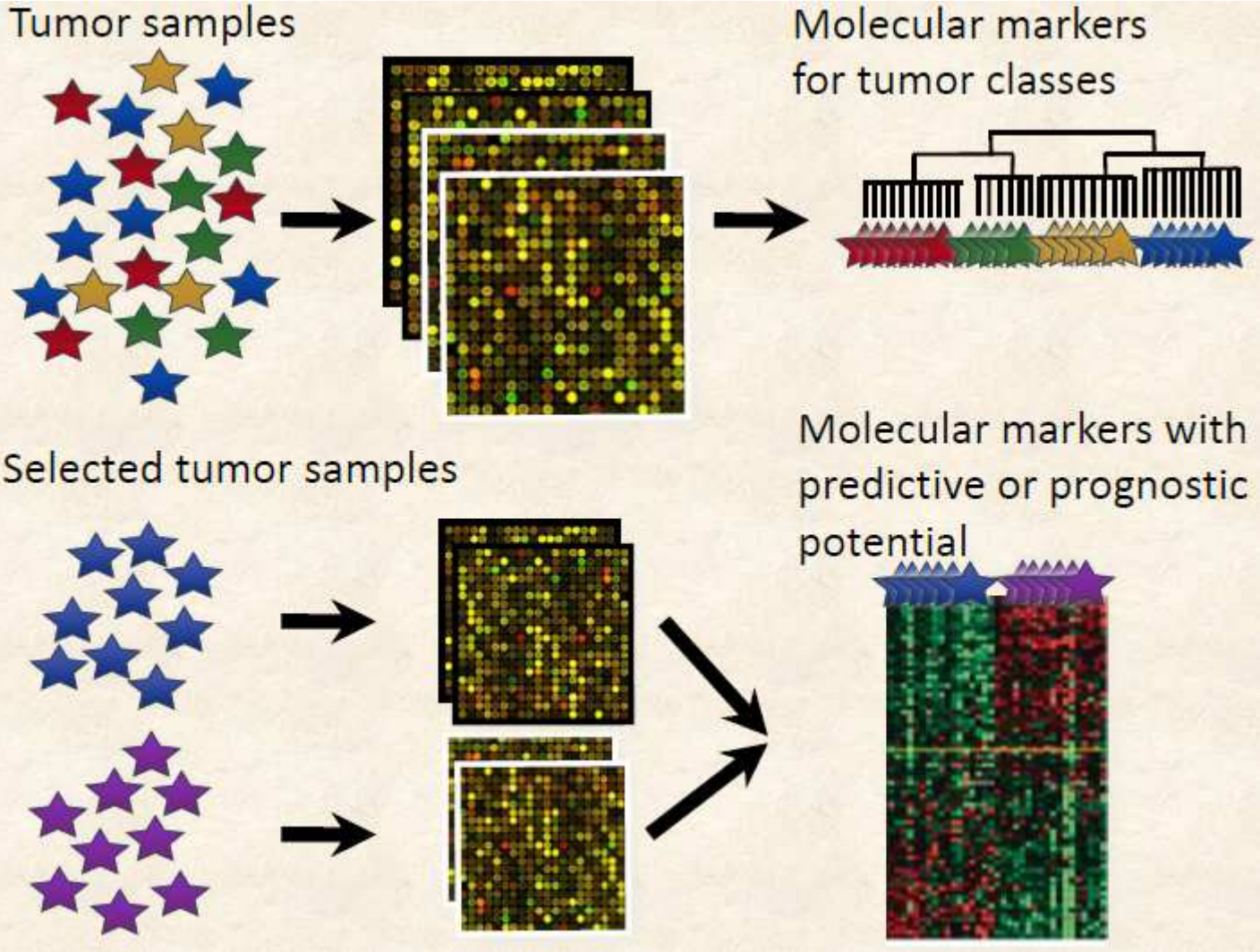


- **CLASSIFICAZIONE MOLECOLARE**
Luminale A e B
HER2
Basal-like

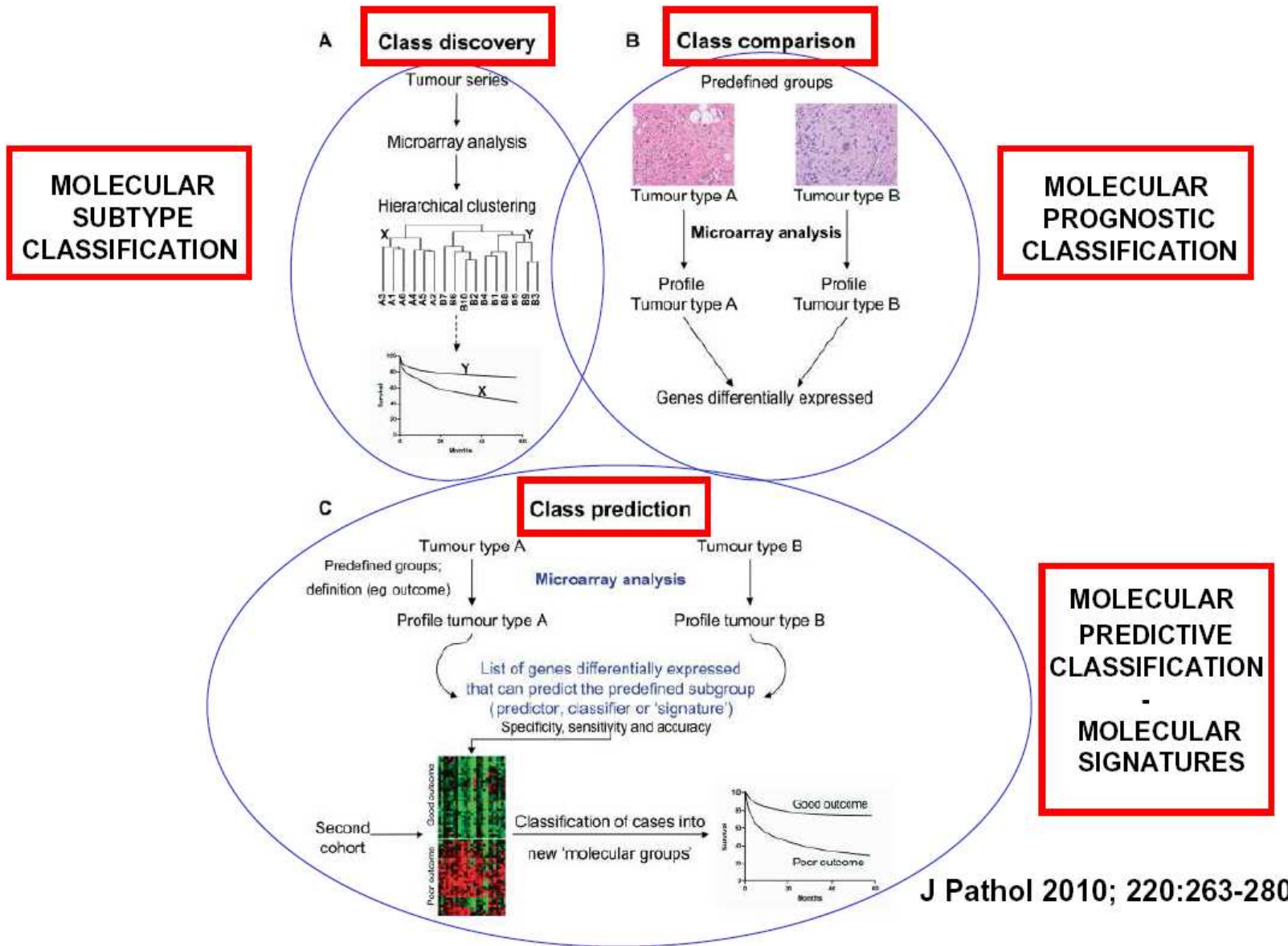


	ACCURATEZZA	UTILIZZO	CLINICO	APPLICABILITA'
CLASSIFICAZIONE WHO	Green	Yellow	Green	Green
St. GALLEN PREDITTIVA	Green	Green	Green	Green
MOLECOLARE	Green	Yellow	Green	Red

Integrazione delle classificazioni istopatologica, predittiva e molecolare



Microarrays-based gene expression profiling studies



MOLECULAR SUBTYPE CLASSIFICATION (1)

2010

ER+

LUMINAL A: high levels of ER, low proliferation rates, low histological grade

LUMINAL B: lower levels of ER, higher proliferation rates, higher histological grade

ER-

BASAL SUBTYPE

ERB-B2/HER2 +: overexpress ERB-B2 or HER2 amplified

[The majority (more than 80%) of molecular-defined HER2 cancers harbour HER2 gene amplification or HER2 3+ IHC expression, not all HER2 amplified tumors fall into the HER2 cluster by molecular analysis. A significant number of HER2 amplified, ER+ cancers actually fall into the luminal B subtype rather than HER2 molecular subtype.

Parker JS et al J Clin Oncol 2009]

NORMAL BREAST-LIKE: this subtype is poorly characterized

[The proponents of the molecular classification suggested that this subtype may be a mere artifact of sample representation (i.e. disproportionately high content of normal tissue contamination. **Parker JS et al J Clin Oncol 2009]**

MOLECULAR SUBTYPE CLASSIFICATION (2)

2010

3 additional **ER-** molecular subtypes:

MOLECULAR APOCRINE: similar to the HER2 subtype, with activation of the androgen receptor signalling.

Farmer P et al Oncogene 2005

INTERFERON-RICH: high expression of interferon regulated-genes, including STAT1 (Signal Transducer and Activator of Transcription1). **Hu Z et al BMC Genomics 2006**

CLAUDIN LOW: tumors with transcriptomic features suggestive of a “cancer stem cell-like” phenotype.

Hennesy BT et al Cancer Res 2009

clinical and biological significance

remains to be determined

MOLECULAR PROGNOSTIC - PREDICTIVE CLASSIFICATION by GENE EXPRESSION SIGNATURES

TABLE 2. Commercially Available Prognostic and Predictive Gene Expression Signatures

Gene Signature	Mammaprint (Agendia)	Oncotype DX (Genomic Health)	EP (Sividon Diagnostics GmbH)	Theros (Biotheranostics)	MapQuant DX (Ipsogen)
Assay	70-gene signature	21-gene recurrence score	11-gene EP score	2-gene HOBX13:IL17R ratio/ 5-gene molecular grade index combined into a Breast Cancer Index	97-gene Genomic Grade Index
Platform	Microarray	qRT-PCR	qRT-PCR	qRT-PCR	Microarray
Tissue type	Frozen or stabilized RNA	FFPE	FFPE	FFPE	Frozen or FFPE
Results	Good vs. poor prognosis	Low vs. intermediate vs. high risk	Low vs. high risk	Low vs. intermediate vs. high risk	GG1 (low grade) vs. GG3 (high grade)
Clinical Indication	Prognosis in N0, < 5 cm diameter, stage I/II, ER + or ER - BC	Prognosis and prediction of benefit from chemotherapy in ER + N0/ 1-3N+ BC on tamoxifen use	ER + HER2 - patients treated with endocrine therapy alone	Prognosis and prediction of response to tamoxifen in ER + BC	Molecular grading in ER +, histologic grade II BC
Level of Evidence*	III	I	I	III	III
FDA approval	Yes	No	No	No	No
ASCO recommended	No	Yes	No	No	No
Randomized trial	MINDACT	TAILORx	No	No	No

*Levels of evidence as defined by Simon et al.⁷²

ASCO indicates American Society of Clinical Oncology; BC, breast cancer; ER, estrogen receptor; EP, endopredict; FDA, Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; GG, Genomic Grade; MINDACT, Microarray In Node-Negative and 1 to 3 positive lymph node Disease may Avoid Chemo Therapy; N+, positive lymph nodes; N0, lymph node negative; qRT-PCR, quantitative reverse-transcriptase PCR; TAILORx, Trial Assigning Individualized Options for Treatment (Rx).

