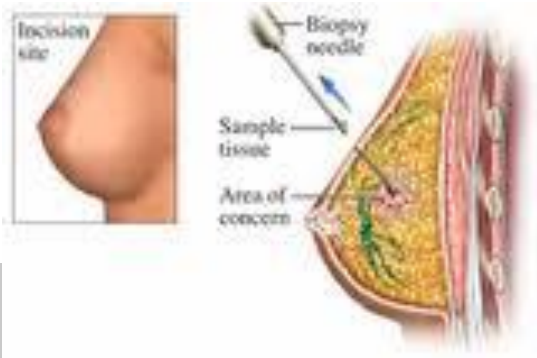
Erken Evre Meme Kanserinde Moleküler Patoloji ve Biyobelirteçler

Dr. Güldal ESENDAĞLI
Gazi Üniversitesi Tıp Fakültesi
Tıbbi Patoloji AD

➢ Doku örneğinin histopatolojik inceleme *öncesi* manipülasyonu çok önemlidir.



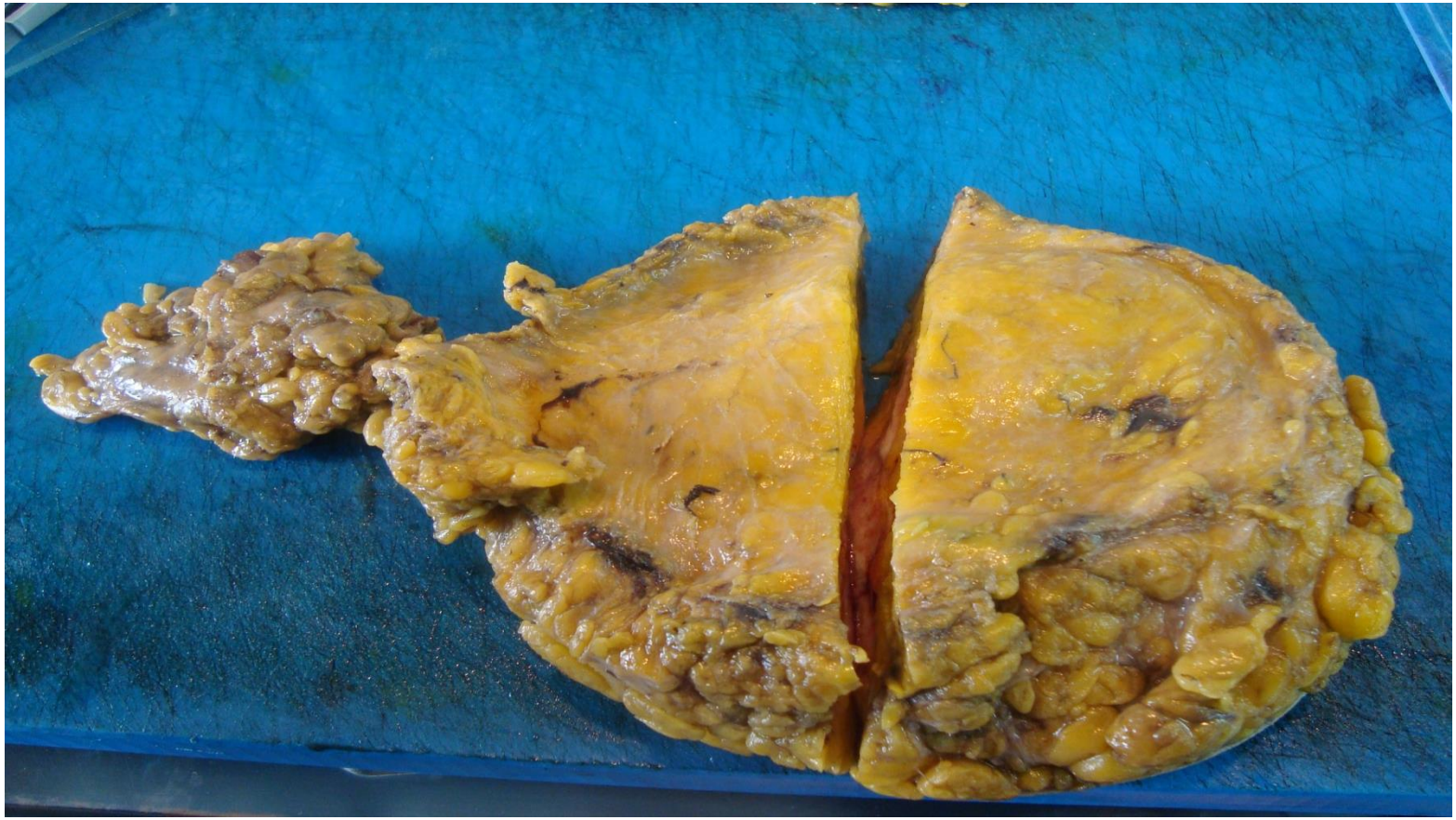
- Kor/tru-cut biyopsiler için sorun yok.

- Büyük materyalin,
  - Ameliyathanede tespit solüsyonuna koyulmadan beklediği süre
  - Dilimlenmeden tespit solüsyonuna koyulduğu durumlarda, solüsyonun tümöre ulaşıncaya kadar geçirdiği süre

SOĞUK İSKEMİ SÜRESİ

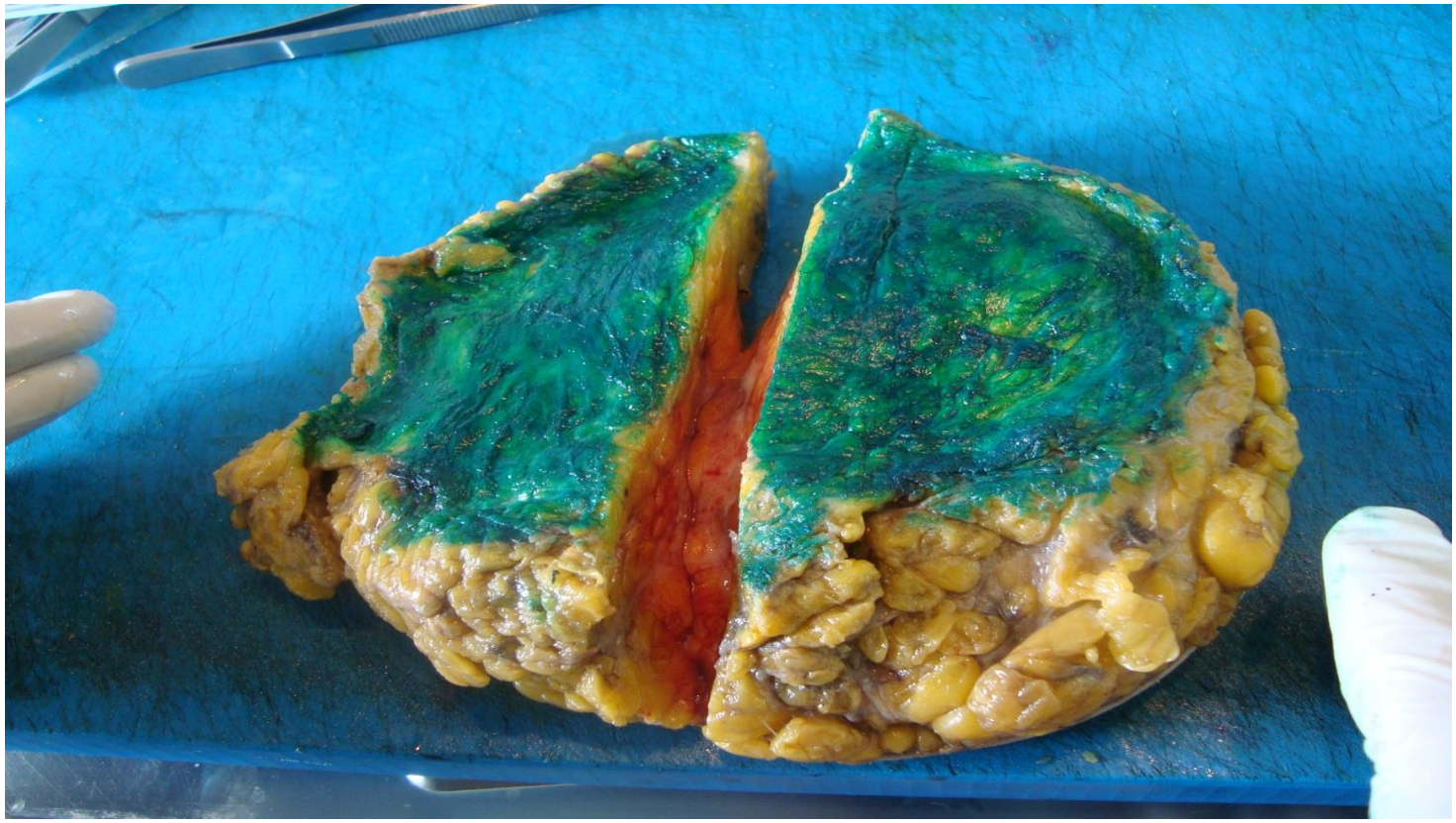
- Materyal, en fazla 1 saat içinde tespit solüsyonuna koyulmalıdır.
- Tespit (fiksasyon) süresi: 6-72 saat

- **Doku örneğinin histopatolojik inceleme *<u>öncesi</u>* manipülasyonu çok önemlidir.**



Kraniokaudal yönde tek kesi

- **Doku örneğinin histopatolojik inceleme *öncesi* manipülasyonu çok önemlidir.**



Kraniokaudal yönde tek kesi → Patolojik incelemede cerrahi sınır oryantasyonunu bozmaz.

