Dal prelievo all'analisi molecolare: certezze e criticità

L'analisi dei fattori prognostico-predittivi di risposta alla terapia

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Anatomia Patologica 2

FATTORI PROGNOSTICI E PREDITTIVI

Biologia del tumore (Aggressività)

- Hormone receptors (ER, PgR)
- Grading (differentiation)
- HER2
- Ki67 (proliferation)
- Vascular invasion
- Gene expression tests (molecular finger print)

(2009, St Gallen)

Carico tumorale (dimensioni e disseminazione)

- Tumor size
- Number of involved lymph nodes
- Distant metastasis

Eighth Edition 2017

uicc
global cancer control
A MEMBERSHIP ORGANISATION
FIGHTING CANCER TOGETHER

TNM CLASSIFICATION OF MALIGNANT TUMORS

AJCC-2017

Breast Cancer—Major Changes in the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual

Immunohistochemically detected tumor markers that are known to have great practical treatment importance are now ESSENTIAL

ER HER2, Histological grade
Number and percentage of involved nodes Tumour size
Presence of lymphatic or vascular invasion
Surgical resection margin status

The eighth edition of the staging system also uses genomic assays when available **to downstage some estrogen receptor-positive, lymph node-negative tumors.**

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CHANGE	DETAILS OF CHANGE	LEVEL OF EVIDENCE
Inclusion of multigene panels (when available) as stage modifiers—21-gene recurrence score (Oncotype Dx)	For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 21-gene (Oncotype Dx) recurrence score less than 11, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0, and the tumor is staged using the AJCC prognostic stage group table as stage I.	I
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Abbreviations: AJCC, American Joint Committee on Cancer; PAM5[illegible]ediction analy[illegible]rray 50.

LA DETERMINAZIONE DEI **FATTORI PROGNOSTICI CLASSICI** DEVE ESSERE OTTIMALE

IL **TEST MOLECOLARE** IN ITALIA ANCORA NON HA IL RIMBORSO DAL SSN

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DEVO ESSERE SICURO DI QUELLO CHE VEDO

DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO

……DEVO ESSERE SICURO DI QUELLO CHE VEDO

Appl Immunohistochem Mol Morphol • Volume 20, Number 6, December 2012

Delay to Formalin Fixation Alters Morphology and Immunohistochemistry for Breast Carcinoma

Thaer Khoury, MD

Alterazioni della morfologia

TABLE 3. General Histomorphologic Alteration Correlation With DFF Time

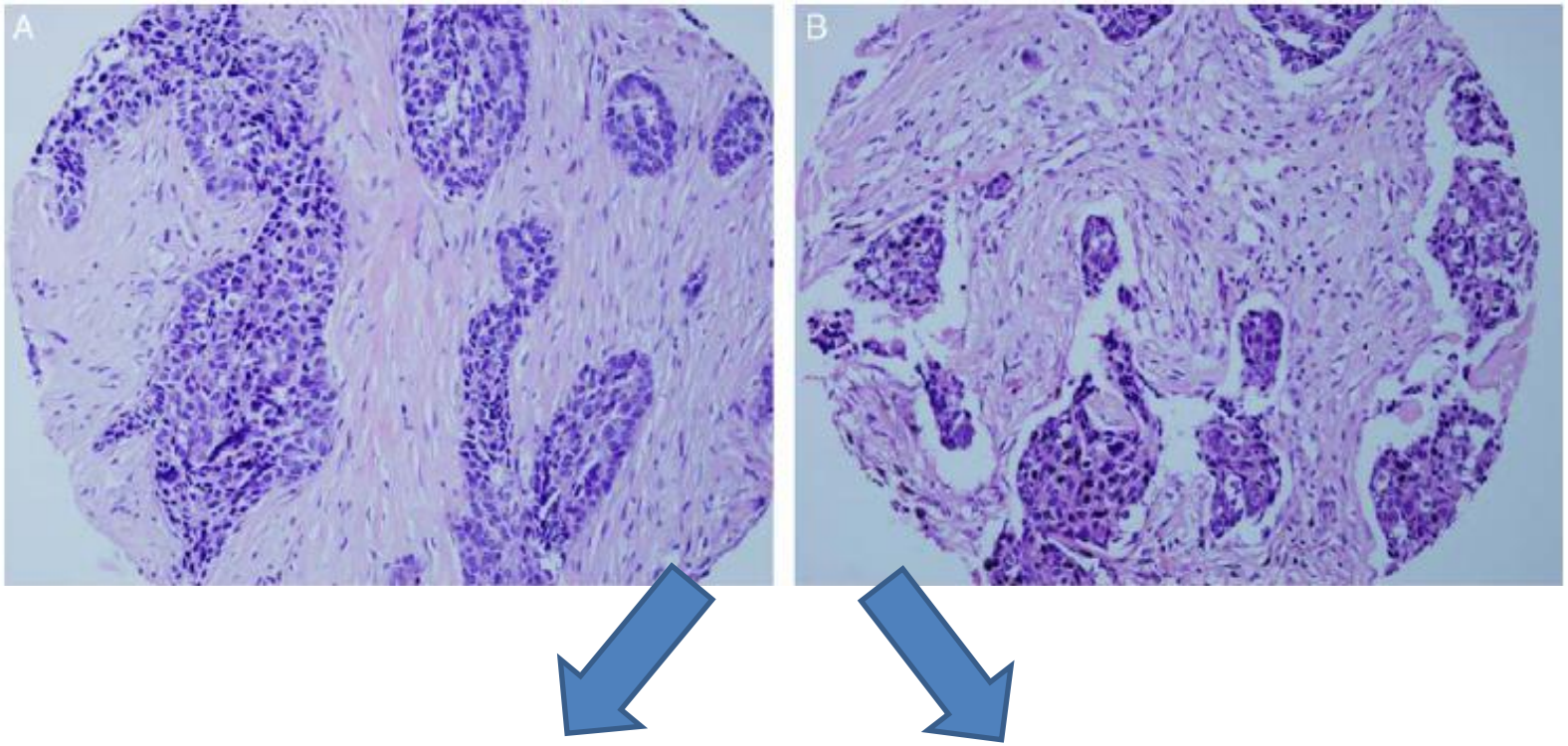
	0 min	10 min	30 min	1 h	2 h	4 h	8 h	ON
#1	No	No	No	No	No	No	Yes*	Yes*
#2	No	No	No	No	No	No	No	Yes
#3	No	No	No	No	No	No	No	Yes
#4	No	No	No	No	No	Yes	Yes	Yes
#5	No	No	No	No	No	Yes*	Yes*	Yes*
#6	No	No	No	No	Yes*	Yes*	Yes*	Yes*
#7	No	No	No	No	Yes	Yes	Yes	Yes
#8	No	No	No	No	No	No	Yes	Yes
#9	No	No	No	No	No	Yes*	Yes*	No
#10	No	No	No	No	No	No	No	No
Total affected	0	0	0	0	2	5	7	8

*Alteration with significance. Note increase the number of affected cases with longer period of DFF.

No indicates morphologic alteration not present; ON, overnight; Yes, morphologic alteration present.