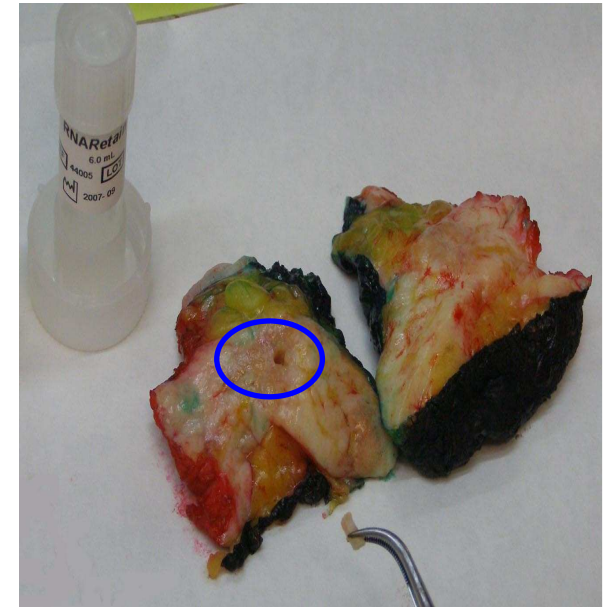
Protocollo operativo percorso casi di carcinoma mammario infiltrante da avviare al test diagnostico-molecolare MammaPrint™



Campionamento **entro un'ora** dall'intervento chirurgico



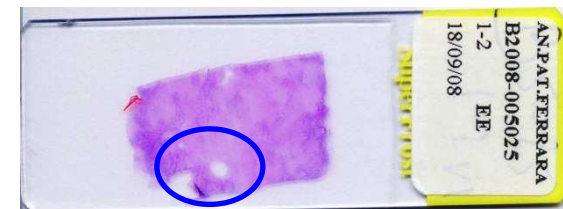
Biopsia 3 mm



Fissato in RNA later



AN PAT



SETTEMBRE 2008

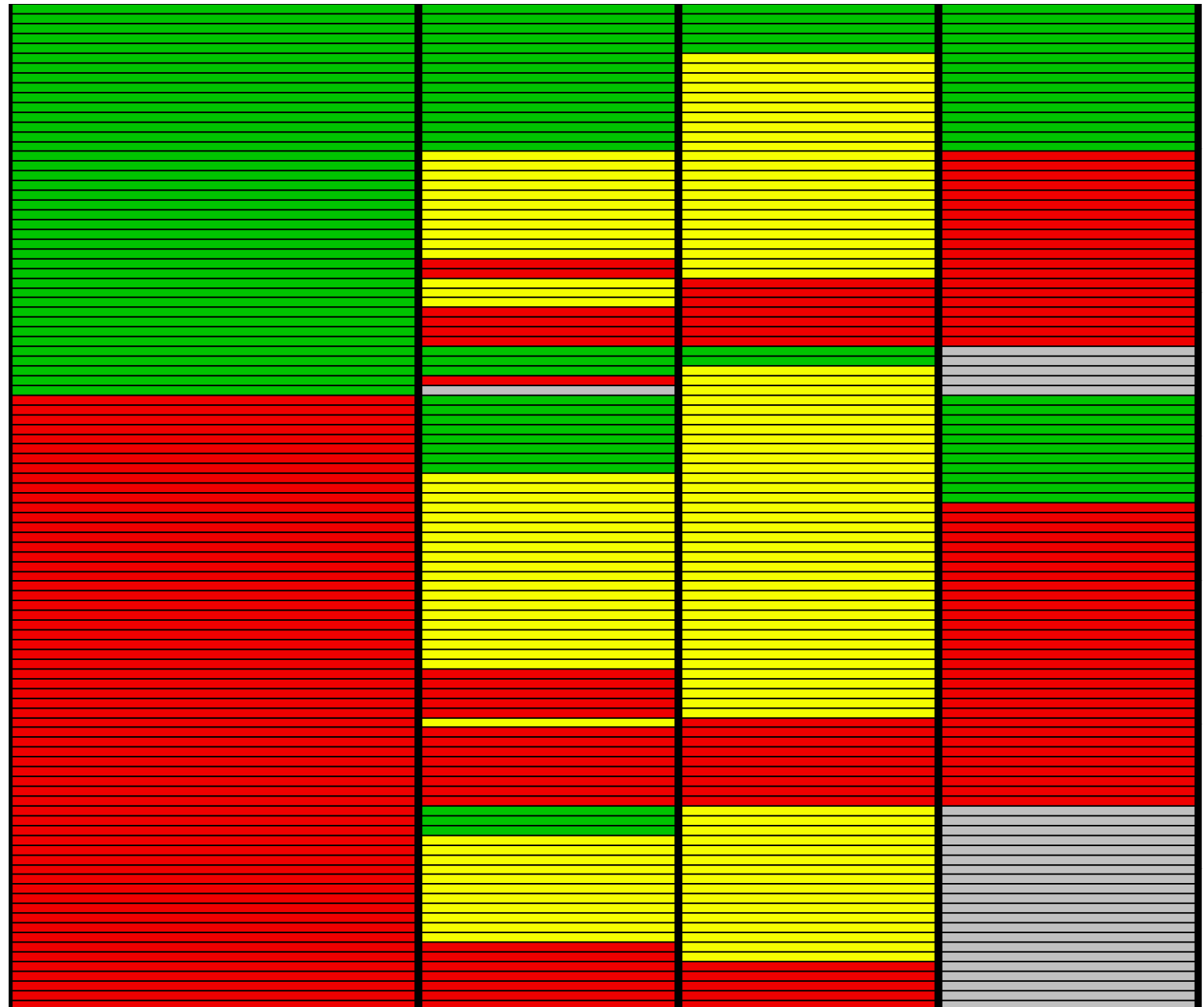
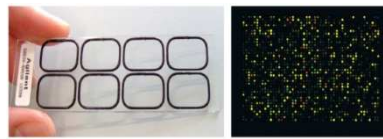
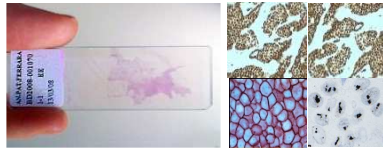
Confronto tra diverse metodiche di valutazione prognostico-predittive : 103 pz

Mammaprint

NPI

St.Gallen

ADJUVANT on LINE



CUSTOMER

Doctor : Italo Nenci
Account : Università di Ferrara
Address : Via fossato di mortara 64 b
Anatomia patologica, dept. di m
City : Ferrara 44100
Country : Italy

SPECIMEN

Requisition # : 00008295
Customer Reference : B/2009/003412
Collection Date : 09-Jun-2009
Test Request Date : 09-Jun-2009
Date Received : 15-Jun-2009
Report Date : 28-Jun-2009
Specimen Type : Surgical Specimen 3

PATIENT

Patient :
DOB : 09-Jan-1900
Patient # :
Gender : Female

Gene Profile Result

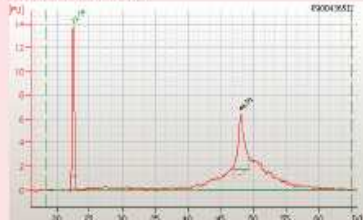
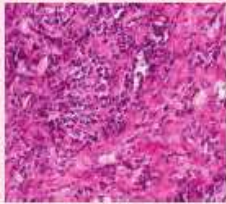
High Risk

The breast cancer tissue sample submitted was analyzed by MammaPrint®, an FDA Cleared IVDMA 70 - Gene Profile of Breast Cancer for Metastatic Risk that has been validated to correlate with high or low outcome risk for distant metastases in women with breast cancer ≤ 5 cm with up to 3 positive lymph nodes.

Clinicopathologic Findings

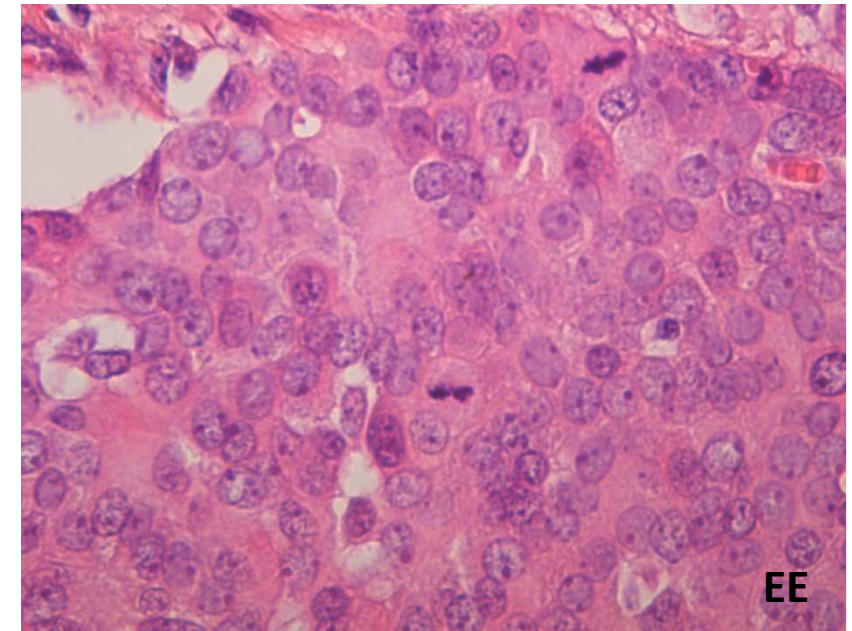
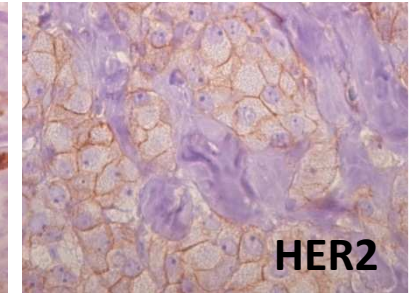
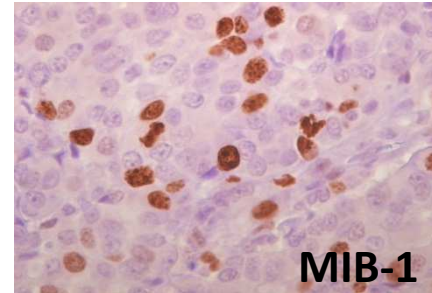
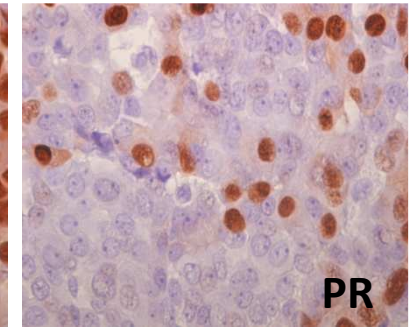
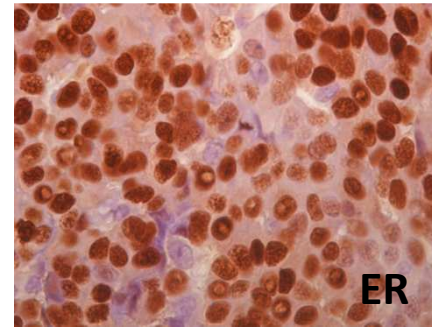
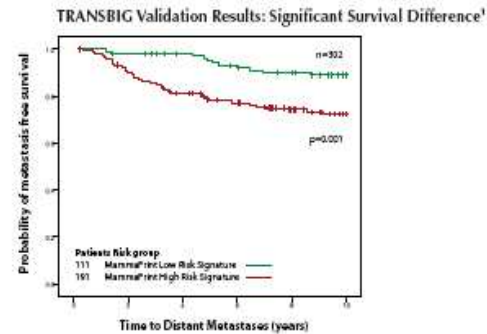
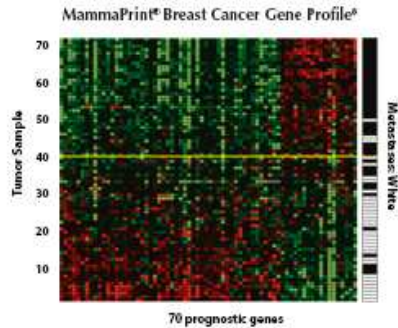
Tumor Cell Percentage: 70%

RNA Integrity Score: 6.1



Assay Description

In the reference group as published¹, lymph node negative patients classified as "low risk" had a 10% chance to develop distant metastases at 10 years without any adjuvant treatment. Patients classified as "high risk" had a 30% chance to develop distant metastases at 10 years without adjuvant treatment. MammaPrint® has been validated in over 1,600 breast cancer patients and shown to provide information independent of clinicopathological risk assessment^{1,2}.