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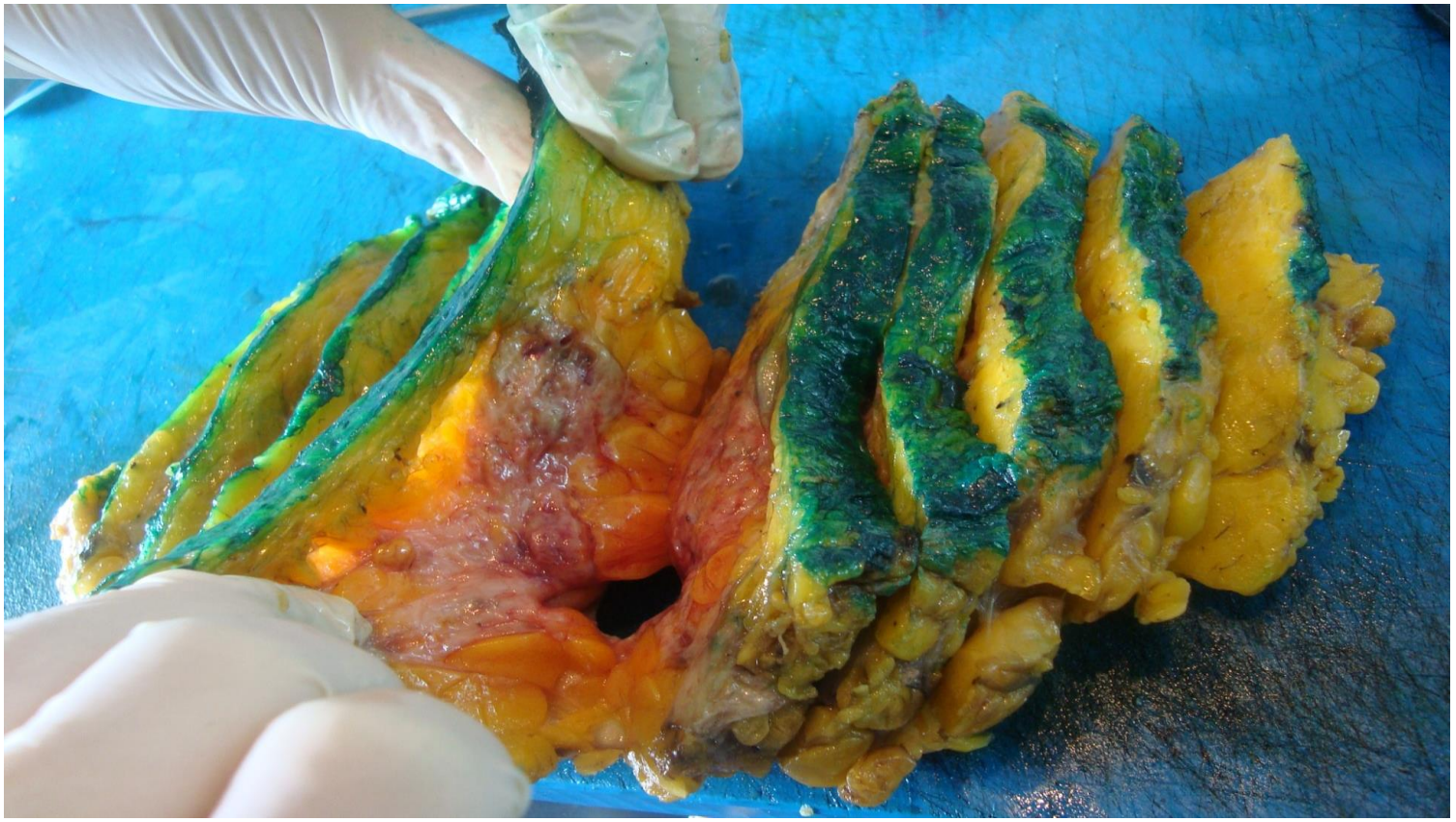


Kraniokaudal yönde tek kesi → Patolojik incelemede cerrahi sınır oryantasyonunu bozmaz.

İnvaziv meme Ca'da prognostik önemi bulunan ve rutinde kullanılan biyobelirteçler

- Hormon reseptörü (**ER, PR**)(+) meme Ca'lar → %75-80
- **c-erbB-2** (+) meme Ca'lar → %15-20
- Üçlü (-) meme Ca'lar → %10-15
- **Ki67**

«c-erbB-2 (+)'lerin yaklaşık yarısı hormon reseptörlerini ko-eksprese eder.»

Meme Kanserinin Moleküler Klasifikasyonu

Gen Ekspresyon Profiline Göre Meme Kanseri Moleküler Alttipleri

- Luminal A
- Luminal B
- HER-2 overekseprese eden
- Bazal benzeri
- Normal meme benzeri