……DEVO ESSERE SICURO DI QUELLO CHE VEDO

ARTEFATTI DA RETRAZIONE TUMORALE (7 CASI SU 8)



ISTOTIPO MICROPAPILLARE?

INVASIONE VASCOLARE?

Appl Immunohistochem Mol Morphol • Volume 20, Number 6, December 2012

Ritardo nella fissazione per ER e PgR

FINO A 4 ORE HA UN IMPATTO MINIMO SULLA LORO DETERMINAZIONE

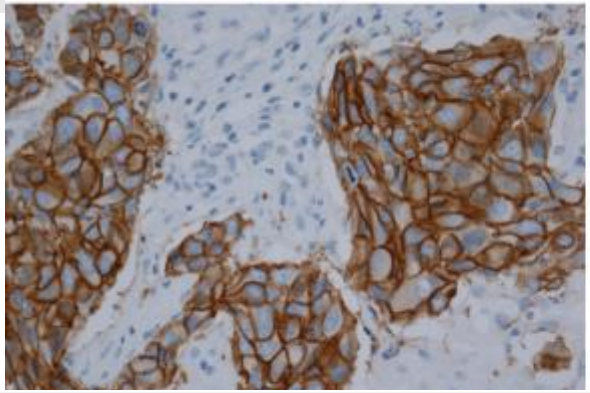
ma

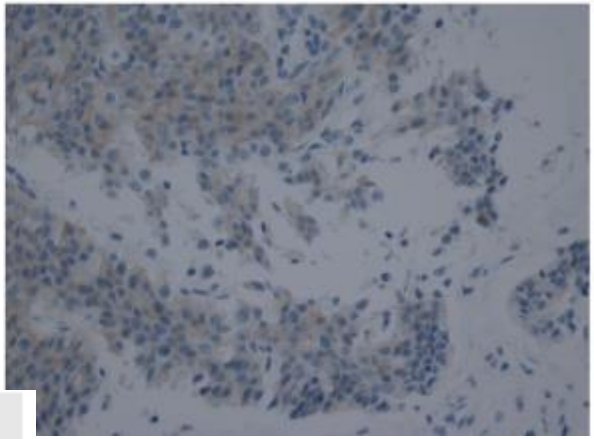
C'è una *diminuzione della positività* (circa 25%) se la cold ischemia è > 2 ore

Mod Pathol. 2013 January ; 26(1):
Am J Clin Pathol 2011;135:592-598
Mod Pathol. 2009; 22:1457–67.
Am J Clin Pathol. 2010; 134:813–9.

Ritardo nella fissazione per HER2

	Delay in fixation/hours					
Tumour	0	1	2	4	8	24
HercepTest						
1	3+, 87%	0+, 0%	0+, 0%	1+, 2%	0+, 0%	
2	3+, 75%	3+, 85%	3+, 87%	3+, 35%	2+, 15%	
3	3+, 80%	3+, 85%	3+, 70%	3+, 87%	3+, 80%	
4	3+, 98%	3+, 98%	3+, 94%	3+, 92%	3+, 85%	
5	3+, 50%	3+, 95%	3+, 78%			





- Delayed fixation can reduce immunohistochemical staining for HER2 potentially compromising treatment decisions.
- This reduced immunohistochemical staining is seen with different antibody systems.
- Delayed fixation appears to have less effect on HER2 in situ hybridisation.
- These results emphasise the importance of incision of mammary carcinomas in surgical specimens.

J Clin Pathol 2014;67:573–575.

The importance of tissue handling of surgically removed breast cancer for an accurate assessment of the Ki-67 index

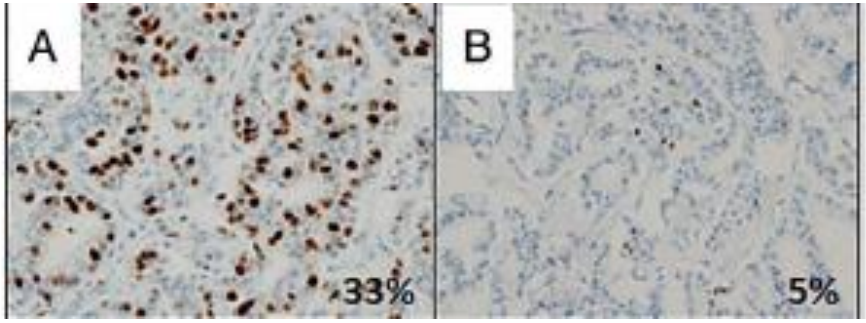
Arima N, *et al. J Clin Pathol* 2016;**69**:255–259.

Table 2 Effect of time of fixation on Ki-67 immunohistochemistry

Conditions	No. of samples	Ki-67 index (mean±SD: %)	p Value (paired t test)
Time to fixation			
Immediate	10	43.1±11.5	0.0284
16 h		36.7±18.4	
Insufficient fixation			
48 h	8	21.8±10.8	0.0177
3 h		8.8±9.4	
Prolonged fixation			
48 h	10	36.3±13.6	vs 48 h
14 days		26.6±15.8	0.0414
28 days		22.4±11.2	0.0042
56 days		14.4±6.5	0.0003

Il ritardo nella fissazione dopo 16 ore ed una fissazione prolungata oltre le 48 ore causano una significativa riduzione del ki67

Ki-67



……..DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!

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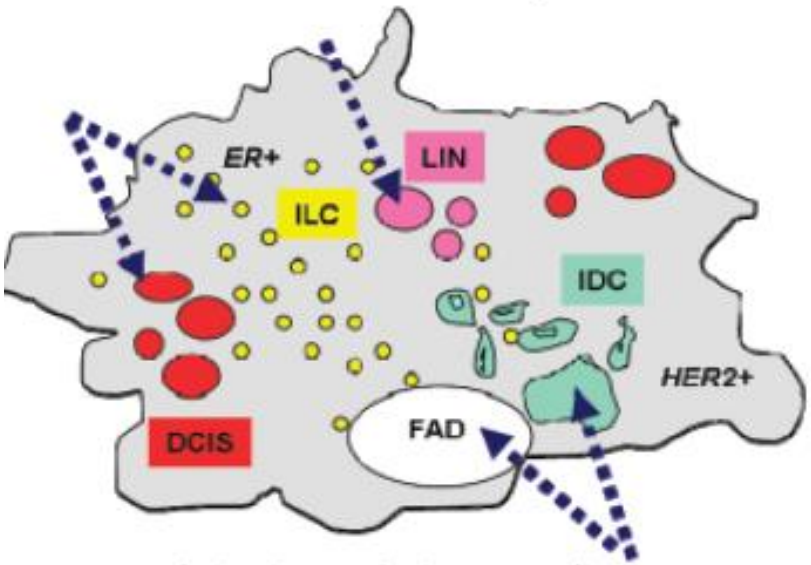
HETEROGENEITY IN BREAST CANCERS