“First-generation” PROGNOSTIC GENE SIGNATURES

2012

- 70-Gene signature (MammaPrint®)
- 21-Gene recurrence score (OncotypeDx®)
- 2-Gene ratio HOXB13/IL17R (H/I) and molecular-grade index (Theros®)
- Genomic grade index (MapQuant Dx®)
- Intrinsic subtypes derived prognostic index (PAM50 BC Intrinsic Classifier®)
- 11-Gene EP score (EP®)

“First-generation” PREDICTIVE GENE SIGNATURES

- 21-Gene recurrence score (OncotypeDx®)

level 1B evidence-for routine clinical use as an adjunct
to clinical and pathological information

“Next-generation” PROGNOSTIC PREDICTIVE GENE SIGNATURES

- Immune response-related gene signature
- Stromal-related gene signature
-

Integrative approach combining
Genetic
Transcriptomic
Proteomic



biology of tumor clinically relevant

Morphologic & IHC Correlates for Molecular Subsets of Breast Cancer

Luminal A

Luminal B

HER2

Basal-like

ER Expression

Proliferation (KI67)

HER2 Over-Expression

Different subsets show distinctive morphologic features

- Grade
- Architecture
- Growth pattern

Can be recognized or suspected in some cases

Proposed IHC Surrogates for Molecular Classification of Breast Cancer

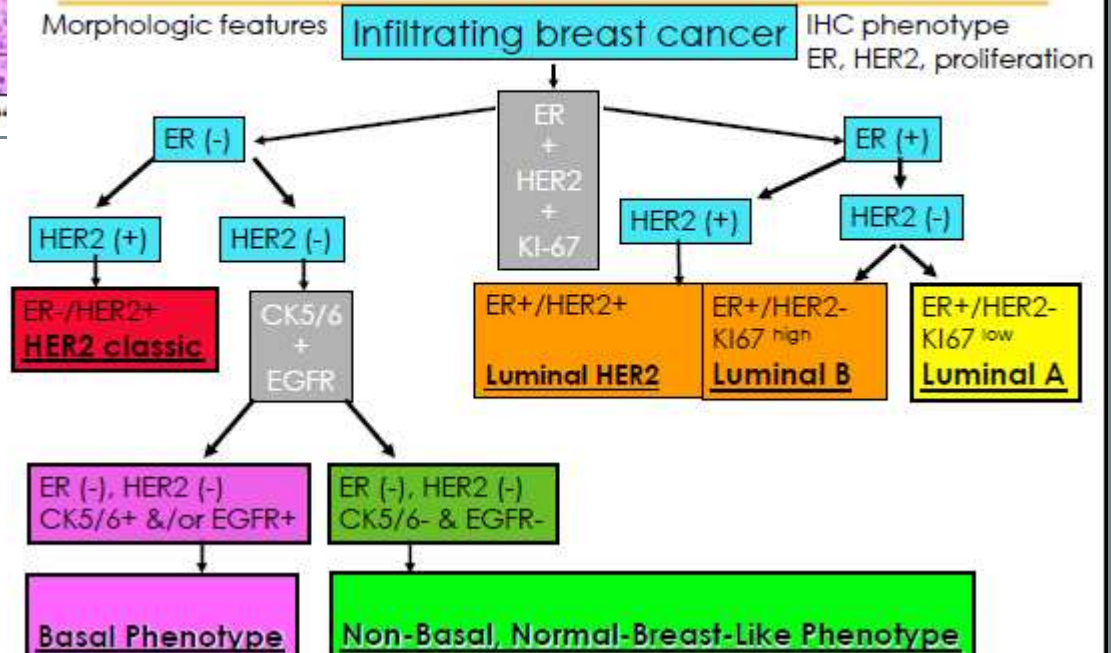


Table 2 – Characteristics of molecular subtypes and assignment of histological special types of breast cancer.

Molecular subtype	ER, PR, HER2	Additional markers	Proliferation cluster microarrays	Histological special types
Basal-like	ER- PR- HER2-	CK5/6+ EGFR+	High	Adenoid cystic Acinic cell Medullary Metaplastic Pleomorphic lobular Secretory
HER2/ER-	ER- PR- HER2+	CK5/6+/- EGFR+/-	High	Apocrine Lobular Micropapillary Pleomorphic lobular
Normal breast-like	ER-/+ PR unknown HER2-	CK5/6+ EGFR+	Low	Medullary Metaplastic
Luminal	ER+ (-) PR+/- HER2- (+)		Low/high	Apocrine IDC osteoclastic Lobular Micropapillary Mucinous Neuroendocrine Pleomorphic lobular Tubular
Molecular apocrine	ER- PR- HER2+/-	AR+ CK5/6+/- EGFR+/-	High	Apocrine Pleomorphic lobular
Claudin-low	ER- PR- HER2-	CLDN low/- CDH1 low/- CK5/6+/- EGFR+/-	High	Metaplastic Medullary (?)
Interferon-related	ER-/+ PR unknown HER2-	STAT1	High	Medullary (?)

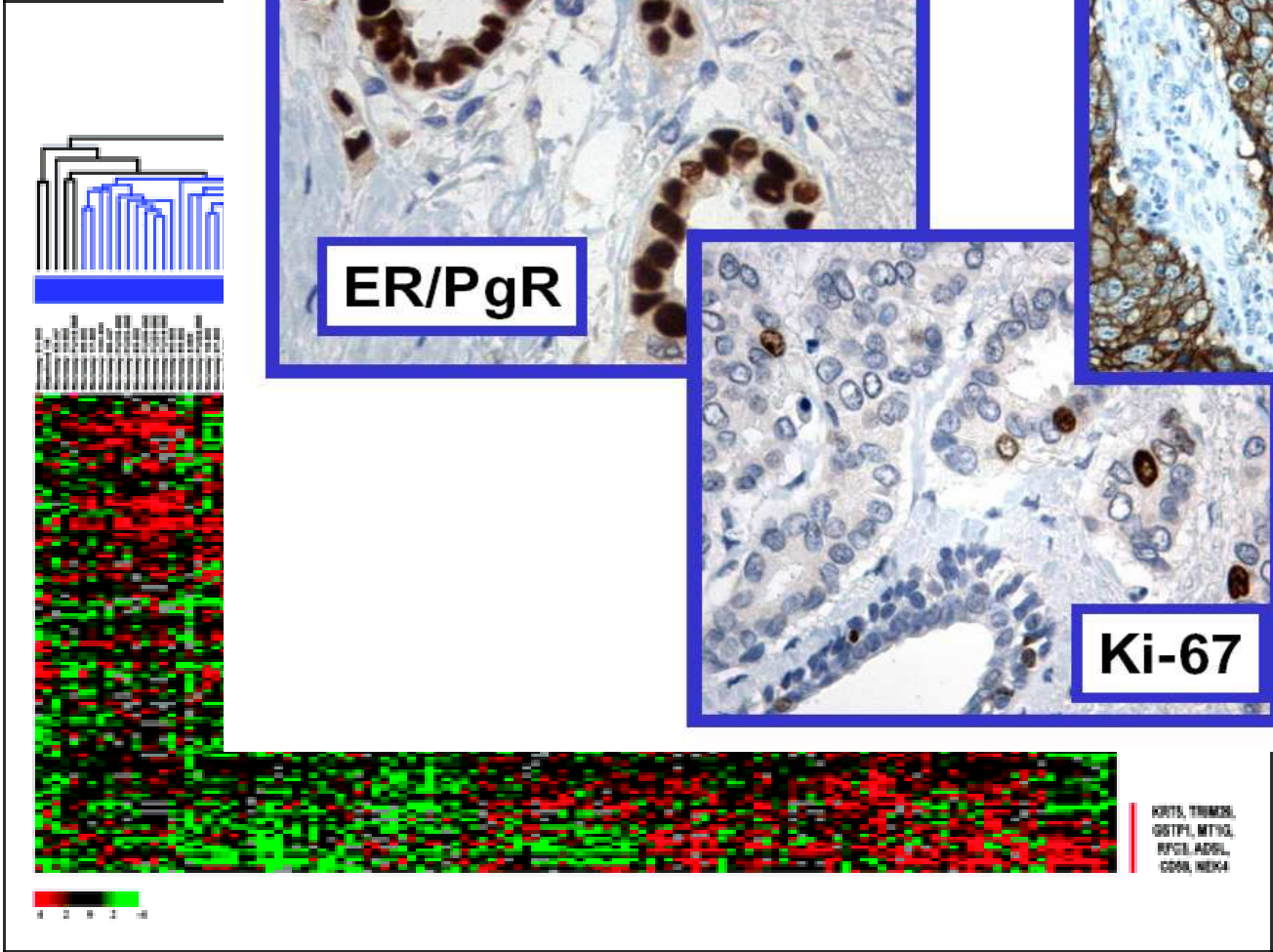
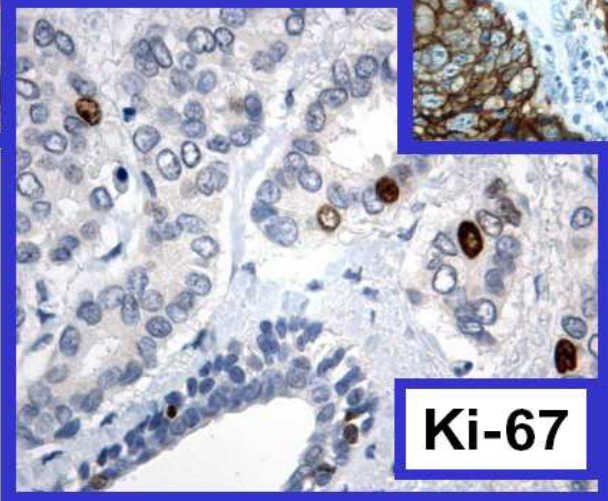
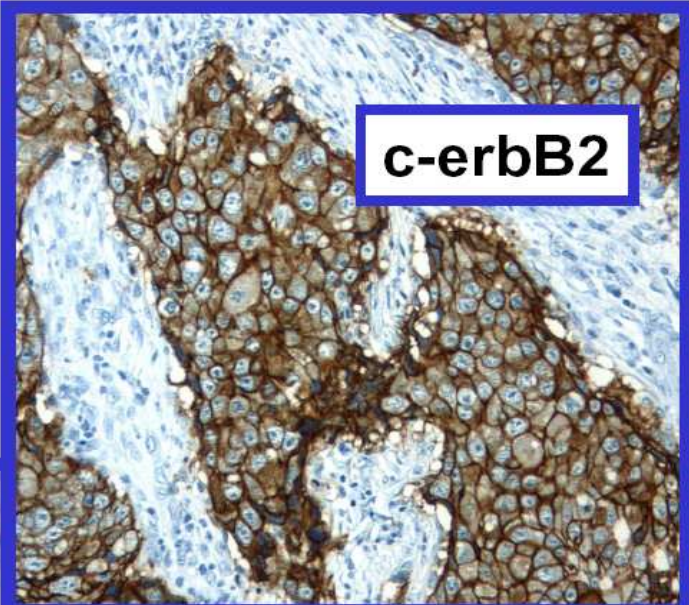
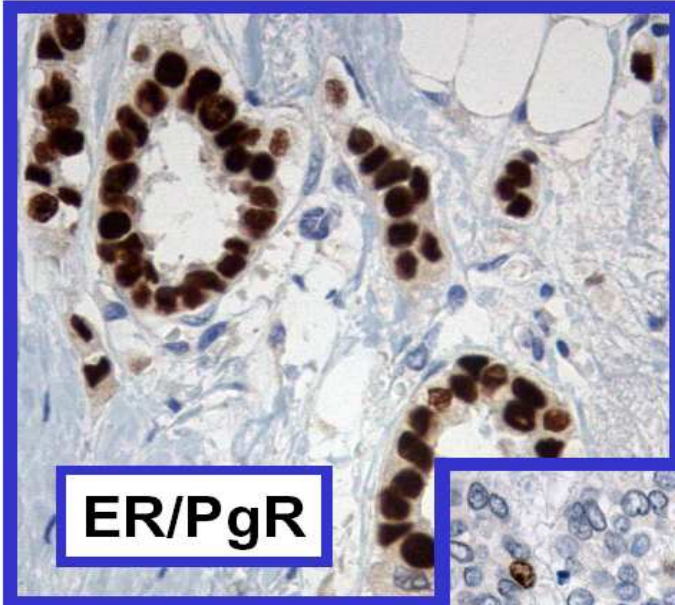
AR: androgen receptor; CDH1: E-cadherin; CLDN: claudin; CK: cytokeratin; EGFR: epidermal growth factor receptor; ER: oestrogen receptor; IDC: invasive ductal carcinoma; PR: progesterone receptor; STAT1: signal transducer and activator of transcription 1. -: negative; +: positive; +/-: occasionally positive; -/+: rarely positive.

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Classificazione immunoistochimica dei sottotipi molecolari *St Gallen 2011*

'Subtype' <i>Surrogate IHC markers</i>	Type of therapy	Notes
'Luminal A' <i>ER and/or PgR positive HER2 negative, Ki-67 low (<14%)*</i>	Endocrine therapy alone	Few require cytotoxics (e.g. high nodal status).
'Luminal B (HER2 neg)' <i>ER and/or PgR positive HER2 negative, Ki-67 high</i>	Cytotoxics + endocrine therapy	Inclusion and type of cytotoxics may depend on perceived risk and patient preference.
'Luminal B (HER2 pos)' <i>ER and/or PgR positive HER2 positive</i>	Cytotoxics + anti-HER2 + endocrine therapy	No data are available to support the omission of cytotoxics in this group.
'HER2 positive (non luminal)'	Cytotoxics + anti-HER2	
'Triple negative (ductal)'	Cytotoxics	Consider DNA disrupting agents.
'Special histological types'* A. Endocrine responsive B. Endocrine non responsive	Endocrine therapy Cytotoxics	Medullary and adenoid cystic carcinomas may not require any adjuvant cytotoxics.