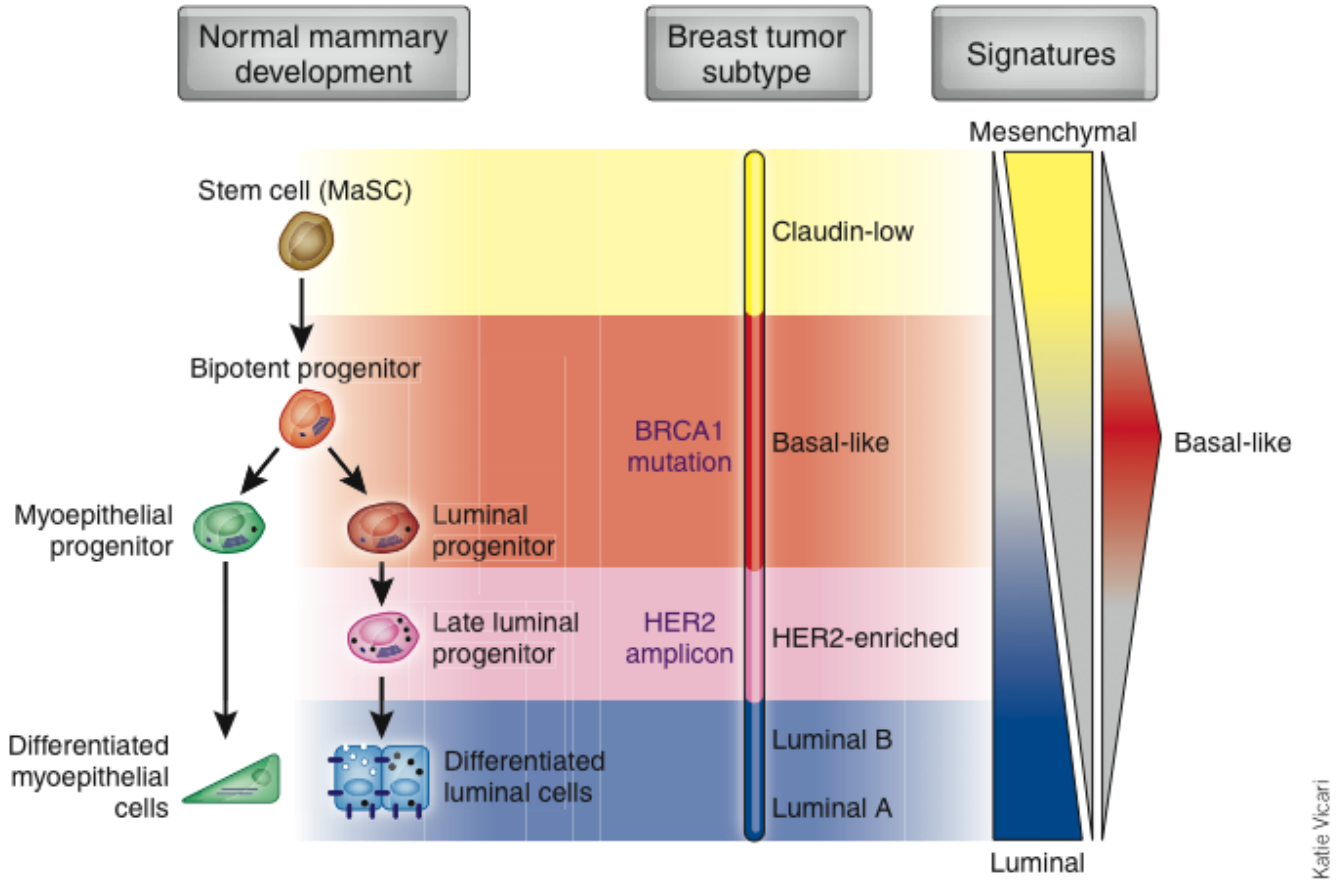
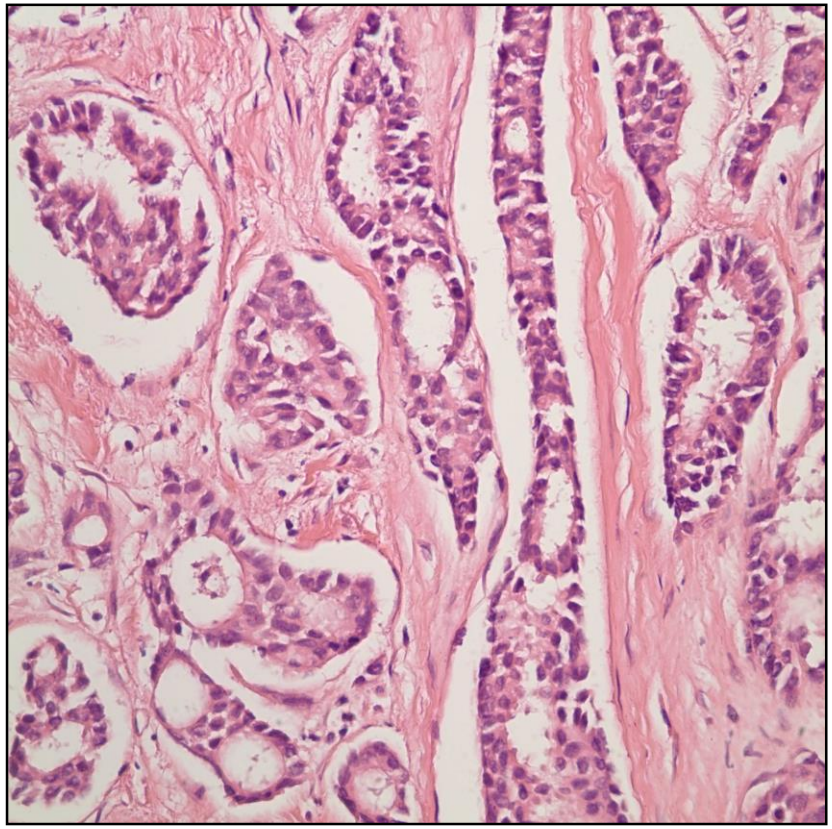
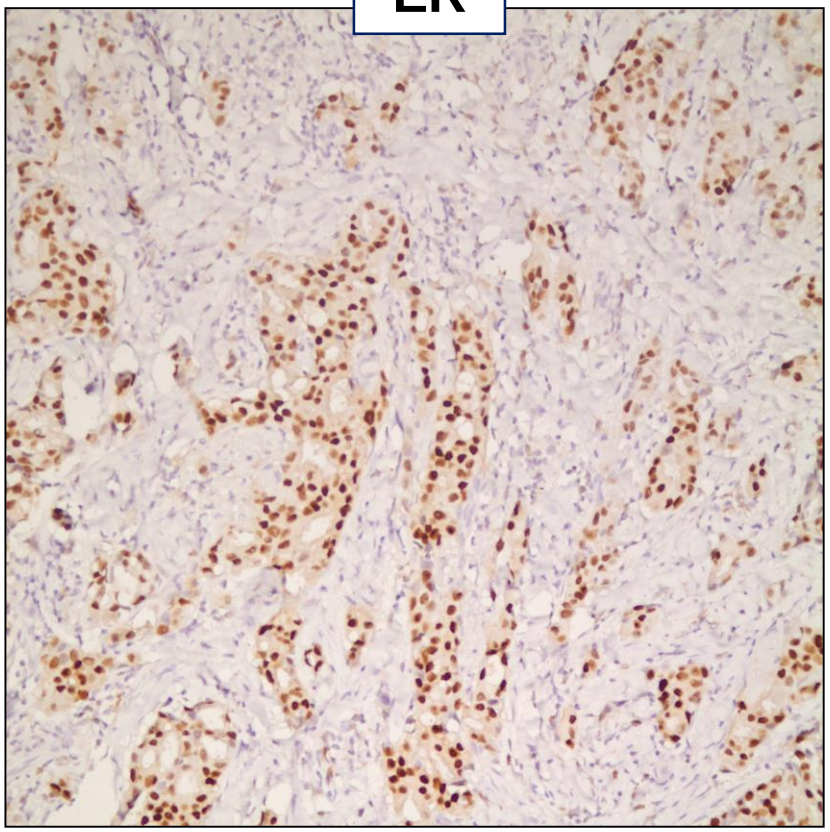


Figure 1 Model of the human mammary epithelial hierarchy linked to cancer subtype. (**a**) Subpopulations of normal breast tissue and potential cells of origin for the intrinsic subtypes of breast cancer; these cells may represent a stage of developmental arrest for a tumor with an origin earlier in the differentiation hierarchy or, alternatively, transformation of a cell type at one specific stage of development. (**b**) The various breast tumor subtypes molecularly compared to subpopulations from normal breast tissue. (**c**) The defining expression patterns of luminal, mesenchymal or claudin-low, and basal-like cells. These molecular patterns may be best represented as gradients of expression, as opposed to a discrete 'on' or 'off' state of expression. The approximate locations of the differentiation blocks imposed by BRCA1 loss and HER2 amplification are suggested by their locations in the differentiation hierarchy.

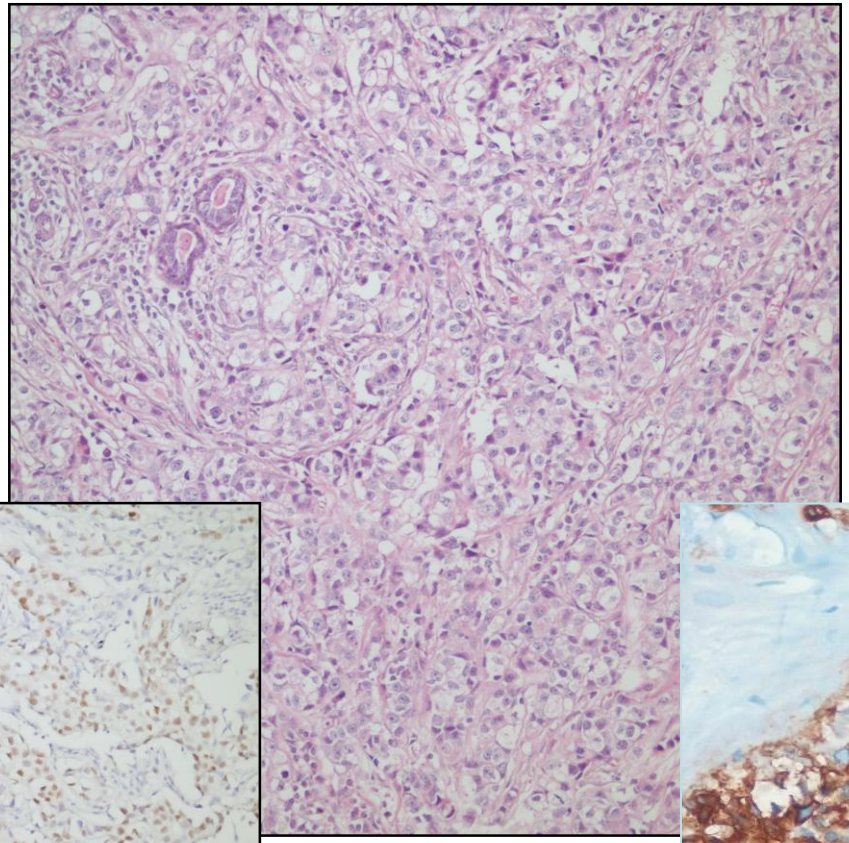
Alex Prat& Charles M Perou Nature Med 2009 :15(8) 842-844