Histotype
Immunophenotype
Gene expression profile



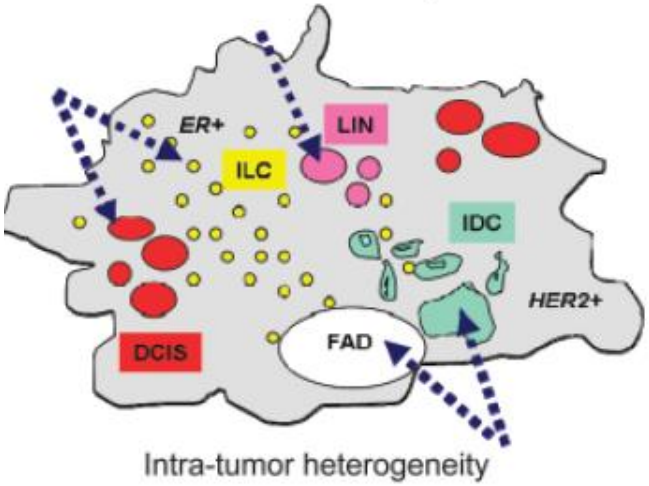
Intra-tumor heterogeneity

J Natl Cancer Inst Monogr 2011;43:86–90

PER LA DETERMINAZIONE DEI FATTORI PROGNOSTICI SCEGLIERE LA SEZIONE TUMORALE CHE PRESENTA MAGGIORE ETEROGENEITA' MORFOLOGICA

COME FACCIO SULLE AGOBIOPSIE?

……DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!



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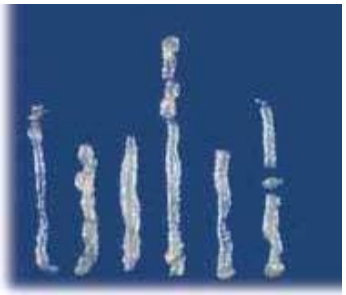

MULTIPLE CORE BIOPSIES FROM DIFFERENT AREAS OF PRIMARY TUMOR

Linee guida MINISTERIALi

Per le agobiopsie sono importanti la **numerosità e le dimensioni** dei prelievi: possono condizionare la rappresentatività della lesione e, quindi, la valenza diagnostica dell'esame istopatologico su esse effettuato.

Senonetwork Italia Onlus

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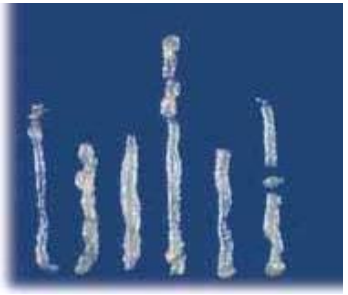
si prende cura di te

……DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!



MULTIPLE CORE BIOPSIES FROM DIFFERENT AREAS OF PRIMARY TUMOR

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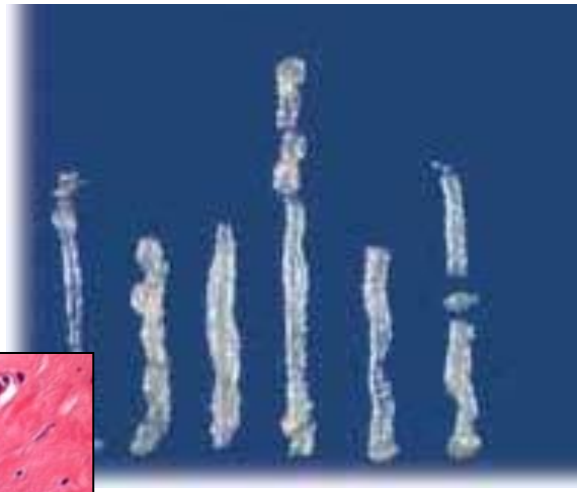
SARANNO SEMPRE DI Più I TUMORI DA SOTTOPORRE A TERAPIA NEOADIUVANTE, SPECIE SE TRIPLI NEGATIVI ED HER2 POSITIVI (ANCHE IN STADIO II, *sec ST Gallen 2017*)