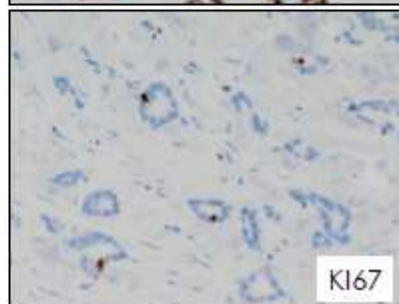
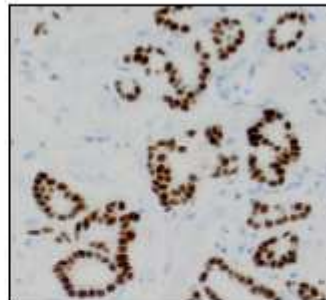
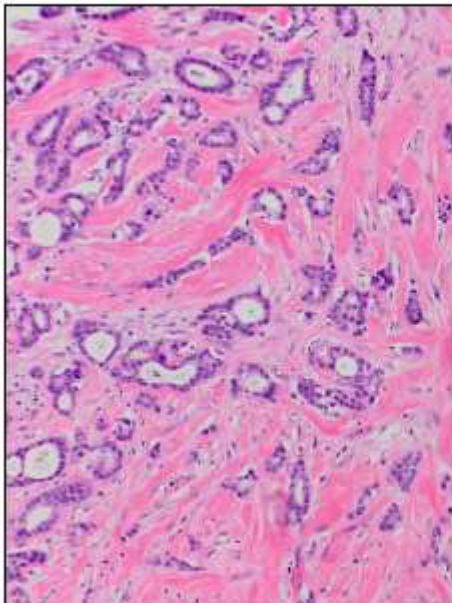
PARAMETRI BIOLOGICI



Luminal A Breast Tumors: Clinical Features

- **Demonstrate higher level of expression of ER & ER related genes**
 - Lower histologic grade
 - Lower levels of proliferation related genes
- **More indolent clinical course**
 - May experience later recurrences
- **Better prognosis compared with Lum-B and other subty**

ER+ Breast Cancer (Luminal A)



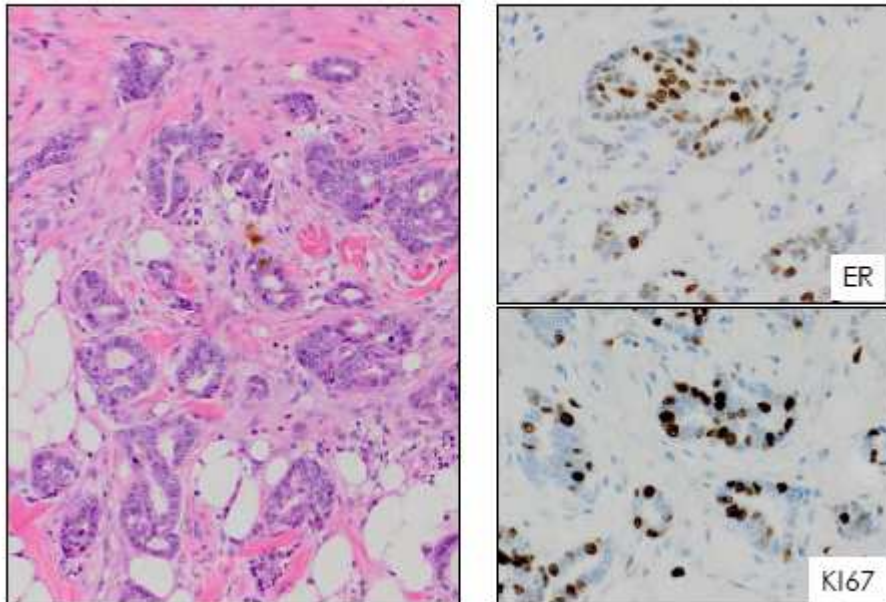
Luminal A Breast tumors

- **Implications for therapy**
 - Good prognosis
 - Respond well to endocrine therapy
 - May be adequately treated with hormonal therapy alone
 - Tend to respond poorly to adjuvant and neo-adjuvant chemotherapy
 - Typically low recurrence score by Oncotype Dx

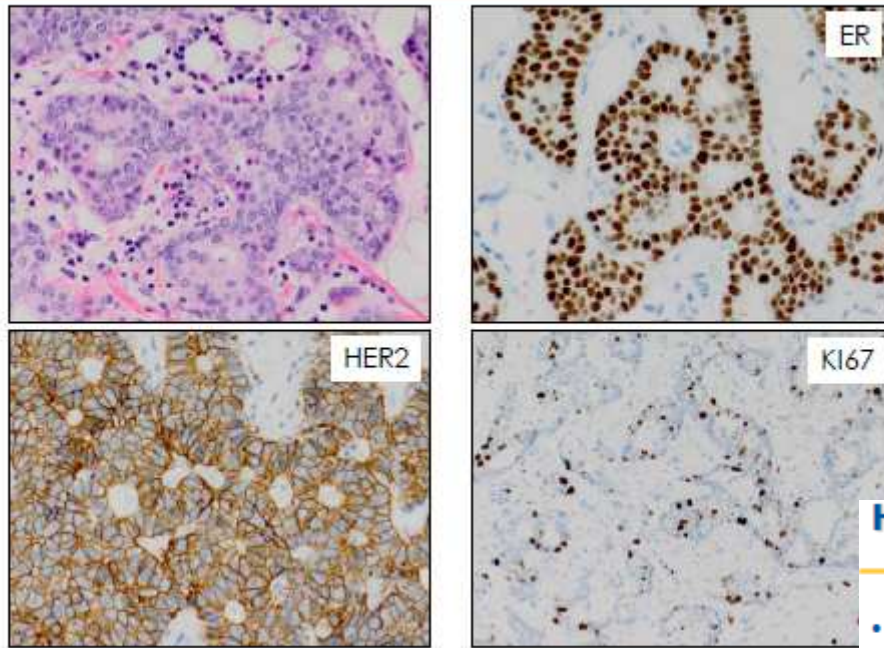
Luminal-B Breast Tumors: Clinical Features

- **Lower levels of ER expression and ER-related genes**
 - May be PR negative
 - May over-express GFR (HER2 & EGFR)
 - 30% of Luminal-B tumors over-express HER2 (IHC & FISH+)
- **Higher histologic grade**
- **More aggressive clinical course, worse prognosis**
 - More likely lymph node positive
- **Higher expression of proliferation related genes**
 - Ki-67 proliferation index may be useful in separating Lum B from Lum A tumors

ER+ Breast Cancer (Luminal-B)



ER+ Breast Cancer (Luminal-B/Luminal-HER2)



Luminal-B/Luminal HER2 Breast Tumors

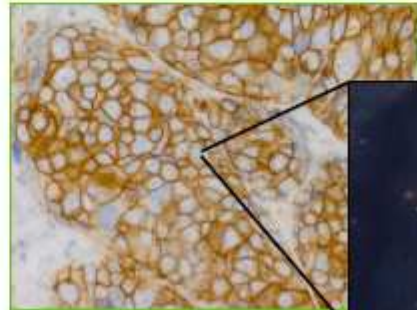
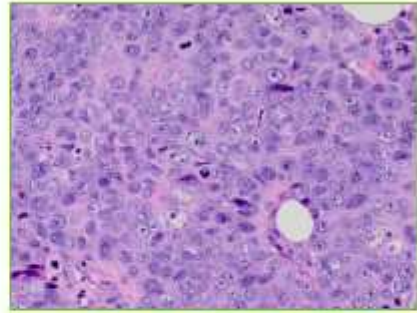
- **Implication for therapy**
 - Aggressive clinical course
 - May be less responsive to tamoxifen
 - More likely to respond to chemotherapy added to endocrine treatment
 - Typically - high Recurrence Score by Oncotype Dx
 - For HER2+ Luminal-B tumors
 - Similar benefit from HER2-targeted therapy compared with HER2+/ER- tumors in adjuvant clinical trials

HER2+ Breast Tumors

- HER2+ breast tumors by GEP are ER-
 - Over-expression of other genes in HER2-amplicon (GRB7, TOP2A)
 - High proliferative index
 - More likely to harbor p53 mutations
 - Higher histologic grade
 - Younger age at presentation
- Aggressive clinical course, poor prognosis

HER2 Classic Breast Cancer Subtype

- 39 year old female
- Size: 4 cm
- Lymph nodes: (2/4)
- ER: negative
- PR: negative
- KI-67: 60%
- P53: positive
- Modified-SBR: 3/3



HER2+ Breast Tumors

- **Implications for therapy**
 - More likely to respond to anthracyclines
 - May be explain by co-amplification of TOP2A
 - Good response to trastuzumab therapy in combination with chemotherapy (adjuvant and metastatic)
 - More likely to show pCR to neo-adjuvant chemotherapy + trastuzumab
 - ER+/HER2+ tumor may show Tam resistance

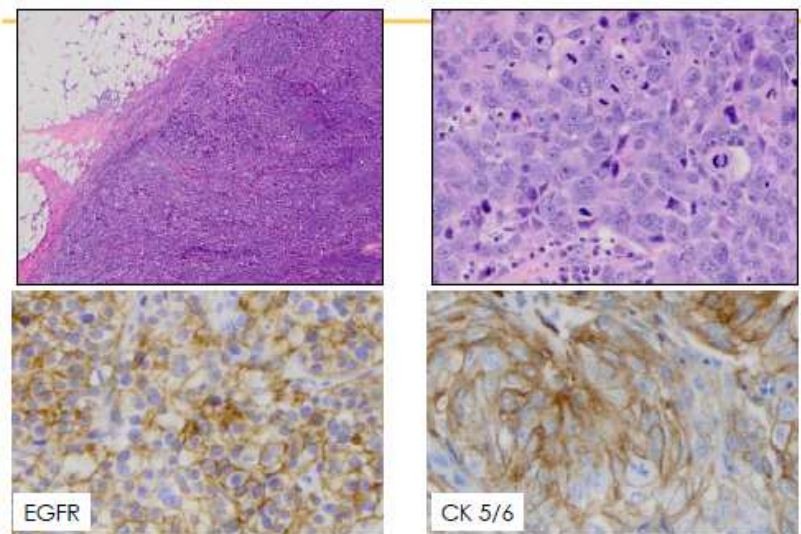
Basal Subtype Breast Tumors: Clinical Features

- **GEP reminiscent of normal myoepithelial cells**
 - Lack of expression of ER and related genes, low HER2
 - High expression of basal cytokeratins (CK5,6,14,17)
 - High expression of proliferation-related genes
 - Amplification/over-expression of EGFR (other GFR), VEGF
- **Aggressive clinical course, poor prognosis**
 - Increase likelihood of early systemic recurrence
 - Visceral recurrence & brain mets more likely than other subtypes
- **Hereditary breast cancer**
 - BRCA1 mutation generally develop basal-like breast tumors
- **Premenopausal African American women 2x the risk of developing basal-like tumors as other group of women**

Basal-Like Carcinoma: Morphologic Features & Immunophenotype

Basal Subtype Breast Tumors

- **Implications for therapy**
 - No role for endocrine therapy or trastuzumab
 - Aggressive clinical course = combination cytotoxic chemotherapy
 - More likely to achieve pCR to neoadjuvant chemo
 - More sensitive to conventional chemo than luminal tumors
 - Anthracyclines + taxane
- **Absence or impaired BRCA1 function-**
 - Loss or impairment of DNA repair
 - Hypersensitivity to DNA damaging agents
 - PARP inhibitors (target DNA repair pathways) in clinical trials



Basal Subtype Breast Tumors

- **Implications for therapy**
 - No role for endocrine therapy or trastuzumab
 - Aggressive clinical course = combination cytotoxic chemotherapy
 - More likely to achieve pCR to neoadjuvant chemo
 - More sensitive to conventional chemo than luminal tumors
 - Anthracyclines + taxane
- **Absence or impaired BRCA1 function-**
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RACCOMANDAZIONI

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IAP)

CAP

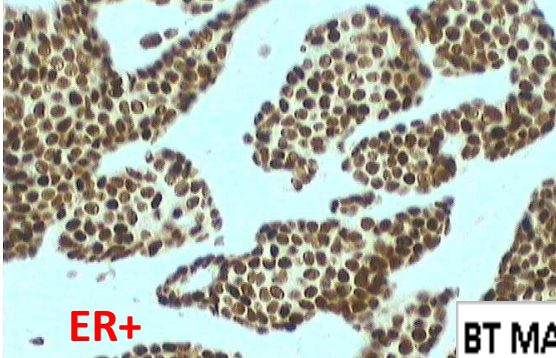
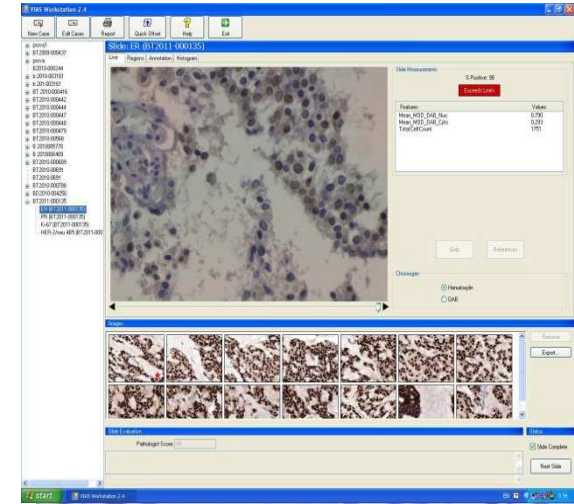
3° Consensus Workshop(AIOM, SIAPEC-

- Determinazione di ER, PR, MIB1 ed HER2 in tutti i carcinomi infiltranti della mammella e sull'eventuale ripresa di malattia.
- Utilizzo di test che siano accurati (sia nella fase pre-analitica che analitica) e riproducibili.
- Impiego di criteri per definire l'adeguatezza del preparato e per la refertazione (computer-assistita).
- I laboratori devono ottenere un'elevata concordanza tra le diverse metodiche .
- Partecipazione a controlli di qualità.
- Training del personale tecnico e medico.