LUMİNAL A

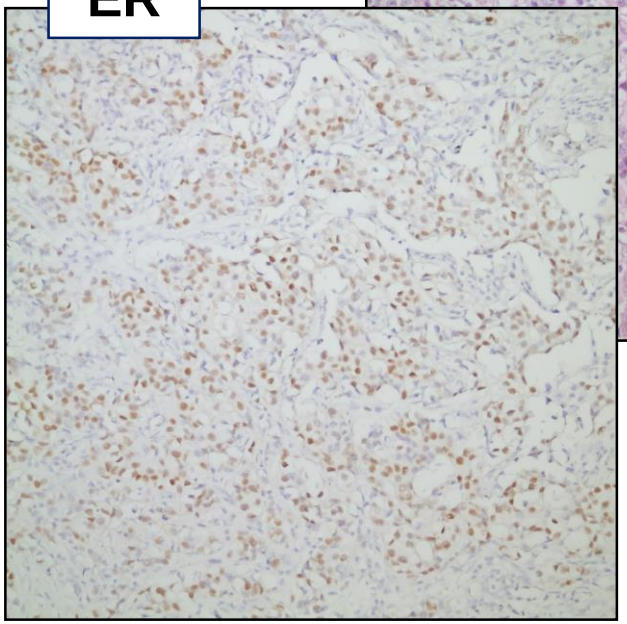




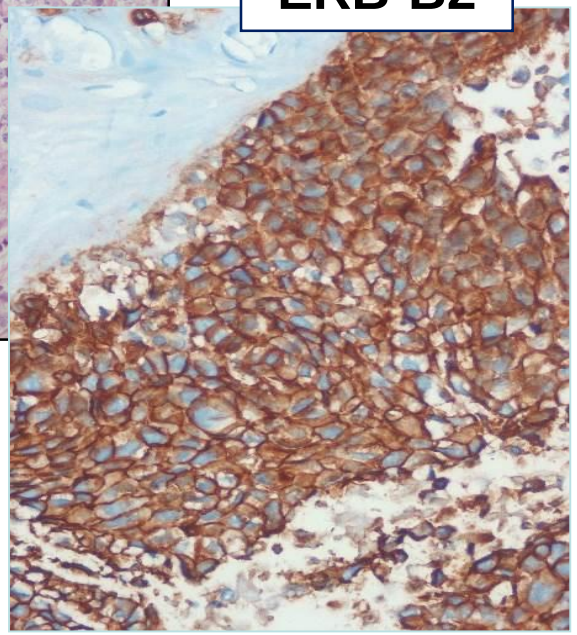
LUMİNAL B



ER

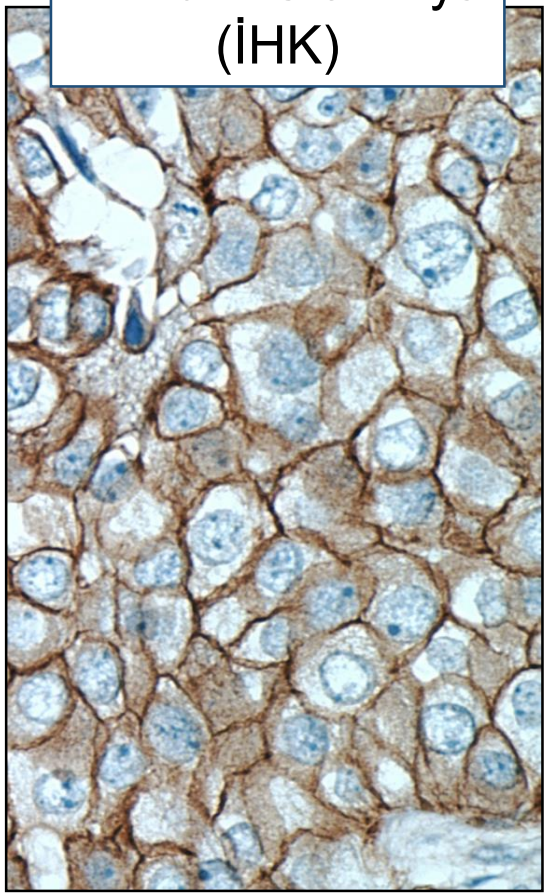


ERB-B2

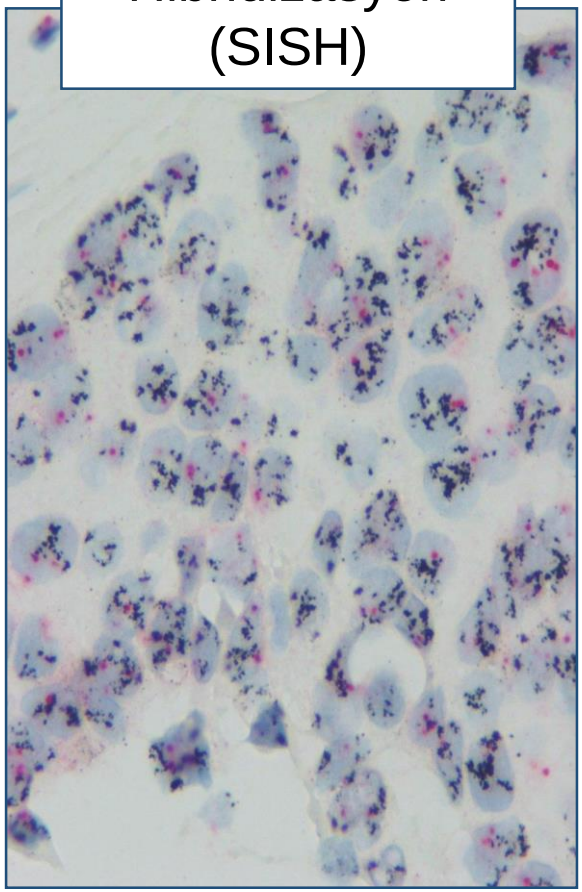


HER2 overeksprese alt tip

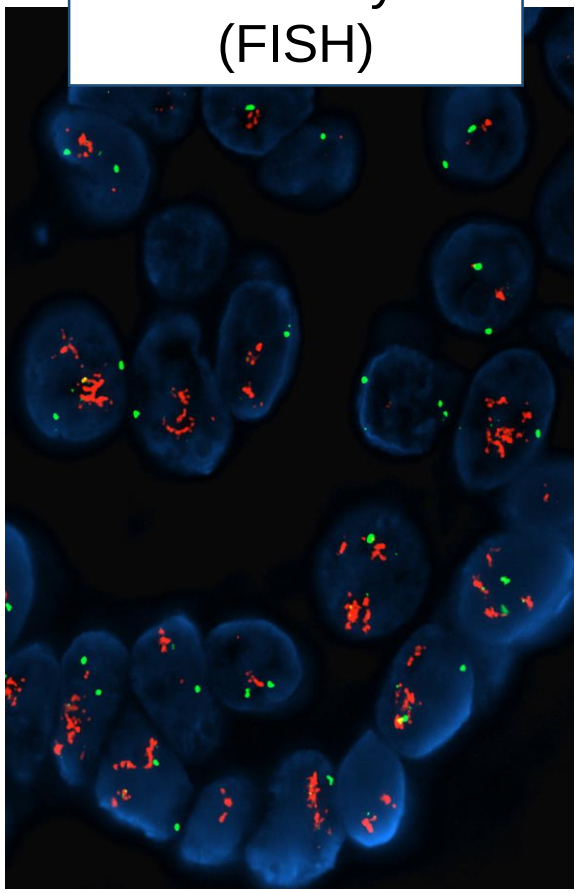
İmmünhistokimya (İHK)



(Gümüş) İn Situ Hibridizasyon (SISH)



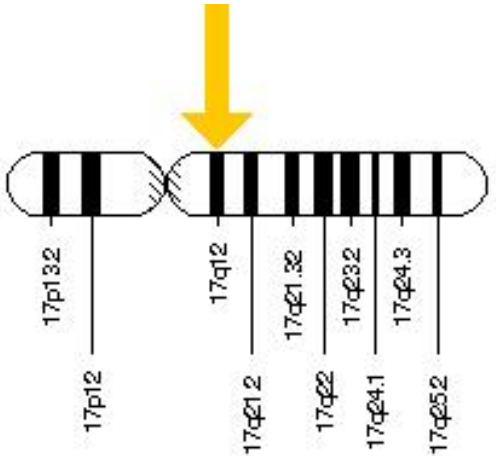
(Floresan) İn Situ Hibridizasyon (FISH)