E' L'UNICO TESSUTO CHE RESTA UTILIZZABILE IN CASO DI RISPOSTA PATOLOGICA COMPLETA ALLA NEOADIUVANTE

IL CAMBIAMENTO DI DETERMINATI MARCATORI PRIMA E DOPO LA CHEMIOTERAPIA HA IMPORTANZA PROGNOSTICA

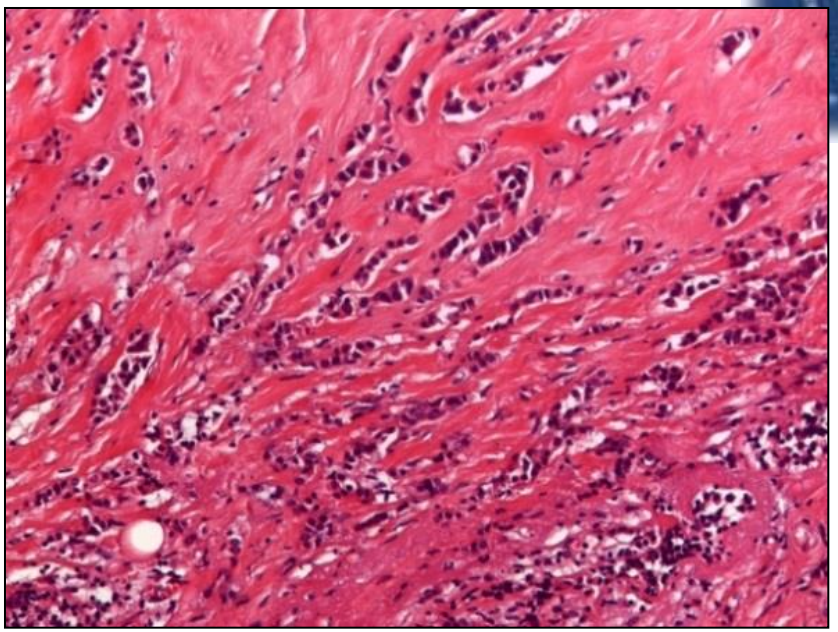
Core biopsy PRE-OPERATORIA

Parametri da riportare nel referto istologico pre-operatorio



Histological type

Lobular carcinoma



GIPAM-SIAPEC Guide Lines 2013

Core biopsy PRE-OPERATORIA

Parametri da riportare nel referto istologico pre-operatorio

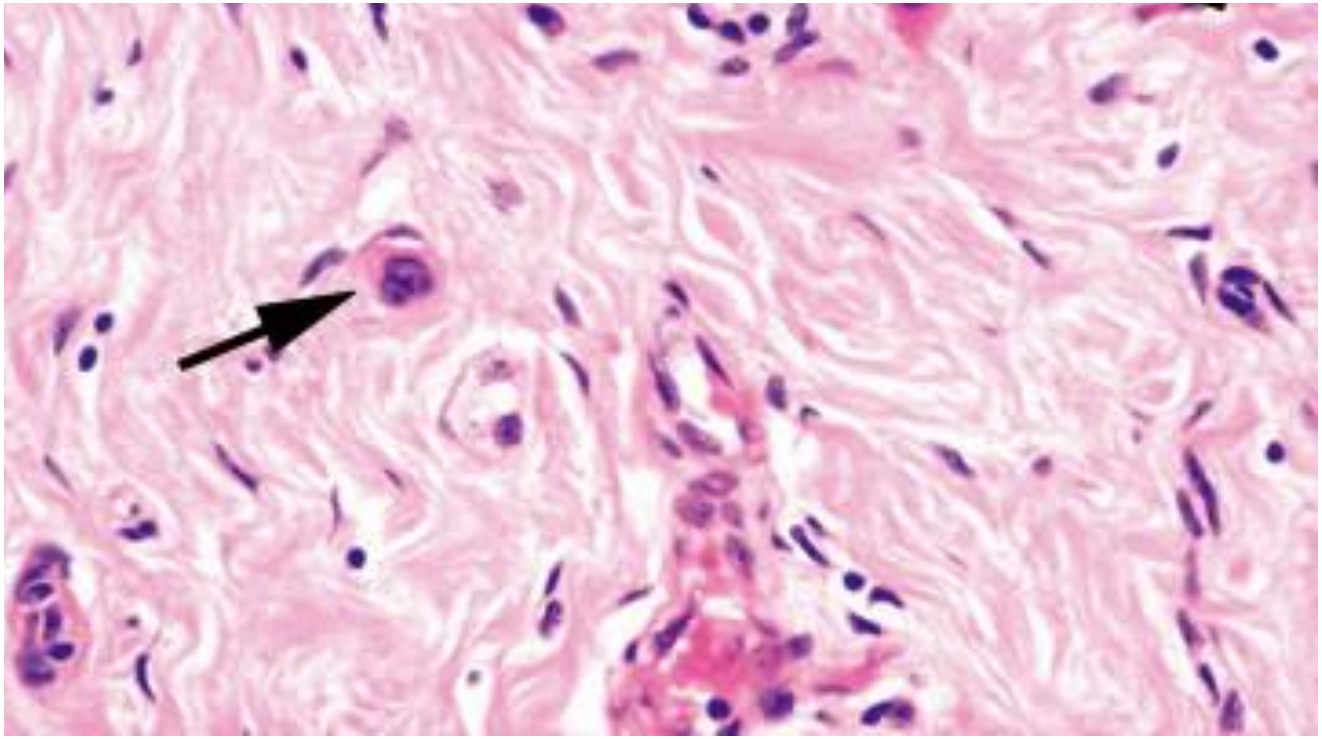
Histological type

Grade of differentiation

	pNR ($n = 74$)			pCR + pPR ($n = 416$)			p values
	Number of cases	Total %	Valid %	Number of cases	Total %	Valid %	
Grade							
1	6	8.1	8.3	27	6.5	6.9	0.007
2	48	64.9	66.7	189	45.4	48.3	
3	18	24.3	25.0	175	42.1	44.8	

Breast Cancer Res Treat (2014) 148:511–523

It is unclear if nuclear atypia after neoadjuvant treatment may indicate the biological aggressiveness of the tumor or the result of the therapy on tissue



We have to evaluate grade of differentiation on pre-treatment sample

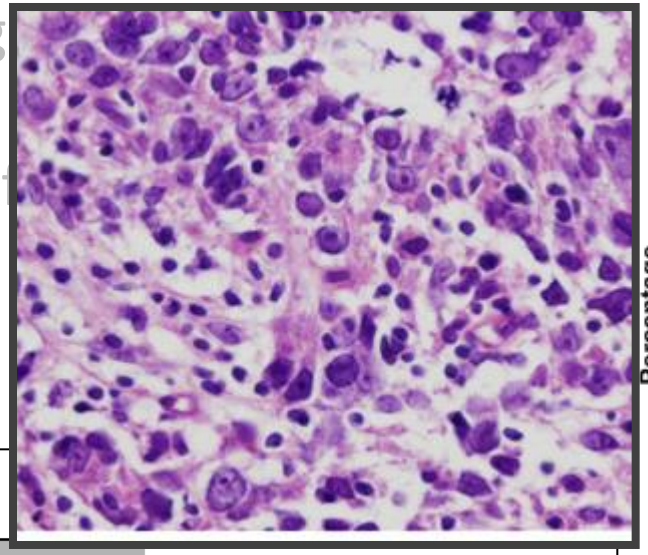
Core biopsy PRE-OPERATORIA

Nuovi parametri da riportare nel referto istologico pre-operatorio

Histolog

Grade of

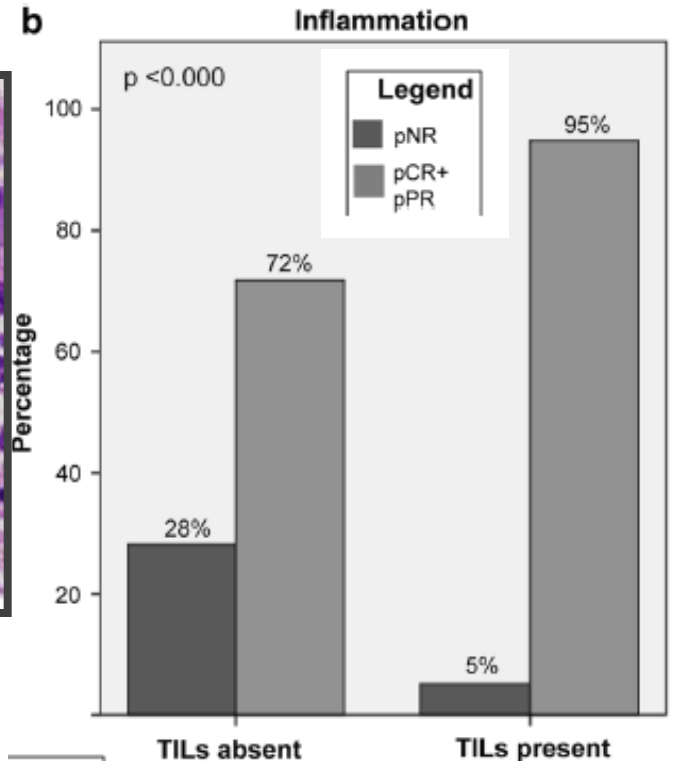
TIL



Breast Cancer Res Treat (2012) 132:793–805
DOI 10.1007/s10549-011-1554-7

CLINICAL TRIAL

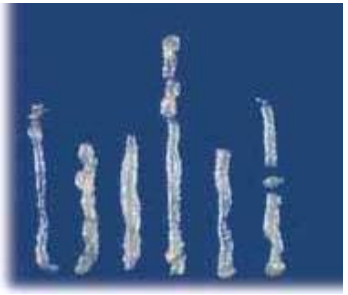
Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer



Breast Cancer Res Treat (2014) 148:511–523

The presence of inflammation is a predictive factor of response to chemotherapy

……DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!