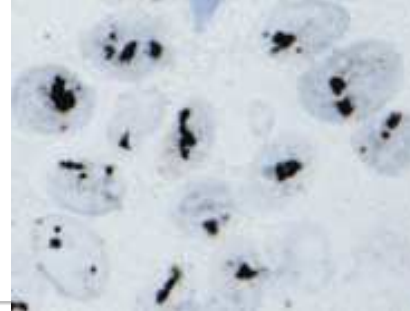
Immunocolorettore automatico Ventana Bechmark XT



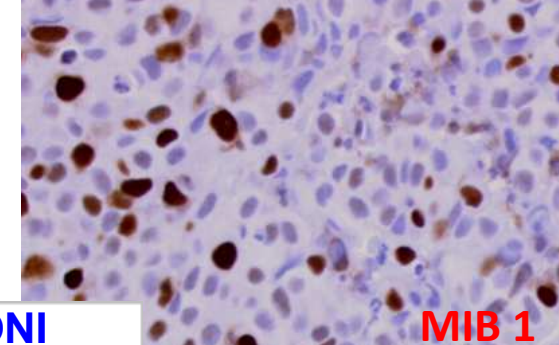
Analizzatore d'immagine computerizzata Ventana VIAS



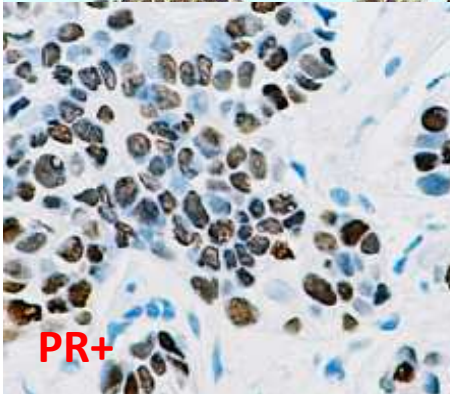
ER+



5456 DETERMINAZIONI

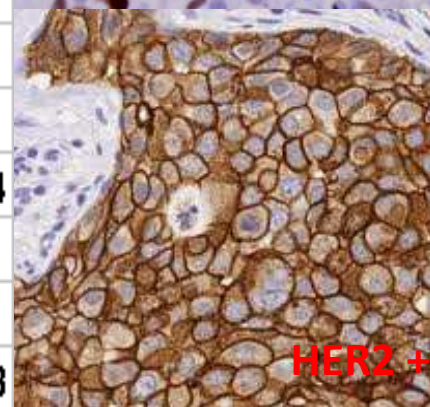


MIB 1



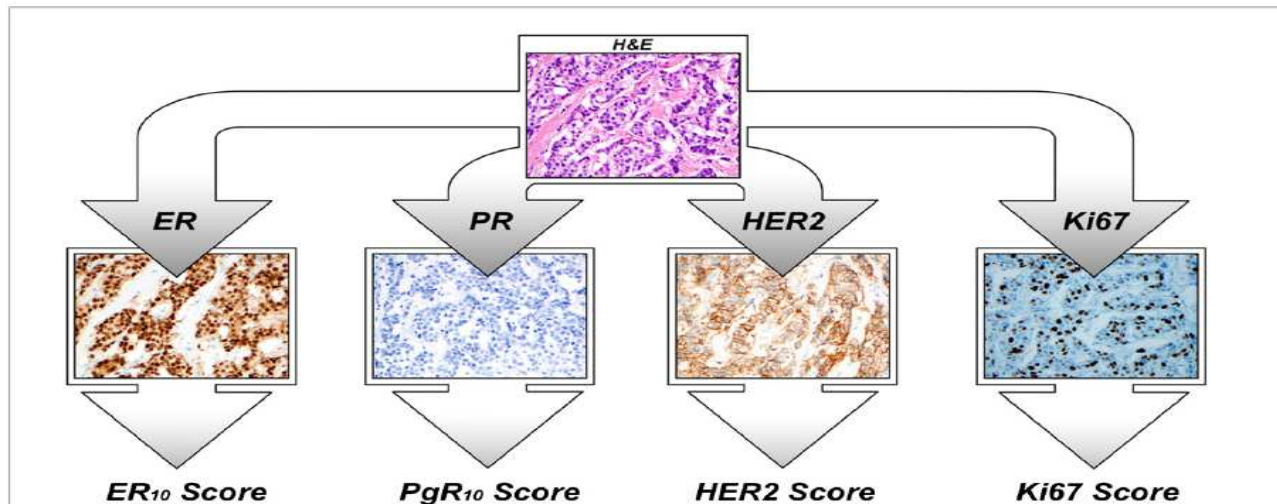
PR+

BT MAMMELLA		5456 DETERMINAZIONI				
Campioni Chirurgici		2010	2011	2012	16-apr-13	Totale
		421	450	375	118	1364
Campioni bioptici (B5)						
		64	61	96	32	253



HER2+

**Sezione Diagnostica Biotecnologica dal 1990: 7170 CASI (28680 determinazioni)
IHC e ISH**



Totale casi: 2906

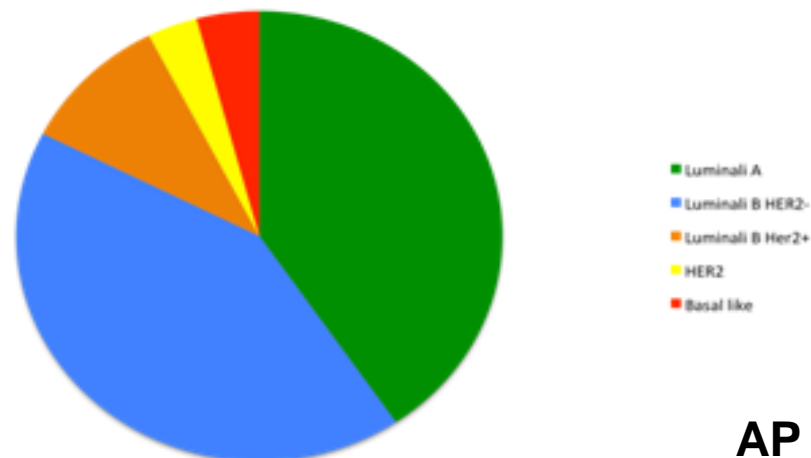
Luminali A (ER+/PR+/Mib<14/Her2<=2): 1177

Luminali B Her2 - (ER+ e/o PR+/Mib1>14/Her2<=2): 1221

Luminali B Her2+ (ER+ e/o PR+/Her2: 3+)= 290

HER2: (ER-/PR-/Her2:3+)= 97

Basal like (ER-/PR-/Her2-)=121



AP FE

LINEE GUIDA INTERNAZIONALI

**ASCO/CAP
2007**

**Sauter et Al.
Revisione
metodologica
2009**

HER2 Test

Technical validation requirements of an assay

Internal and external quality assurance schemes:

Fixation – selection of test – bias in analysis

**ASCO/CAP
2010**

**Analytical validation
Fitzgibbons 2010**

ER-PGR Test

Technical validation requirements of an assay

**Technical validation requirements for
ER e PGR**

**Ki67 - breast study
Group - Dowsett 2011**

Ki67 Test

Preanalytical validity

GUIDELINE QUESTIONS

The overall purpose of this guideline is to improve the accuracy of hormone receptor testing and the utility of ER and PgR as prognostic and predictive markers for assessing in situ and invasive breast carcinomas. Therefore, this guideline addresses two principal questions regarding ER and PgR testing. Findings are listed in Table 1.

1. What is the optimal testing algorithm for determining ER and PgR status?
 - 1.1. What are the clinically validated methods that can be used in this assessment?
2. What strategies can ensure optimal performance, interpretation, and reporting of established assays?
 - 2.1. What are the preanalytic, analytic, and postanalytic variables that must be controlled to ensure that assay results reflect tumor ER and PgR status?
 - 2.2. What is the optimal internal quality management regimen to ensure ongoing accuracy of ER and PgR testing?
 - 2.3. What is the regulatory framework that permits application of external controls such as proficiency testing and on-site inspection?
 - 2.4. How can internal and external control efforts be implemented and their effects measured?

The Panel also reviewed a few special questions.

1. Should immunohistochemistry (IHC) of ER/PgR be performed in ductal carcinoma in situ (DCIS) or recurrent breast cancer specimens?
2. Does PgR expression in breast cancer correlate with or influence the choice of endocrine therapy?

Indicazioni

- Tutti i carcinomi della mammella invasivi di nuova diagnosi
- se pTm: su almeno uno, preferibilmente quello di maggiori dimensioni
- recidive
- CDIS di nuova diagnosi
(National Surgical Adjuvant Breast and Bowel Project (NSABP) B-24 clinical trial)

Ruolo PR

- Valore predittivo indipendente da ER, specialmente nelle donne in post-menopausa
(Ogawa Y, Breast Cancer 2004; Yamashita H, Breast Cancer 2006)

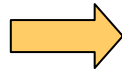
Cosa incide sulla preservazione del mio tessuto?

SALA CHIRURGICA

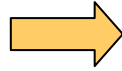
ANATOMIA PATOLOGICA



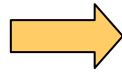
PREPARAZIONE DEL CAMPIONE



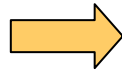
Asportazione/Fissativo



Spessore/tipo di tessuto



Tempo di fissazione



Temperatura del processo



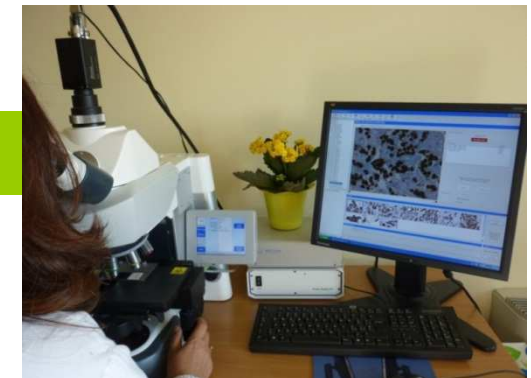
Processazione post-fissazione



OUT



IN

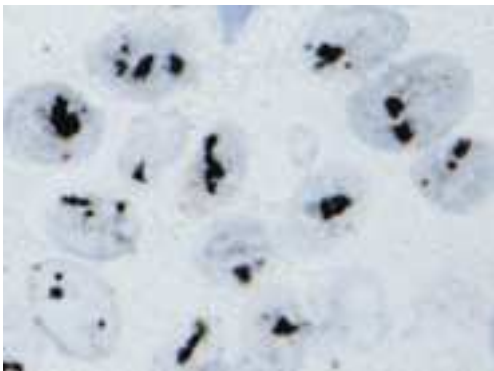


HER2 testing in breast cancer: an overview of current techniques and recent developments

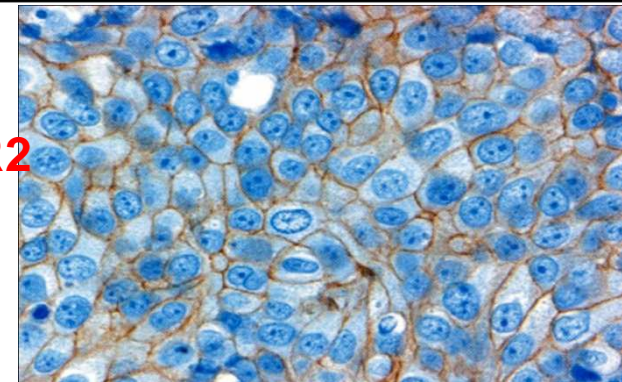
2012

Table 1 Advantages and disadvantages of commonly used technologies for HER2 testing