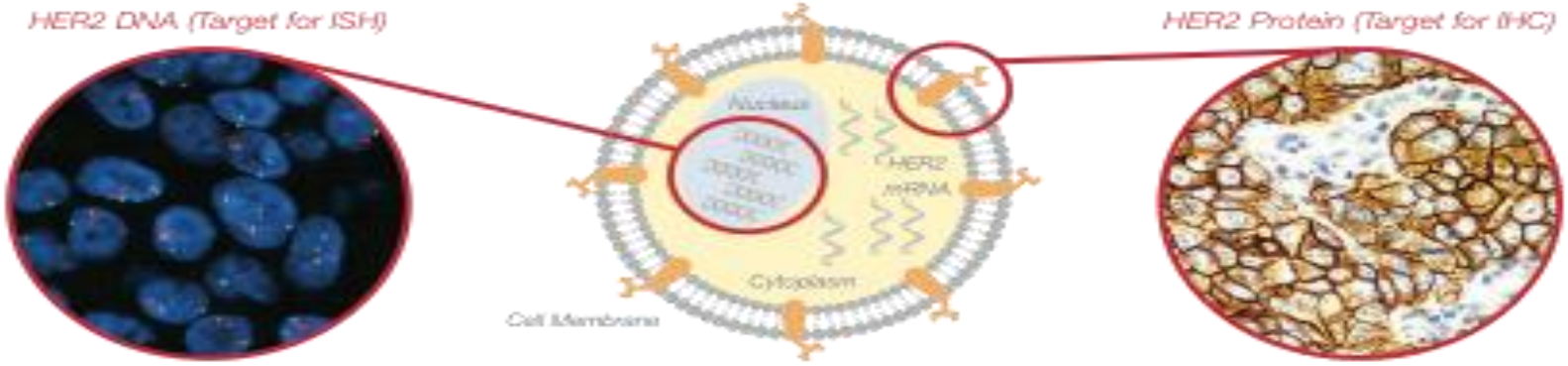
HER 2 NEDİR?

- **HER2 bir onkogendir.**
- **Meme kanserinde proliferasyon, migrasyon ve invazyona neden olur.**
- **HER2 gen amplifikasyonu → HER2 (c-erbB-2) protein overekspresyonu ile yakından ilişkilidir.**

ERBB2 Geni (*17q12*)



erbB-2 = HER2/Neu = EGFR-*2*)



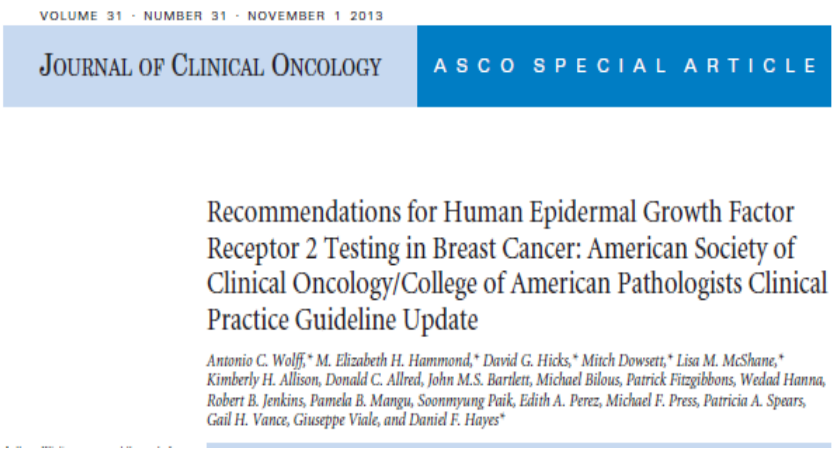
Amplified Result, Score ≥ 2
Breast cancer specimen stained with *HER2* IQFISH pharmDx™.

Positive Result, Score 3+
Breast cancer specimen stained with HercepTest™.

Figure 2: IHC and ISH targets for HER2 testing

HER2
İHK DEĞERLENDİRME KRİTERLERİ

2007



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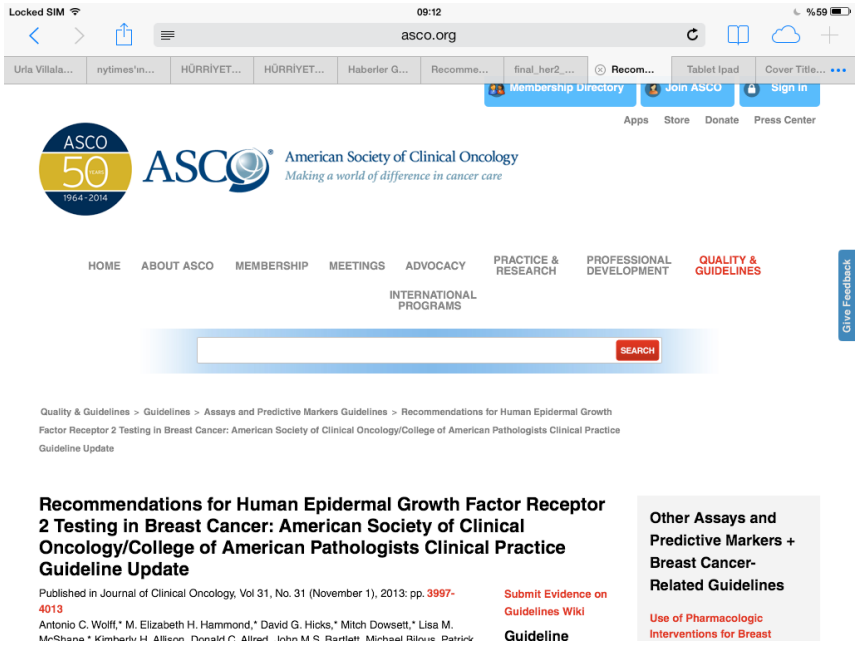
JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

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

Antonio C. Wolff, M. Elizabeth H. Hammond,* David G. Hicks,* Mitch Dowsett,* Lisa M. McShane,* Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes**

2013



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Published in Journal of Clinical Oncology, Vol 31, No. 31 (November 1), 2013: pp. 3997-4013

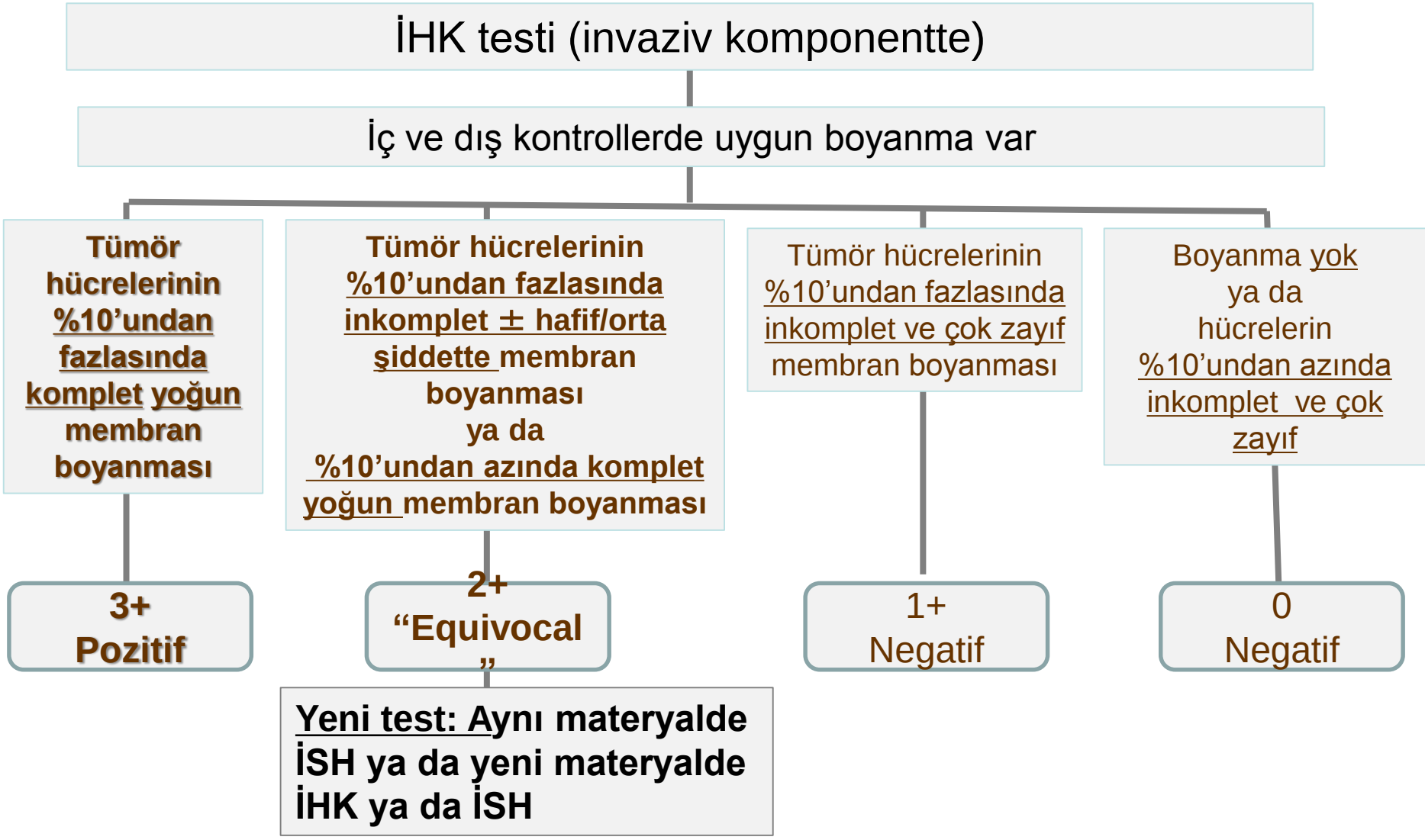
Antonio C. Wolff,* M. Elizabeth H. Hammond,* David G. Hicks,* Mitch Dowsett,* Lisa M.