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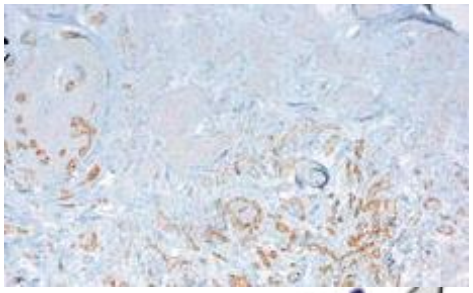
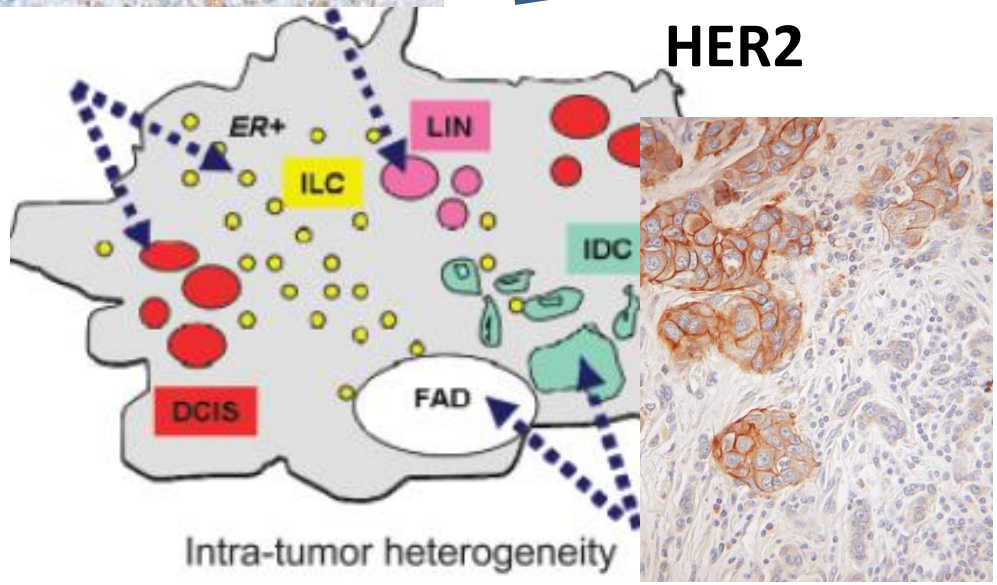
ER, PR, HER2 and KI67 DEVONO ESSERE RITESTATI, SE POSSIBILE DOPO TRATTAMENTO NEOADIUVANTE

ER/PR



HER2



Loss or change of some parameters (HER2 or Ki67) following neoajuvant treatment may have an impact on prognosis

Only some neoplastic clones may be selected by treatment

E SE SONO IN CONDIZIONI OTTIMALI……..DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO

-……DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!

Controlli di qualità sui fattori prognostici

rete oncologica
PIEMONTE
VALLE D'AOSTA

"CONTROLLI DI QUALITA' DEI MARCATORI PROGNOSTICO - PREDITTIVI "DEL CARCINOMA MAMMARIO DELLA ANATOMIE PATOLOGICHE"

Insieme al microscopio multiplo, su stesse sezioni colorate in Istituti diversi

CONFRONTO
INTERISTITUZIONALE
SU VETRINO VIRTUALE
IN PATOLOGIA MAMMARIA
CORE BIOPSY, HER2, Ki-67

SIAPEC - IAP
GIPaM
Gruppo Italiano
Patologia Mammaria

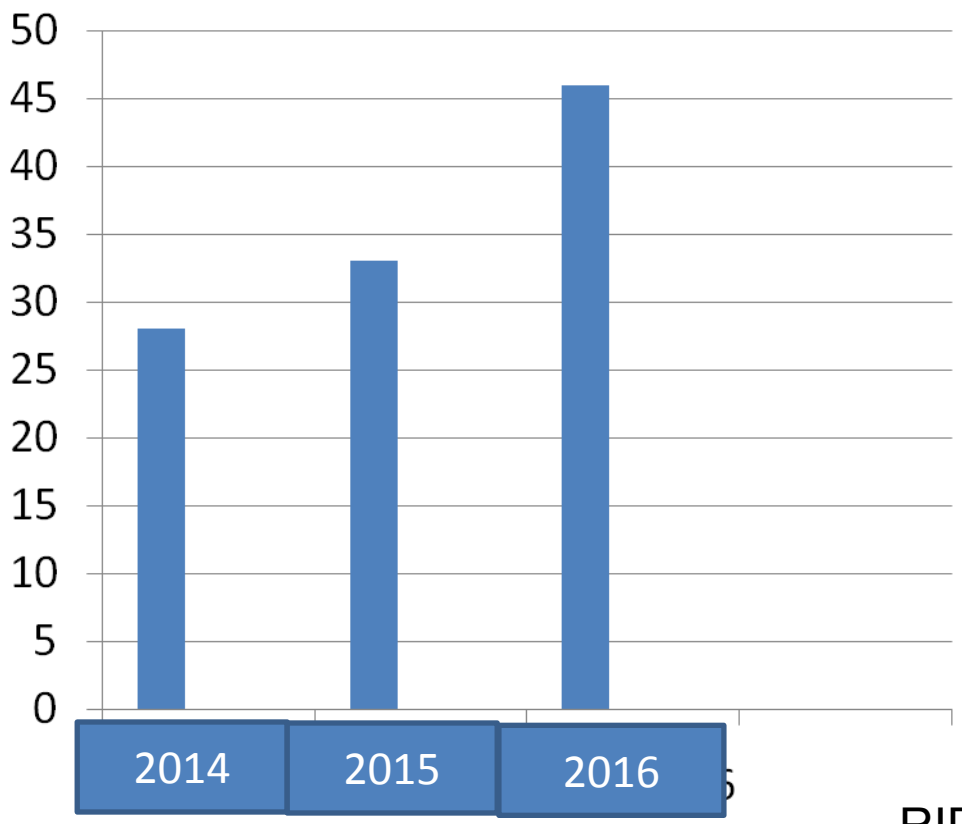
Analisi su vetrino virtuale

NordIQC

Spedizione del vetrino e dei risultati via posta

-……DEVO ESSERE SICURO DI QUELLO CHE INTERPRETO!