Method	Advantages	Disadvantages
IHC	<ul style="list-style-type: none"> ● Widely practiced ● Relatively easy, quick and cheap Can be automated ● Long-term storage of materials ● Morphology seen in same section 	<ul style="list-style-type: none"> ● Susceptible to numerous variations in testing protocol ● Interpretation is subjective and semi-quantitative
FISH	<ul style="list-style-type: none"> ● Interpretation is quantitative ● Resolves IHC 2+ cases ● Partial automation available ● Less affected by pre-analytical factors and handling than IHC 	<ul style="list-style-type: none"> ● More expensive than IHC ● Signal decays over time ● Areas of invasive breast carcinoma may be difficult to identify ● Does not allow for simultaneous assessment of cell morphology ● Few pathologists and technicians are trained in the methodology and its interpretation ● Overnight hybridisation required
SISH	<ul style="list-style-type: none"> ● Fully automated ● Less affected by pre-analytical factors and handling than IHC ● Requires only standard light microscope ● Staining remains stable for a long period of time ● Morphology seen in same section ● Both HER2 and CEP17 bright-field viewable on same slide ● Rapid turnaround 	<ul style="list-style-type: none"> ● Results more difficult to interpret by inexperienced staff ● Some methods require separate slides for HER2 gene and CEP17 copy numbers



**Dual SISH InformHER2
Ventana**



HER2: analisi e interpretazione dei risultati

VOLUME 24 · NUMBER 19 · JULY 1 2006

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

HER2 Testing by Local, Central, and Reference Laboratories in Specimens From the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial

Edith A. Perez, Vera J. Suman, Nancy E. Davidson, Silvana Martino, Peter A. Kaufman, Wilma L. Lingle, Patrick J. Flynn, James N. Ingle, Daniel Visscher, and Robert B. Jenkins

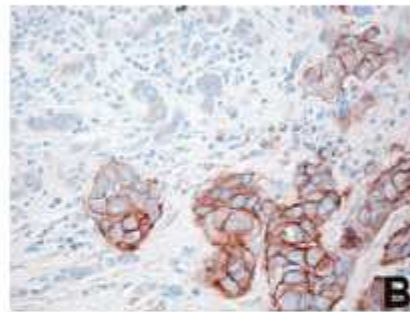
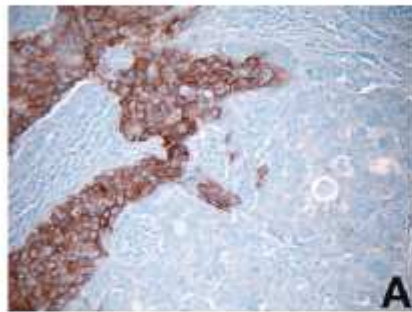
Conclusion

There was a high degree of discordance between local and central testing for IHC and FISH, but a high degree of agreement between central and reference laboratories. These findings support the importance of using high-volume, experienced laboratories for *HER2* testing to improve the process of selecting patients likely to benefit from trastuzumab therapy.

5456 DETERMINAZIONI

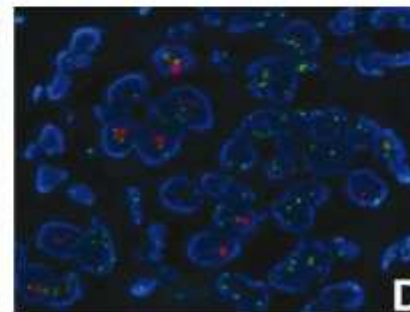
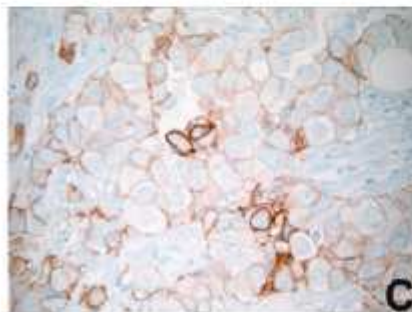
BT MAMMELLA					
Campioni Chirurgici					
2010	2011	2012	16-apr-13	Totale	
421	450	375	118	1364	
Campioni bioptici (B5)					
64	61	96	32	253	

Hot topic: Eterogeneità di HER2



raro

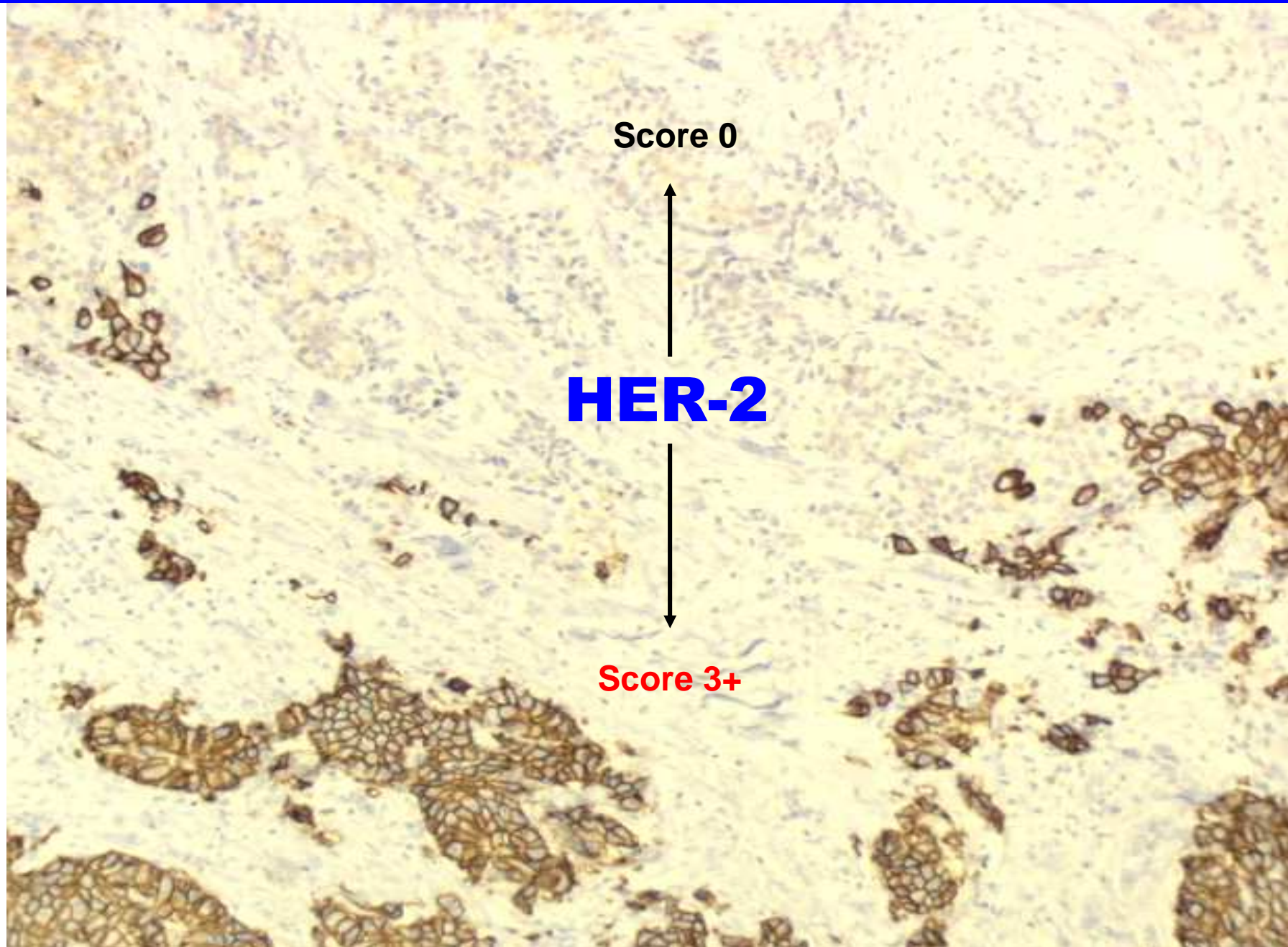
2 CLONI
1 CLONE 100% score 3+
1 CLONE 100% score 0/1+



frequente

1 CLONE
2% score 3+
30% score 2+
68% score 0

ETEROGENEITA' CLONALE



Hot topic: eterogeneità di ki-67



Box 1. Recommendations for Ki67 assessment in breast cancer

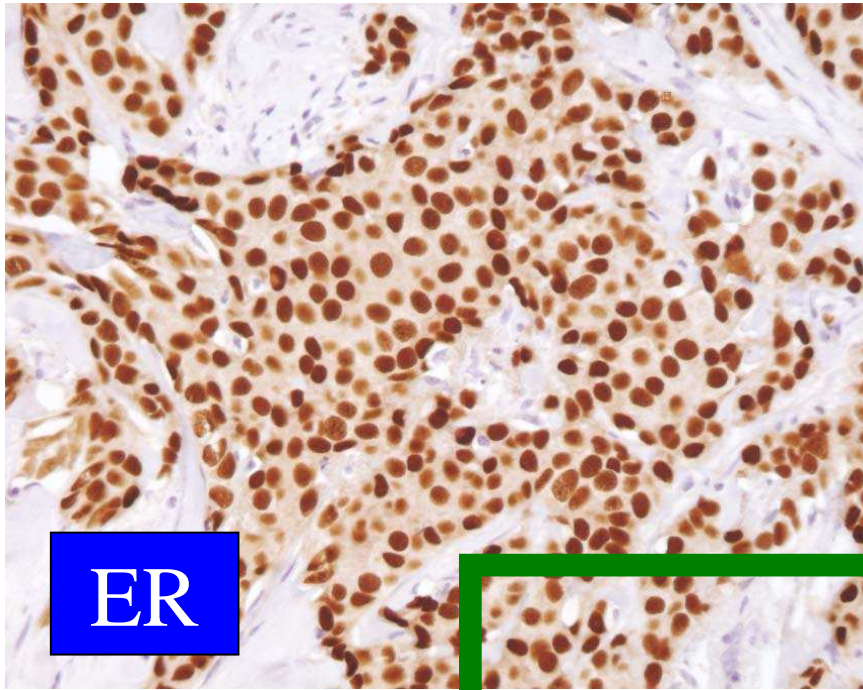
Interpretation and scoring

- In full sections, at least three high-power (x40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
- For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
- If pharmacodynamic comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
- If there are clear hot spots, data from these should be included in the overall score.
- Only nuclear staining is considered positive. Staining intensity is not relevant.
- Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
- Image analysis methods for Ki67 remain to be proven for use in clinical practice.



Dowsett M, JNCI 2011

Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group



ER

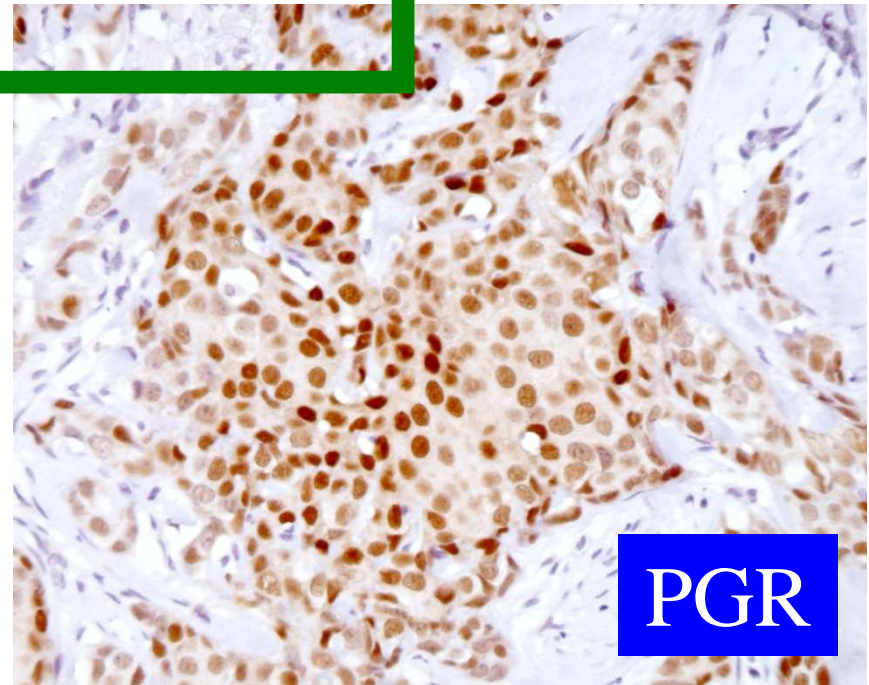
Consensus Meeting StGallen

DEVO VEDERE OGNI ESPRESSIONE

(Sensibilità e specificità)

DEVO QUANTIFICARE ESATTAMENTE

(Sistemi di valutazione quantitativa)