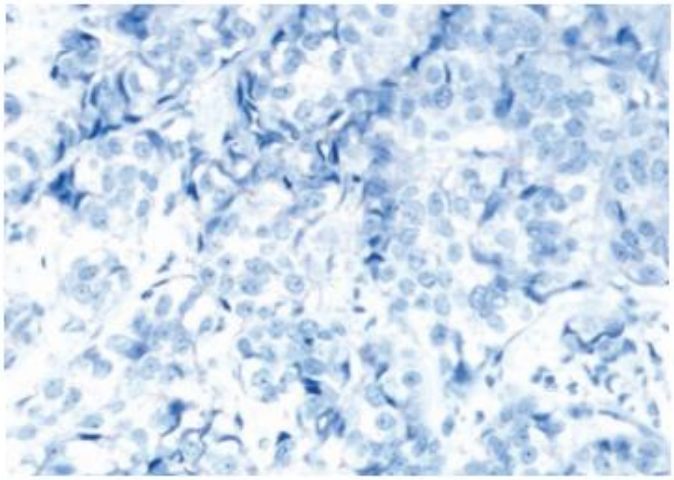
Submit Evidence on Guidelines Wiki

Guideline

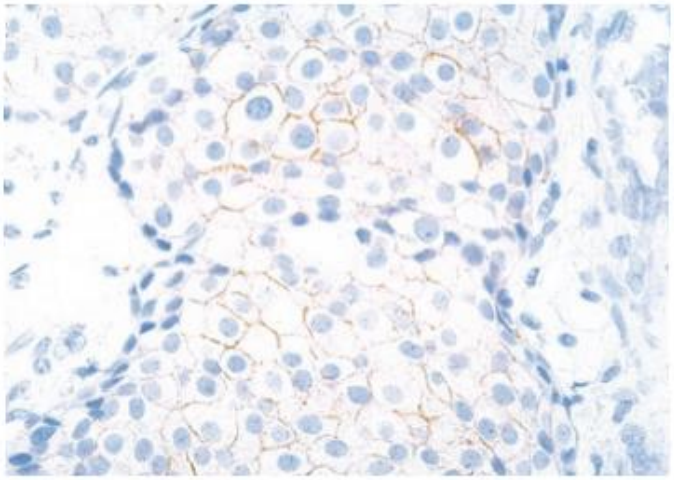
Other Assays and Predictive Markers + Breast Cancer-Related Guidelines

Use of Pharmacologic Interventions for Breast

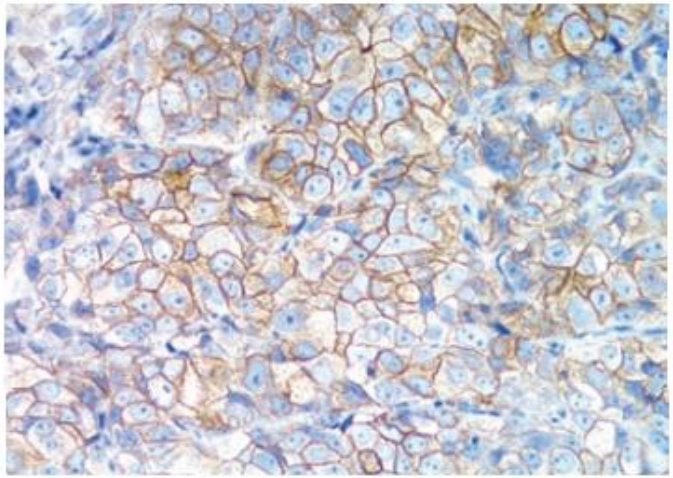

İHK testi (invaziv komponentte)
İç ve dış kontrollerde uygun boyanma var
Tümör hücrelerinin %10'undan fazlasında komplet yoğun membran boyanması
Tümör hücrelerinin %10'undan fazlasında inkomplet ± hafif/orta şiddette membran boyanması ya da %10'undan azında komplet yoğun membran boyanması
Tümör hücrelerinin %10'undan fazlasında inkomplet ve çok zayıf membran boyanması
Boyanma yok ya da hücrelerin %10'undan azında inkomplet ve çok zayıf
3+ Pozitif
2+ "Equivocal"
1+ Negatif
0 Negatif
Yeni test: Aynı materyalde İSH ya da yeni materyalde İHK ya da İSH



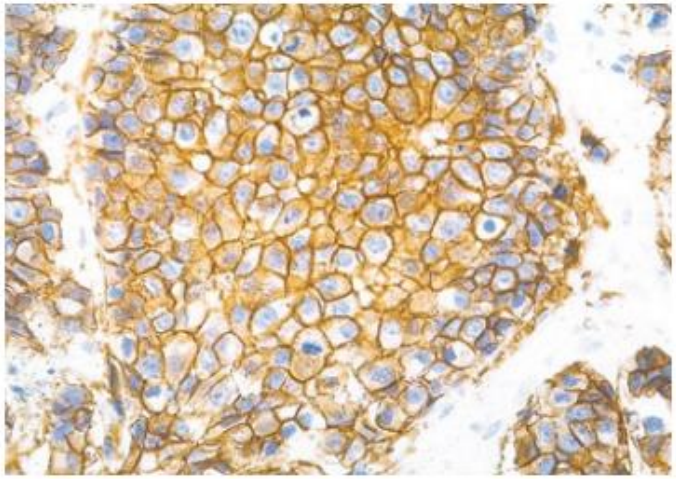
Score: 0 (20x)



Score: 1+ (20x)



Score: 2+ (20x)



Score: 3+ (20x)

HER 2 TESTLERİ

İn Situ Hibridizasyon (İSH)

- FISH
- CISH
- SISH

Birbirleriyle konkordansları çok yüksek

✓Konvansiyonel “bright-field” değerlendirilir
✓Solmayan, saklanabilir sinyaller

İHK (FİSH ile karşılaştırıldığında)

✓Hızlı
✓Konvansiyonel ‘bright-field’ mikroskopta bakılır
✓Boya solmaz
✓Tümör morfolojisi ile korele değerlendirilebilir
✓Tekniği daha kolay

ASCO/CAP 2013 hala İHK ve FİSH’i daha ön planda tutuyor

Üçlü (-) Meme Ca

- **ER(-), PR(-), c-erbB-2 (-)**
- Tüm meme tümörlerinin **~%16'sı**
- Çoğu invaziv duktal Ca (NOS) (%80.9)

  (geri kalanlar metaplastik ve tükürük bezi benzeri meme Ca)
- Çoğu grade 3 (%91)

Rakha EA ve ark Cancer 2007;109:25-32

Bazal benzeri alt tip ve üçlü (-) meme Ca

- Bazal benzeri meme Ca'ların çoğu ER, PR, c-erbB-2 (-) → Ancak, bu belirteçlerle (+) olabilen küçük bir Ca grubu da bildirilmekte.
- Üçlü (-) meme Ca ile bazal benzeri meme Ca arasında tam bir örtüşme yok.