PARTECIPANTI AI CONTROLLI DI QUALITA' GIPAM



RIPRODUCIBILITA' DIAGNOSTICA
ANALISI DI CONCORDANZA

Ajcc 2017

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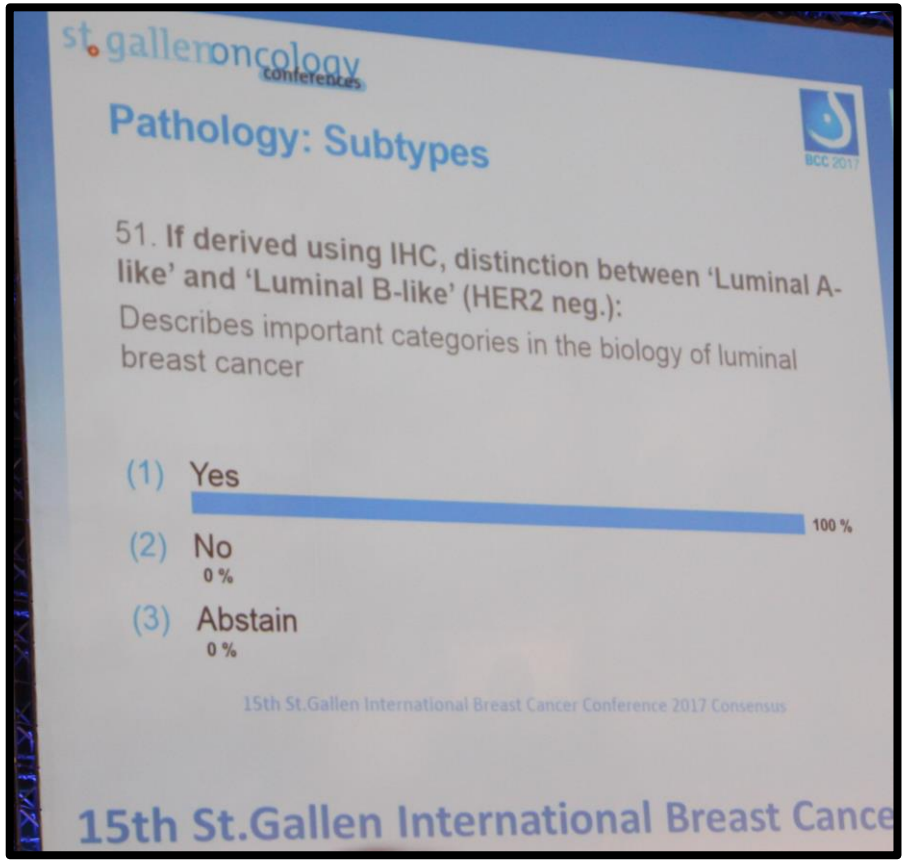
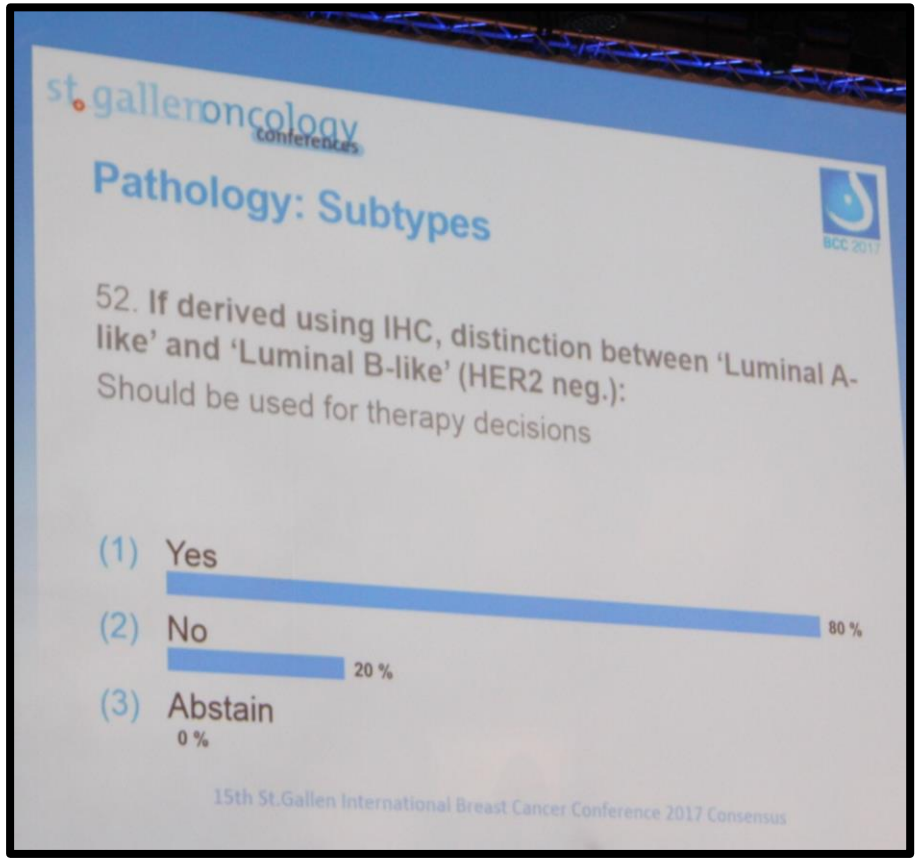
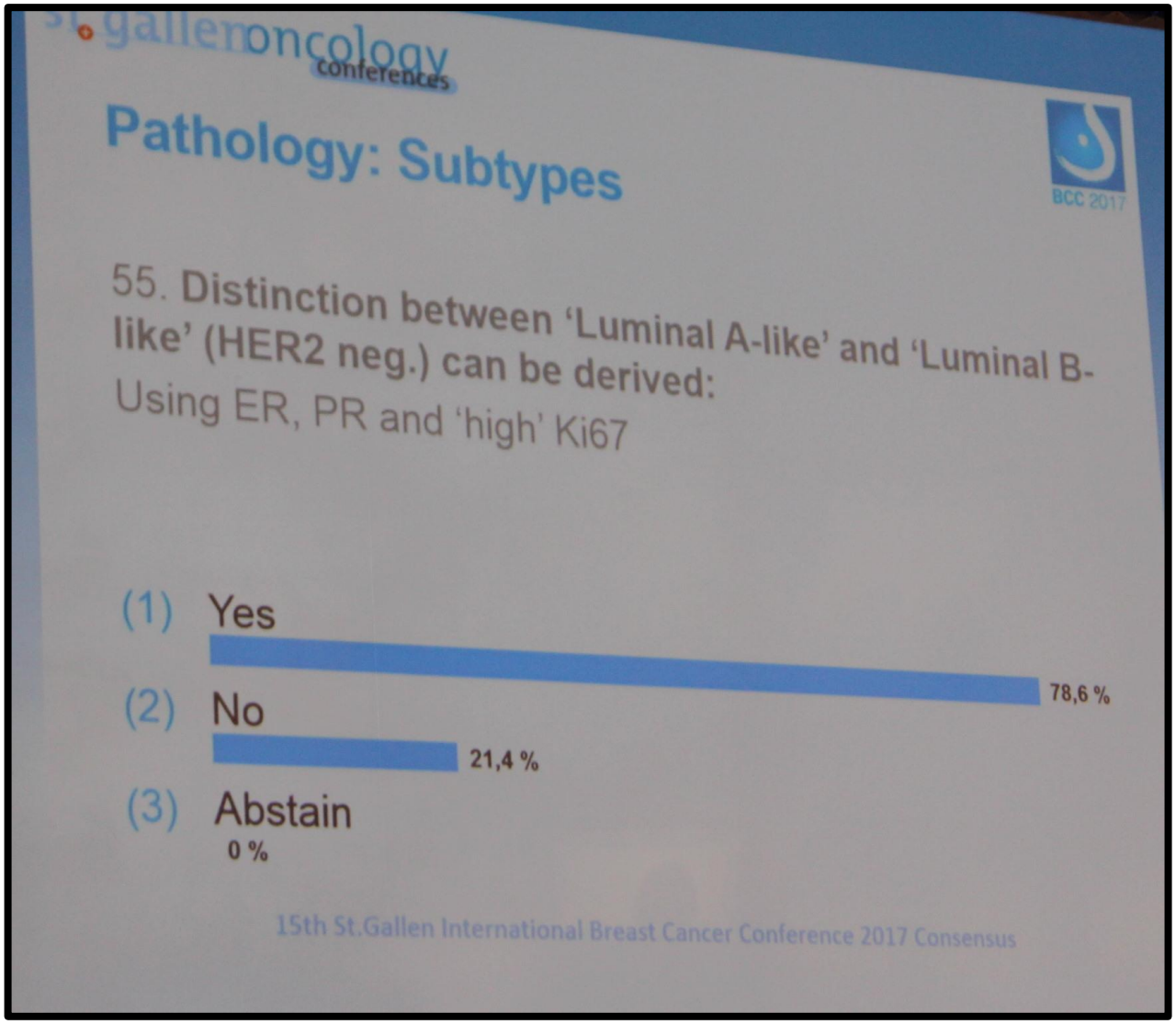
SURROGATO IMMUNOISTOCHIMICO (ER, PR HER2, KI67)
RESTA VALIDO PER ST GALLEN 2017