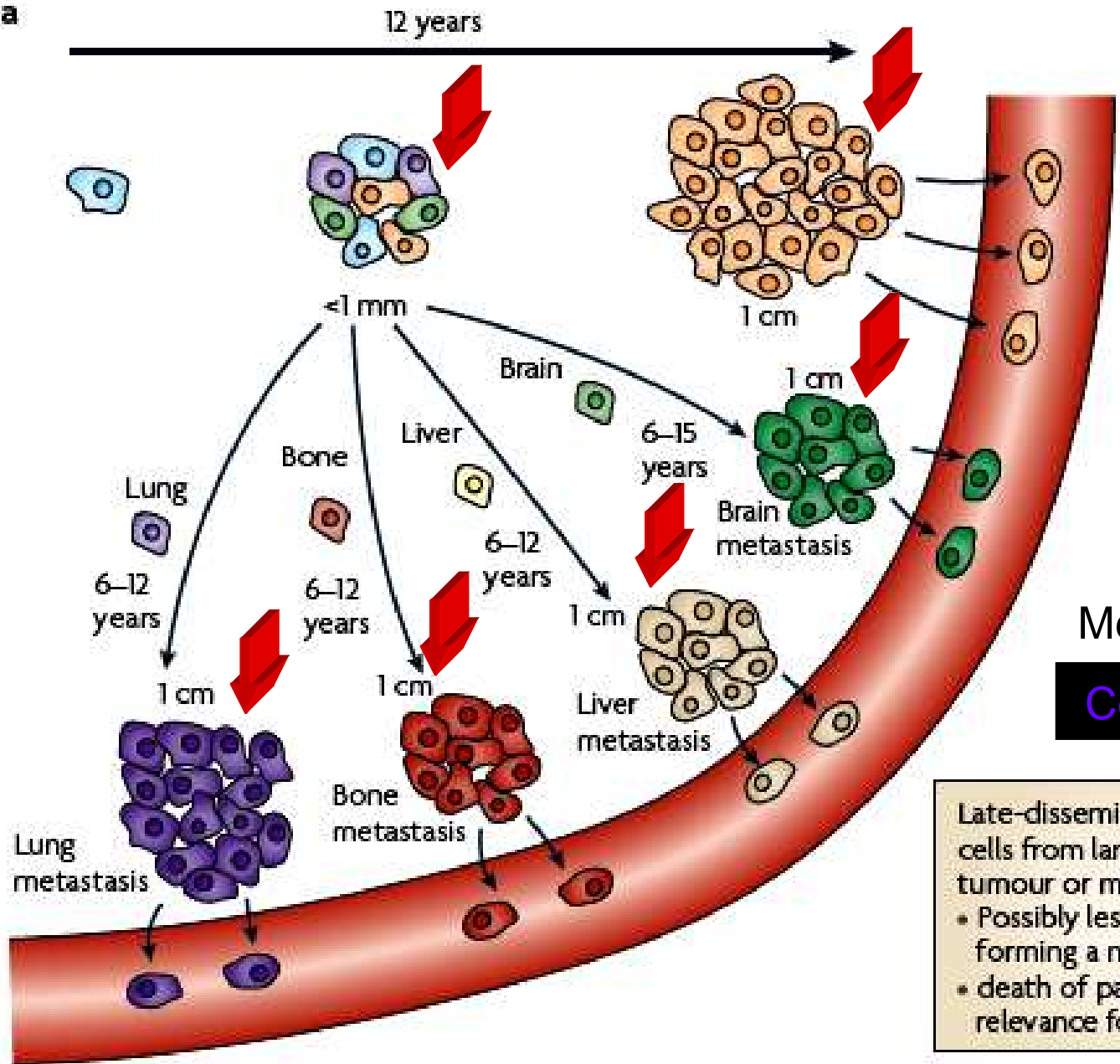


PGR

Modello della Progressione Parallela (Disseminazione metastatica precoce e tardiva)

a

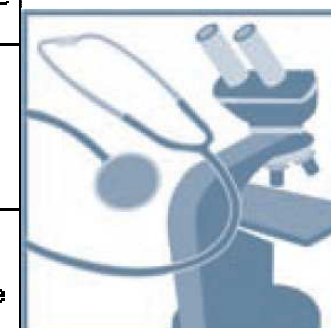
**BIOPROFILO
RIPETUTO??**



Metastasi multiclonali
Colori diversi tra loro

Late-disseminating tumour cells from large primary tumour or metastasis
 • Possibly less capable of forming a metastasis or
 • death of patient before relevance for disease

U.O Oncologia- U.O Anatomia Patologica		
Titolo	Fase	Pazienti
GIM-3 Studio di fase III di confronto tra anastrozolo, letrozolo ed exemestane e tra strategia sequenziale (2 anni di terapia con tamoxifen seguiti da 3 anni di terapia con inibitori delle aromatasi) verso strategia upfront (5 anni di terapia con inibitori delle aromatasi) nel trattamento adiuvante del carcinoma mammario postmenopausa	III	Carcinoma mammario ormonosensibile in pazienti in postmenopausa
ShortHer - Trattamento adiuvante con Herceptin per 3 mesi vs 12 mesi, in associazione con chemioterapia, nelle pazienti carcinoma mammario HER2 positive.	III	Pazienti sottoposte a trattamento chirurgico della neoplasia mammaria, candidate a chemioterapia
OPTIMA - Sequenza ott [redacted] on carcinoma mammario metastatico ricadute dopo terapia adiuvante con inibitori delle aromatasi: studio randomizzato tra due diverse sequenze di terapia endocrina.	III	Donne in postmenopausa, affette da carcinoma della mammella metastatico ormonoresponsivo, ricadute dopo terapia adiuvante con AI
GIM-8 - Studio Randomizzato con disegno fattoriale di confronto tra Fulvestran: ± Lapatinib ± Inibitori Aromatasi nel carcinoma mammario metastatico in progressione dopo terapia con Inibitori Aromatasi	III	Pazienti con carcinoma mammario metastatico in progressione dopo inibitori delle aromatasi
LUX - Studio in aperto, randomizzato, di fase III, con BIBW 2992 e vinorelbina contro trastuzumab e vinorelbina in pazienti con cancro metastatico della mammella, con sovra espressione di HER2, che abbiano fallito un precedente trattamento con trastuzumab.	III	Pazienti con cancro metastatico della mammella, con sovra espressione di HER2, che abbiano fallito un precedente
T.P.Call - Lapatinib più letrozolo o capecitabina come prima linea di trattamento del carcinoma mammario [redacted] Studio di fase IIIb randomizzato con cross-over programmato	III	Pazienti con carcinoma mammario metastatico ER-/HER2+, in trattamento di prima linea, senza progressione rapida di malattia
TH3RESA - Studio di fase III randomizzato, multicentrico, a due bracci, in aperto, per valutare l'efficacia di T-DM1 rispetto al trattamento scelto dal medico in pazienti con carcinoma della mammella metastatico HER2 positivo, che [redacted] ricevuto almeno due precedenti regimi di terapia anti-HER2	III	Pazienti con carcinoma della mammella metastatico, HER2-positivo, che hanno ricevuto almeno due precedenti regimi di terapia anti-HER2
EOS - Follow-up a 6 mesi di pazienti trattate per carcinoma mammario metastatico in line with accepted clinical guidelines for the cancer.	osservazionale	Donne con carcinoma mammario metastatico confermato, alle quali è stato prescritto un trattamento di chemioterapia



MAMMELLA

External Quality Controls



PROGRAMMA DI RICERCA REGIONE-UNIVERSITÀ 2010-2012
AREA 2 – RICERCA PER IL GOVERNO CLINICO

Sintesi della valutazione preliminare

Titolo della Proposta: **Ottimizzazione metodologica dei test molecolari per i geni HER2, KRAS, BRAF, EGFR, CKIT nei pazienti con tumori solidi da sottoporre a terapie oncologiche personalizzate**

Codice della Proposta: 1a203-19

Azienda proponente: Azienda Ospedaliero-Universitaria di Bologna

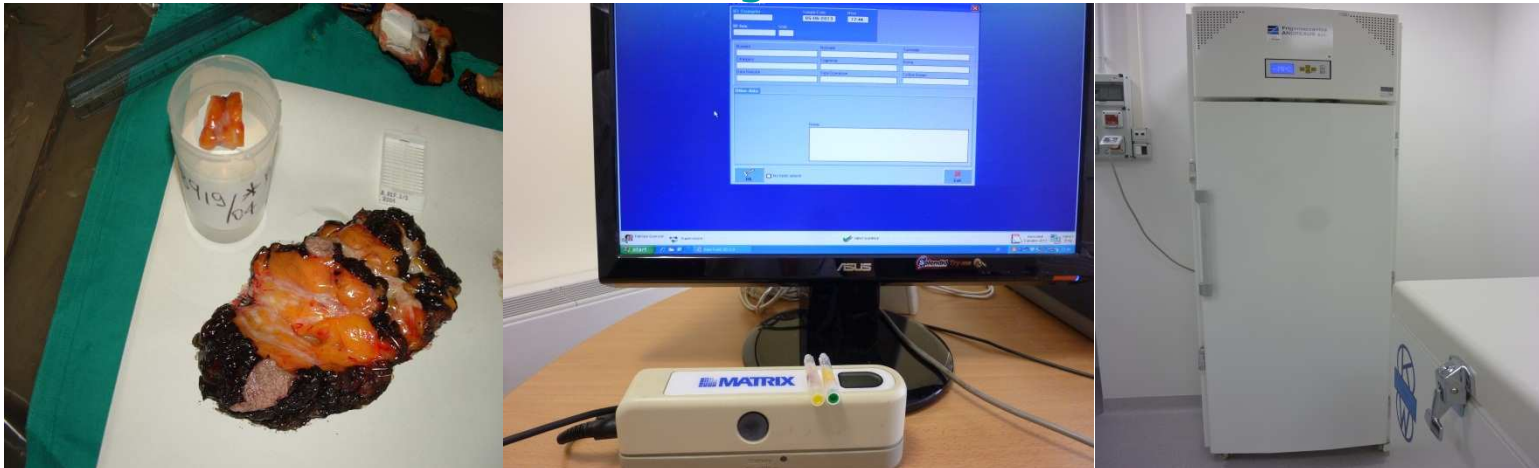
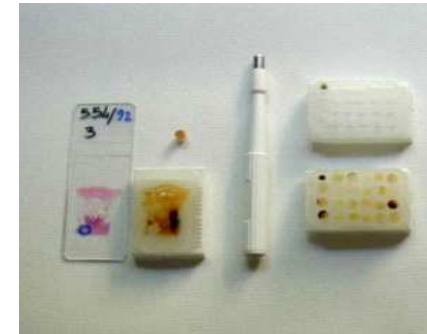
Principal Investigator: Dr. Michelangelo Fiorentino

PARTICIPATING CENTERS :

- | | |
|--|--------------------------|
| - BOLOGNA: Anatomia Patologica, AOU | Dr. D. Santini |
| - MELDOLA: Istituto Romagnolo per lo Studio dei Tumori | Dr. D. Calistri |
| - FERRARA: Anatomia Patologica, AOU di Ferrara | Prof. P. Querzoli |
| - Forlì: U.O. Anatomia Patologica, AUSL | Dr. L. Medri |
| -Modena: Anatomia Patologica, AUO | Dr. S. Bettelli |

Altri centri IHC Ca Mammella: RIMINI AUSL; PIACENZA AUSL; IMOLA AUSL; PARMA AOU; REGGIO EMILIA IRCCS; ANCONA AOU