Cleator S ve ark Lancet Oncol. 2007;8(3):235-44

Bazal benzeri alt tip

Histopatoloji

- İnvaziv duktal (NOS) veya metaplastik
- Derece (Grade): 3
- Coğrafik nekroz
- İtici (ekspansil) invazyon sınırı
- Stromal hümoral immün (lenfoplazmositer) yanıt

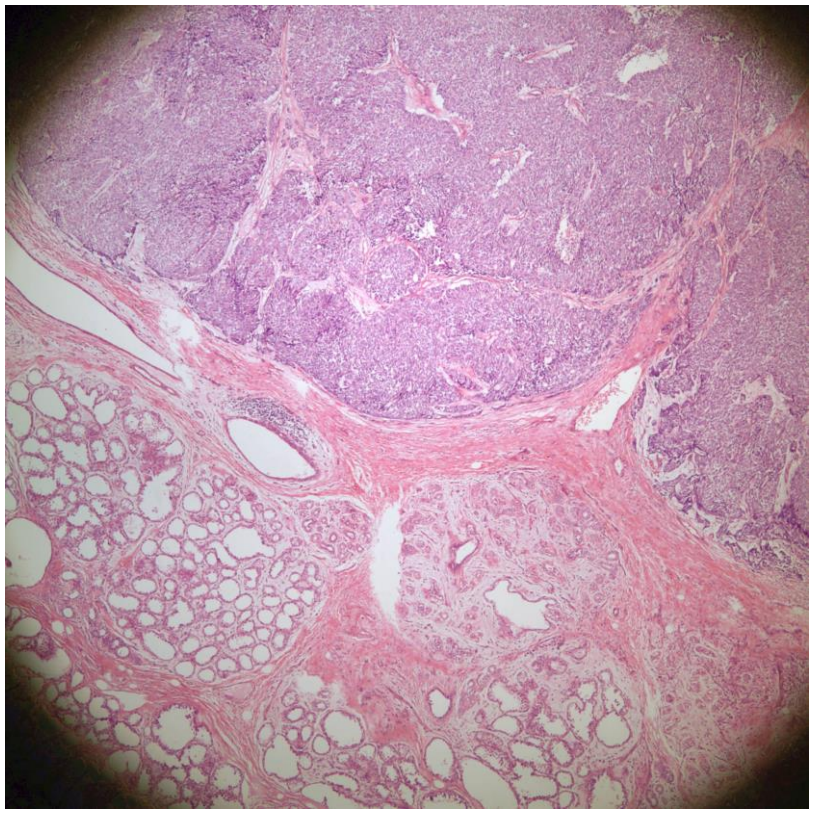
İmmünfenotip

- ER (-)
- PR (-)
- c-erbB-2 (HER2) (-)
- Bazal sitokeratinler (+) (CK 5/6, CK 14, CK 19)
- EGFR (HER1) (+)
- c-KIT (+)

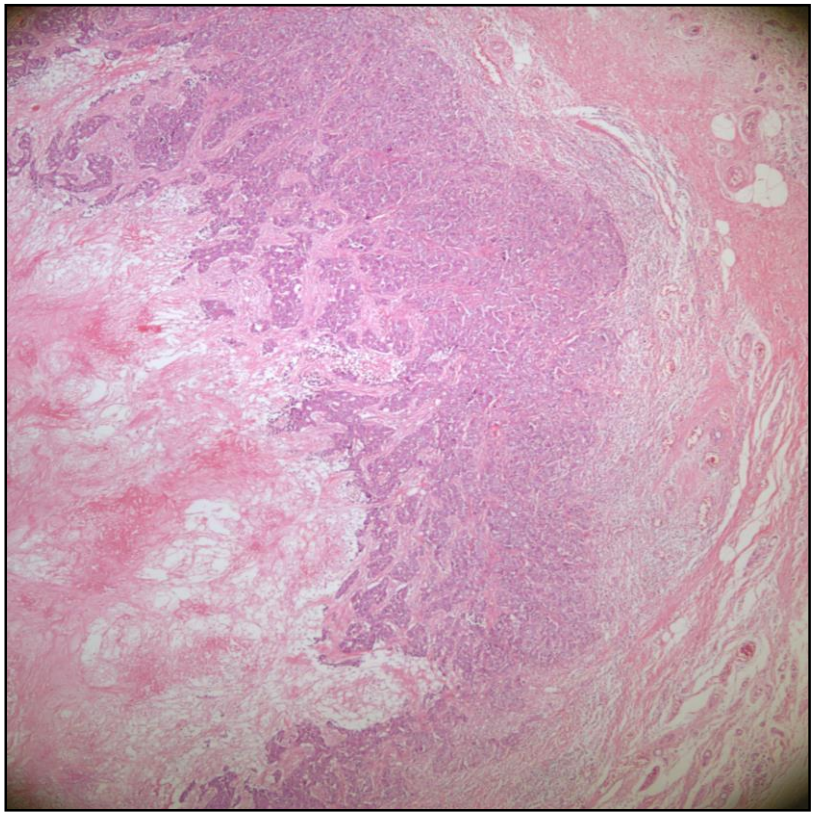
Livasy CA et al.Mod Pathol. 2006 Feb;19(2):264-71
Nielson TO et al Clin Cancer Res. 2004;10(16):5367-74