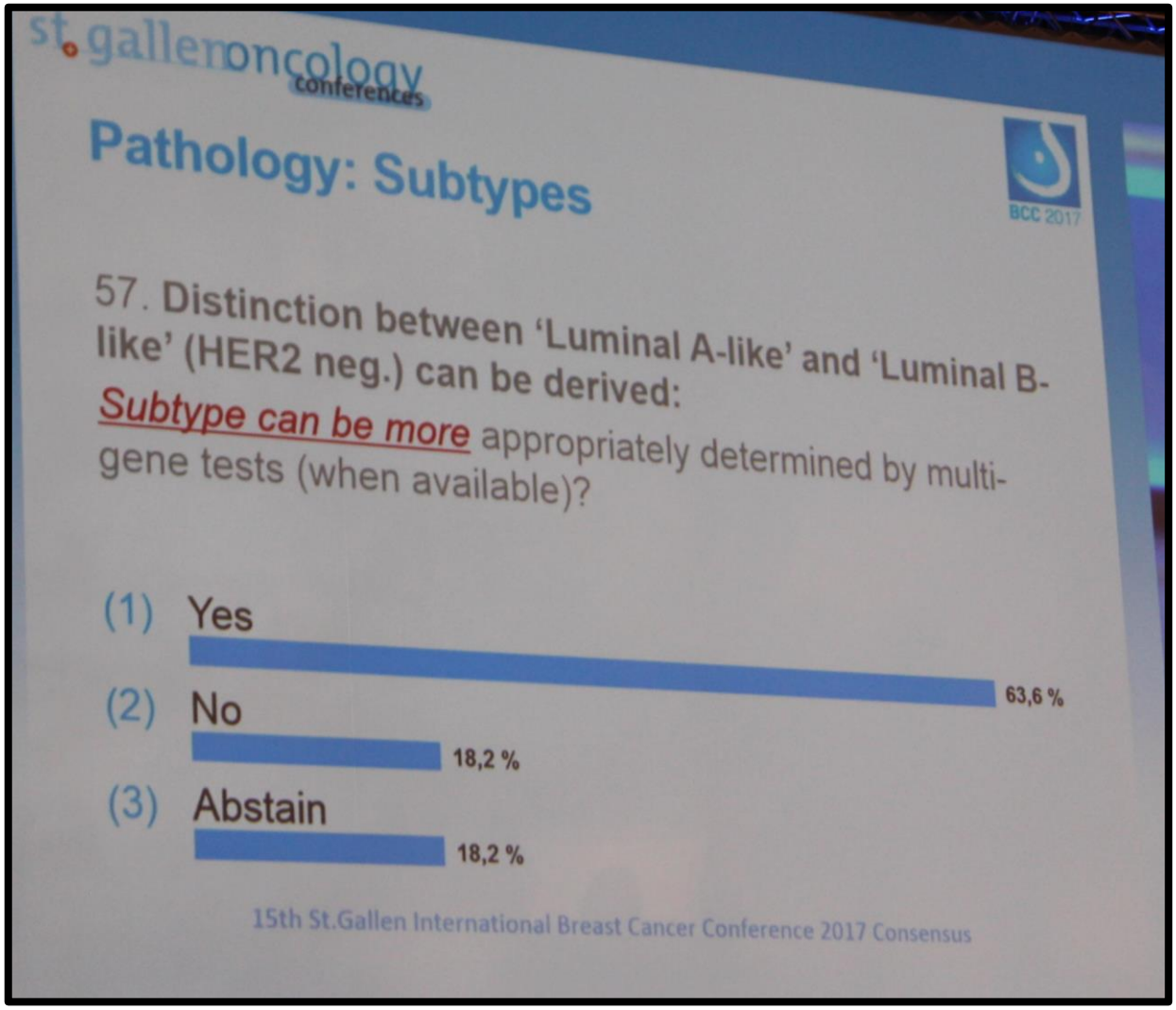




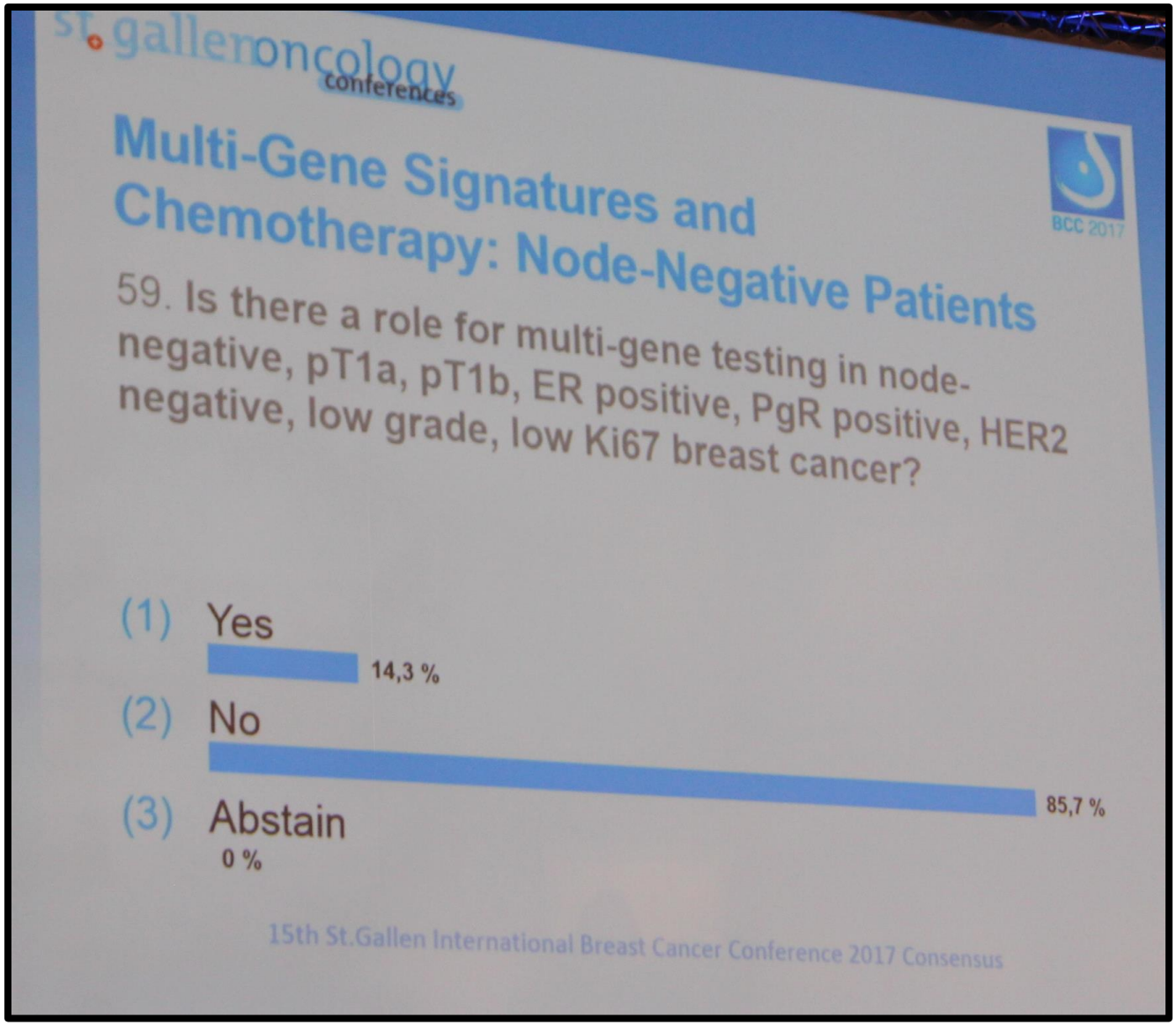
LA DISTINZIONE FRA LUMINALE A E LUMINALE B SI PUO' FARE CON I CLASSICI MARCATORI IMMUNOISTOCHIMICI