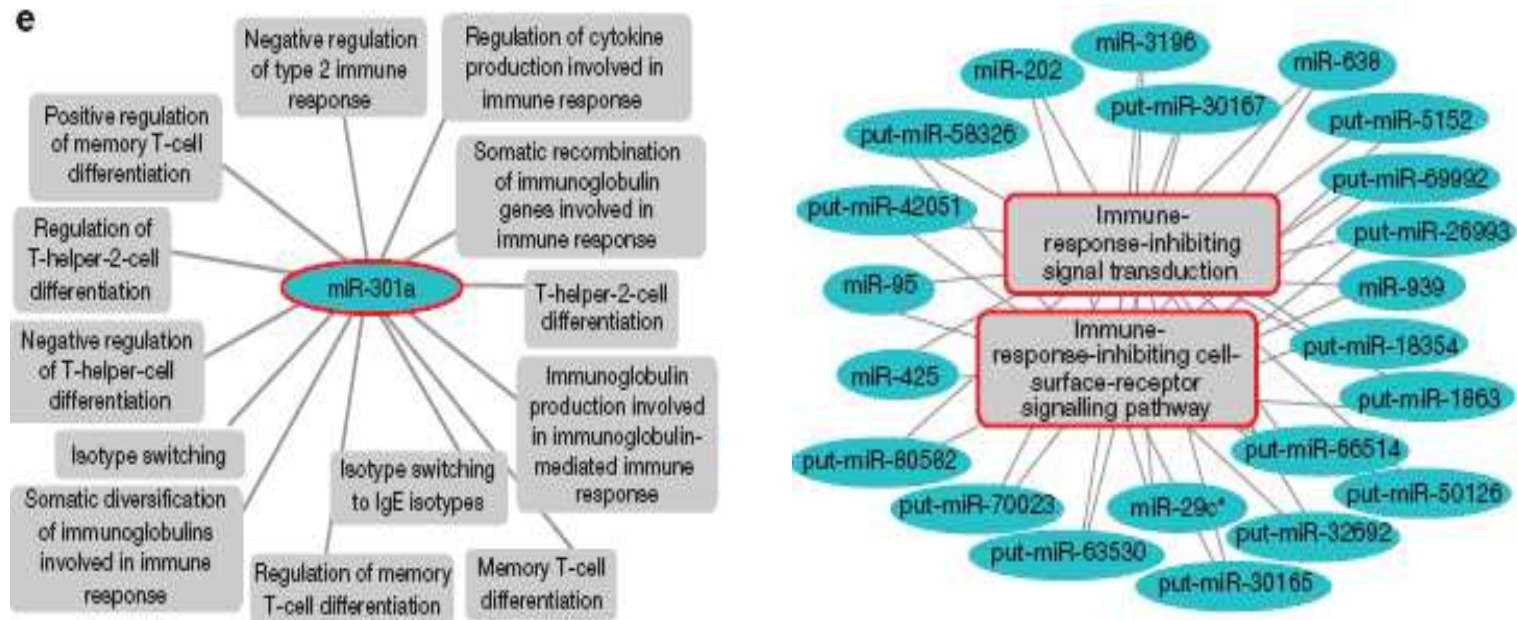
- I profili di espressione genica e i microRNA sono oggi di straordinario interesse e potenzialità
- Disponibilità di test diagnostico-molecolari
- Mammaprint® , Oncotype DX®
- Ruolo “centrale” del patologo
 - Banche di tessuto fresco/congelato/fissato/incluso



- Laboratori di diagnostica molecolare
- Formazione di patologi-molecolari
- Formazione di biotecnologi
- Database relazionale per la gestione dei dati morfologici, bio-molecolari e clinici

The shaping and functional consequences of the microRNA landscape in breast cancer

Dvinge H, 2013



MicroRNAs: toward the clinic for breast cancer patients.
Ferracin M, Querzoli P, Calin GA, Negrini M.
Semin Oncol. 2011

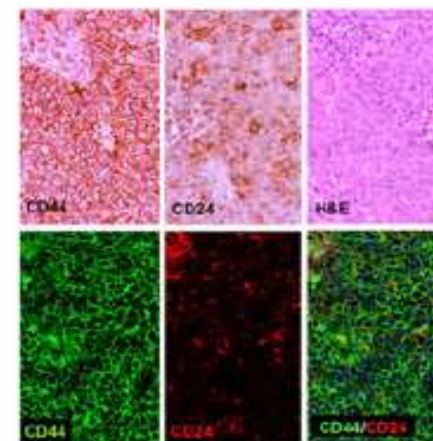
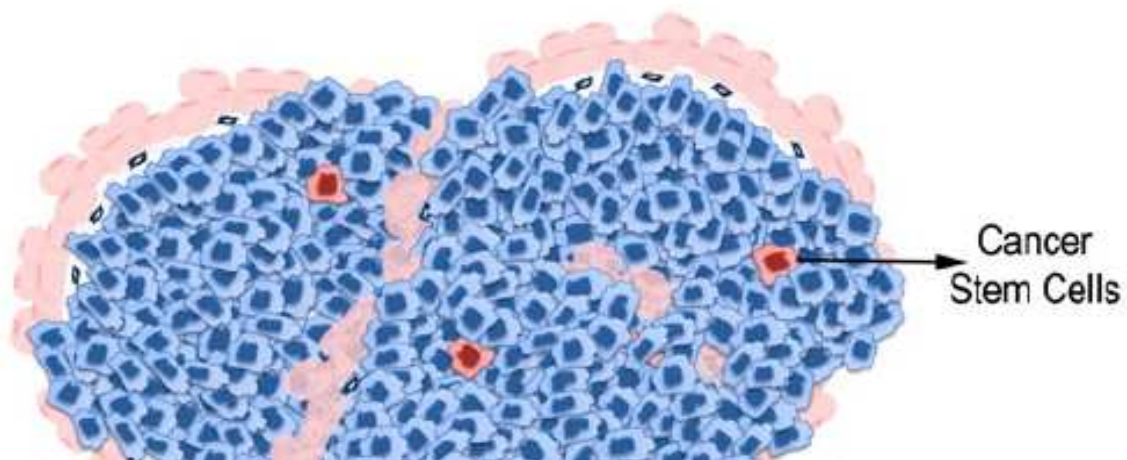
Priority Report

MicroRNA Gene Expression Deregulation in Human Breast Cancer

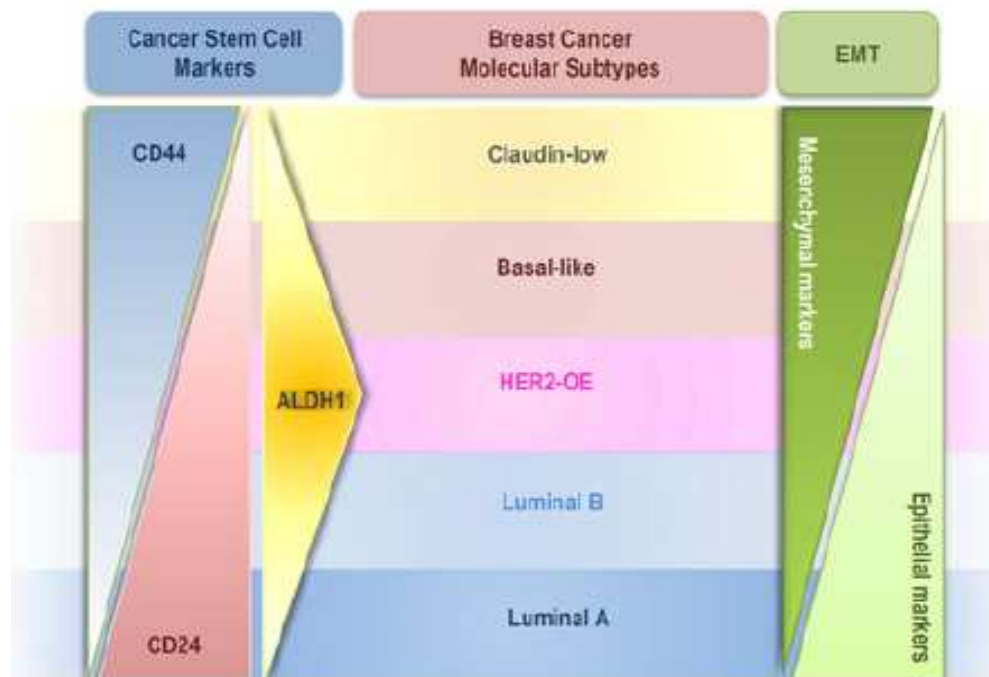
Marilena V. Iorio,¹ Manuela Ferracin,² Chang-Gong Liu,¹ Angelo Veronese,² Riccardo Spizzo,² Silvia Sabbioni,² Eros Magri,² Massimo Pedriali,² Muller Fabbri,¹ Manuela Campiglio,³ Sylvie Ménard,³ Juan P. Palazzo,⁴ Anne Rosenberg,⁵ Piero Musiani,⁶ Stefano Volinia,¹ Italo Nenci,² George A. Calin,¹ Patrizia Querzoli,² Massimo Negrini,² and Carlo M. Croce¹

¹Comprehensive Cancer Center, Ohio State University, Columbus, Ohio; ²Dipartimento di Medicina Sperimentale e Diagnostica, e Centro Interdipartimentale per la Ricerca sul Cancro, Università di Ferrara, Ferrara, Italy; ³Molecular Targeting Unit, Department of Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy; Departments of ⁴Pathology, Anatomy and Cell Biology and ⁵Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania; and ⁶Ce.S.I. Aging Research Center, Chieti, Italy

Breast Carcinoma

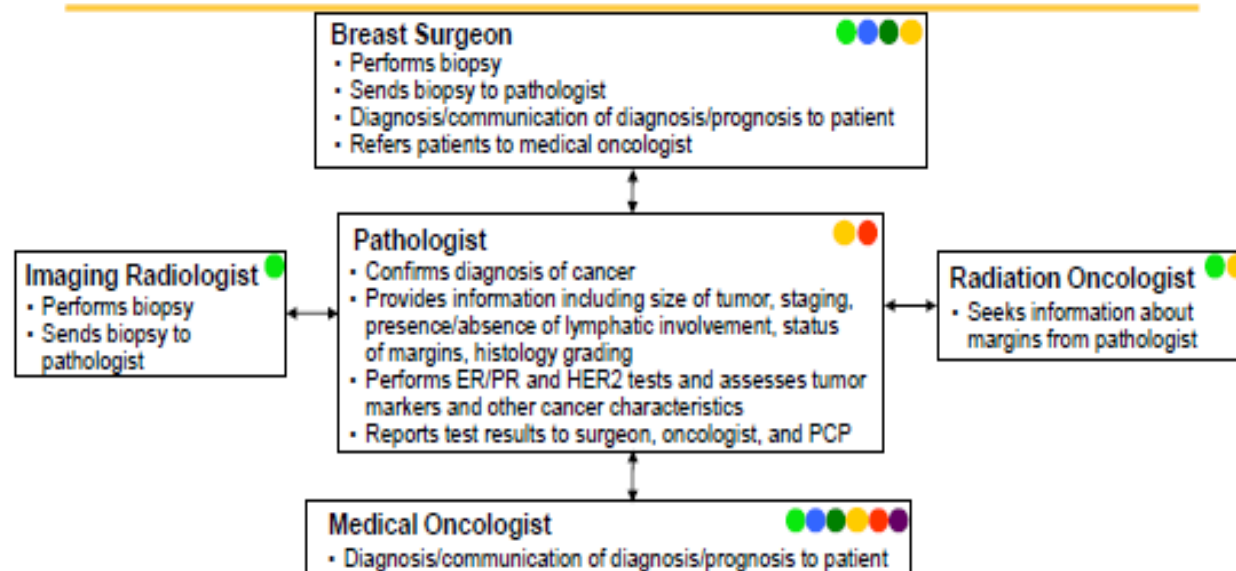


Immunohistochemistry
Immunofluorescence



Schmitt F et al, 2012

Breast Cancer Treatment: Multidisciplinary Team Approach



- Patient contact
- Patient advisor
- Treatment decision-maker
- Treatment advisor
- Directly impacts initiation of treatment
- Directly impacts treatment stop

Opportunities for Pathology Community

- Serve as consultant and treatment advisor - team
- Directly impact treatments
- Directly impact patient outcomes

PCP=primary care physician.

Hicks DG et al. *Arch Pathol Lab Med.* 2008;132:1226-1227.

IMPATTO CLINICO DELLA “-OMICS REVOLUTION”: IL CONTRIBUTO DELL’ANATOMIA PATOLOGICA

- **Classificazione** dei tumori in base alla loro diversa espressione genica.
- Definizione di gruppi di pazienti a diversa **prognosi**.
- Valutazione dell’efficacia di specifiche **terapie**.

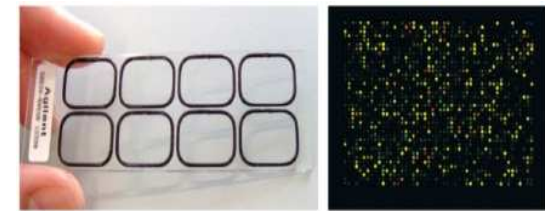
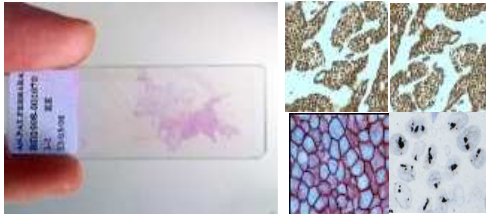
- Profili di espressione genica, morfologica ed immunofenotipica (TMAs) devono essere considerati complementari.
- Utilizzo dei microarrays per selezionare nuovi parametri prognostico-predittivi
- Identificazione del prodotto dei geni e disponibilita’ di nuovi anticorpi da utilizzare su TMAs (applicabili a casistiche fissate ed incluse).

Percorso Diagnostico-Terapeutico Assistenziale



Incontro settimanale con discussione di tutti i casi con diagnosi B5-B4-B3 e nuovi casi operati . Condivisione delle scelte cliniche su ogni singolo caso. Aggiornamento culturale. Controllo del processo assistenziale per individuare e risolvere le criticità

Integrazione tra la tassonomia morfologica e molecolare. Nuova classificazione ad impatto prognostico e terapeutico



patrizia.querzoli@unife.it