LA DISTINZIONE FRA LUMINALE A E LUMINALE B SI PUO' FARE CON I CLASSICI MARCATORI IMMUNOISTOCHIMICIMA E' PIU' PRECISA SE USIAMO UN TEST MOLECOLARE



SEMPRE? QUANDO USARE I TEST MOLECOLARI?



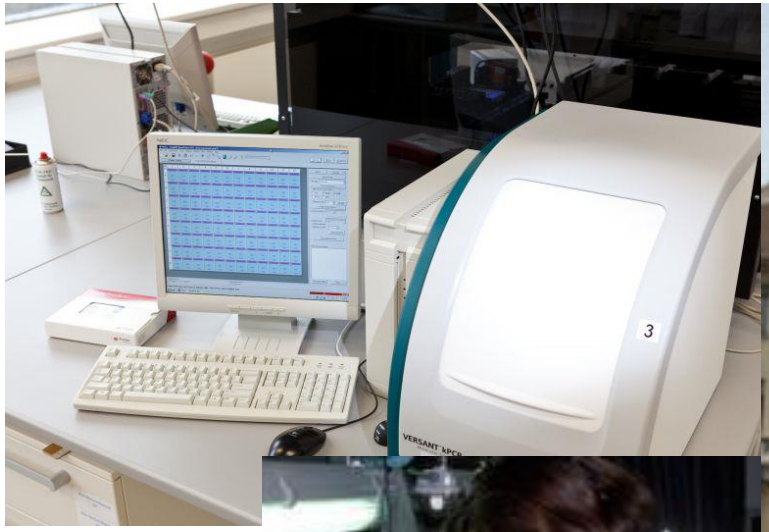
TEST MOLECOLARI IN TUMORI ER+/HER2-

ONCOTYPE DX
MAMMAPRINT
ENDOPREDICT
PAM50

NICE

Associazione Italiana di Oncologia Medica

Estate 2014: ENDOPREDICT ALLE MOLINETTE
LA NOSTRA ESPERIENZA



IL MOTTO E' *RISOLVERE* I DUBBI ….NON CREARNE ALTRI!

COMMISSIONE DI ONCOLOGI
DELLA RETE ONCOLOGICA DEL PIEMONTE E VALLE D'AOSTA

PROTOCOLLO PROPOSTO

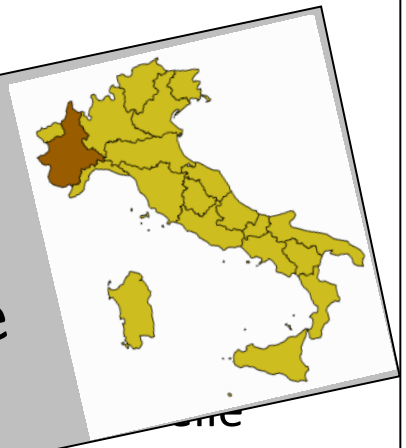
Sono candidabili al test molecolare pazienti che abbiano tutte queste caratteristiche

•Tumori ≤ 2 cm di diametro

•Coinvolgimento

•T ... ativo intermedio (Ki67 15-30%)

Circa 10% DELLE PAZIENTI
300 pazienti/anno in Piemonte



SOTTOPOSTO AL **NUCLEO TECNICO HTA IRES**
CON IL COMPITO DI VALUTARE TALE TECNOLOGIA IN TERMINI DI COSTO-BENEFICIO ANALIZANDONE L'IMPATTO SUI BISOGNI DI SALUTE NONCHE' SULLE SCELTE DI POLITICA SANITARIA E GESTIONE AZIENDALE.

TOTALE CASI: 67

Low risk: 29 (44%)

High risk: 38 (66%)

Casistica selezionata

ENDOPREDICT

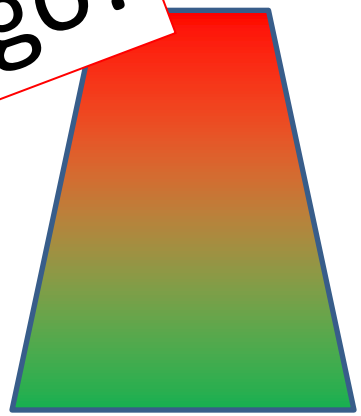
		LOW RISK	HIGH RISK
pT	1	25	31
	2	4	7
G	1	1	1
	2		34
	3		3
N			21
			17
		28	38
		1	
	≥ 10	25	32
	< 10	4	6
Ki67	< 10	1	1
	10-20	13	12
	> 20	15	25
EP SCORE	> 5	13	36
	< 5	16	2

Qualcosa manca al patologo

Oncologi: cosa scegliete?

	Tipo di terapia?		COHEN'S K	AGREEMENT
	HT alone	HT plus CT		
Senza Endopredict	31	25	[illegible] 26.47)	
Con Endopr[illegible]	[illegible]	26	58% (Z: 24.32)	

Qualcosa manca all'oncologo!



15 cases

- 6 cases FROM HT+CT to HT alone
- 9 cases FROM HT alone to HT+CT

CONCLUSIONI

NELLA DETERMINAZIONE DEI FATTORI PROGNOSTICO-PREDITTIVI DI RISPOSTA AL TRATTAMENTO:

IL PATOLOGO DEVE ESSERE SICURO DI QUELLO CHE VEDE
-ATTENZIONE ALLA FASE PRE-ANALITICA

IL PATOLOGO DEVE ESSERE SICURO DI COME INTERPRETA I DATI
-ATTENZIONE ALLA ETEROGENEITA'
-DEDIZIONE AI CONTROLLI DI QUALITA'

IL PATOLOGO, L'ONCOLOGO ED IL TEAM MULTIDISCIPLINARE DEVONO ESSERE CONSAPEVOLI CHE QUALCOSA STA CAMBIANDO E NON POSSIAMO FARNE PU' A MENO!

GRAZIE!