Bazal benzeri alt tip Histopatoloji

İtici invazyon sınırı

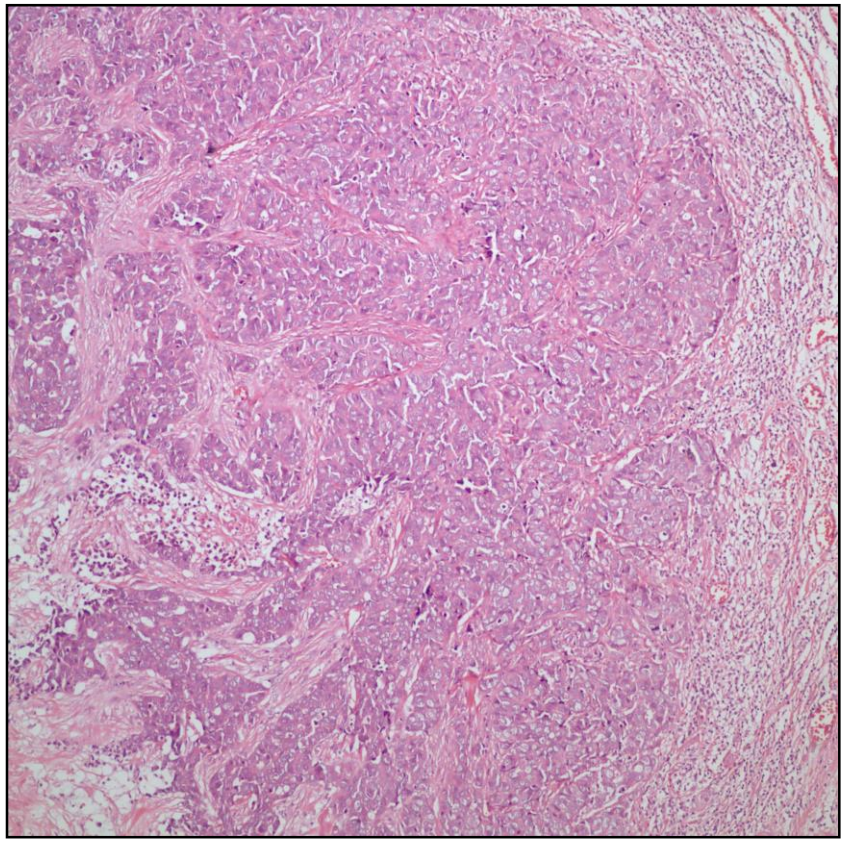


Coğrafik nekroz

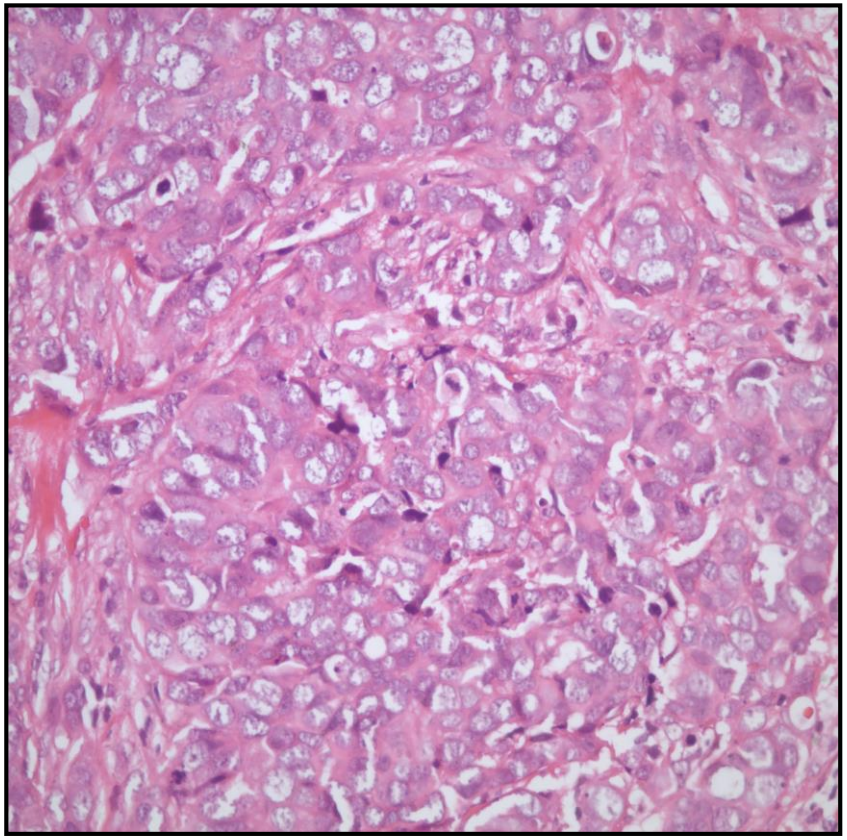


Bazal benzeri alt tip Histopatoloji

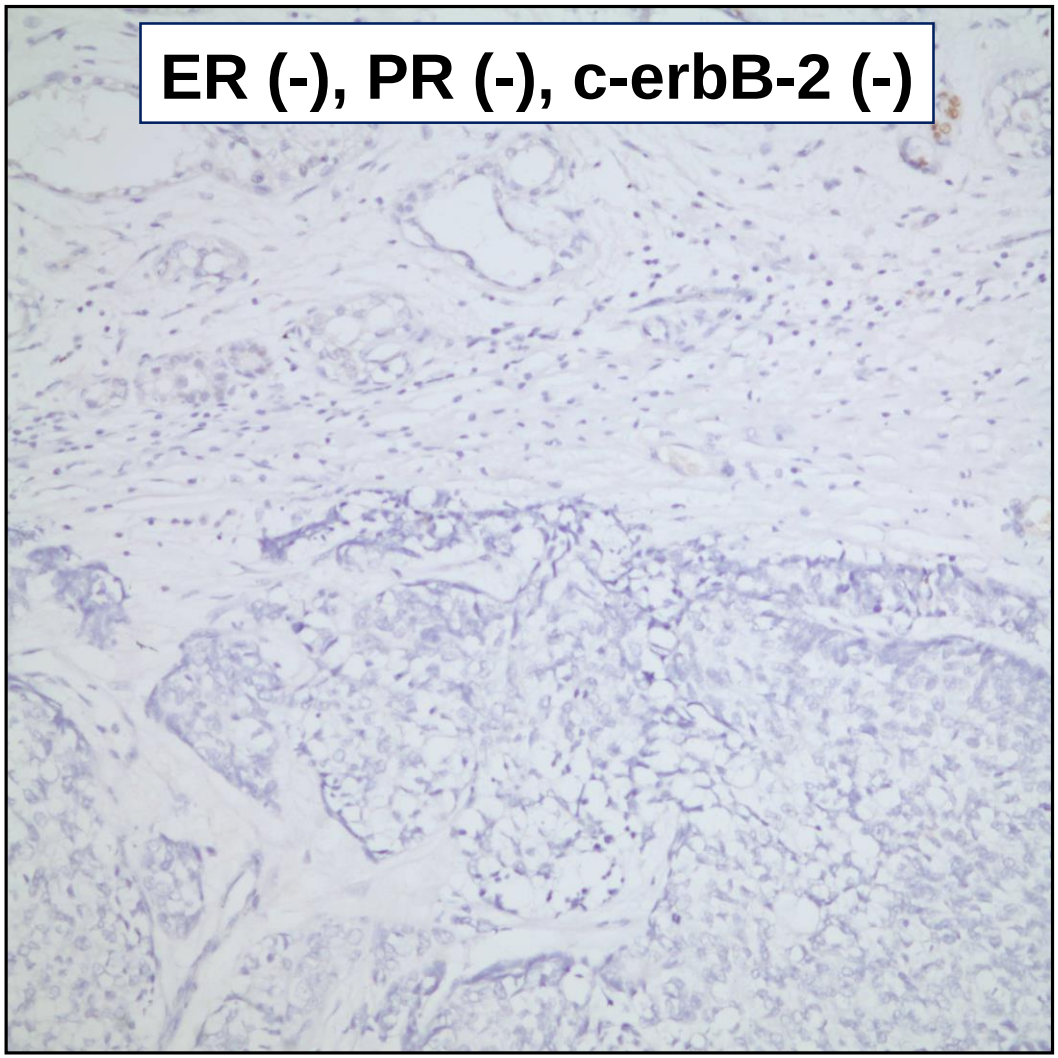
Stromal immün (lenfoplazmositer) yanıt



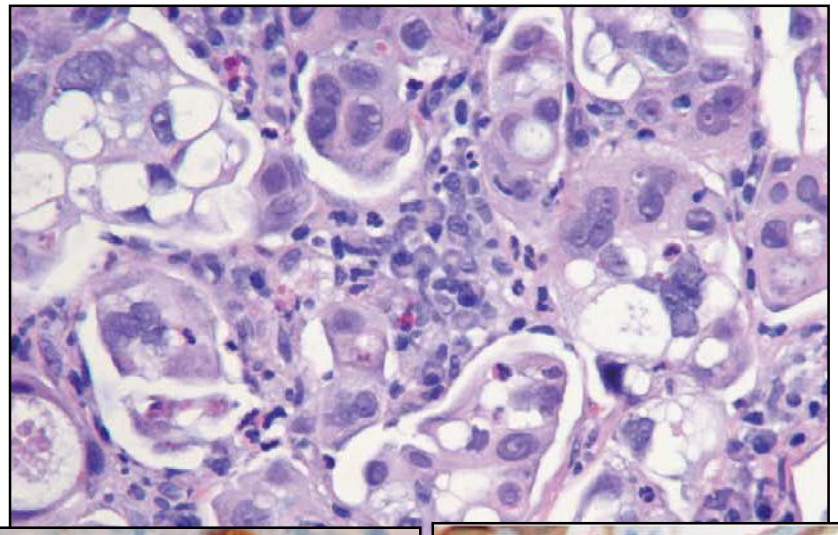
Derece (Grade): 3



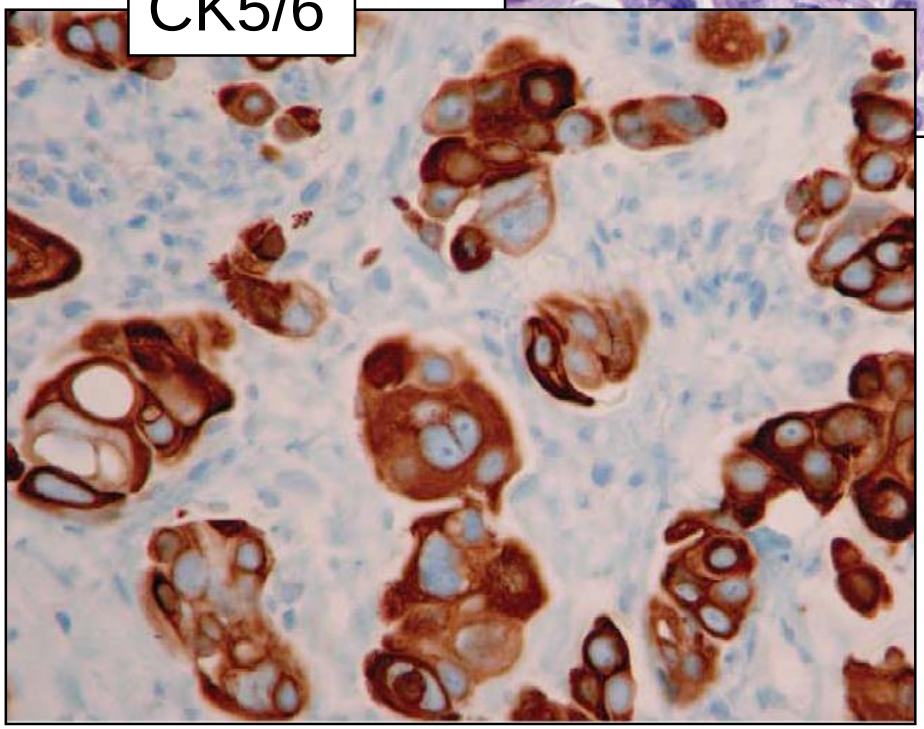
Bazal benzeri alt tip
İmmünfenotip



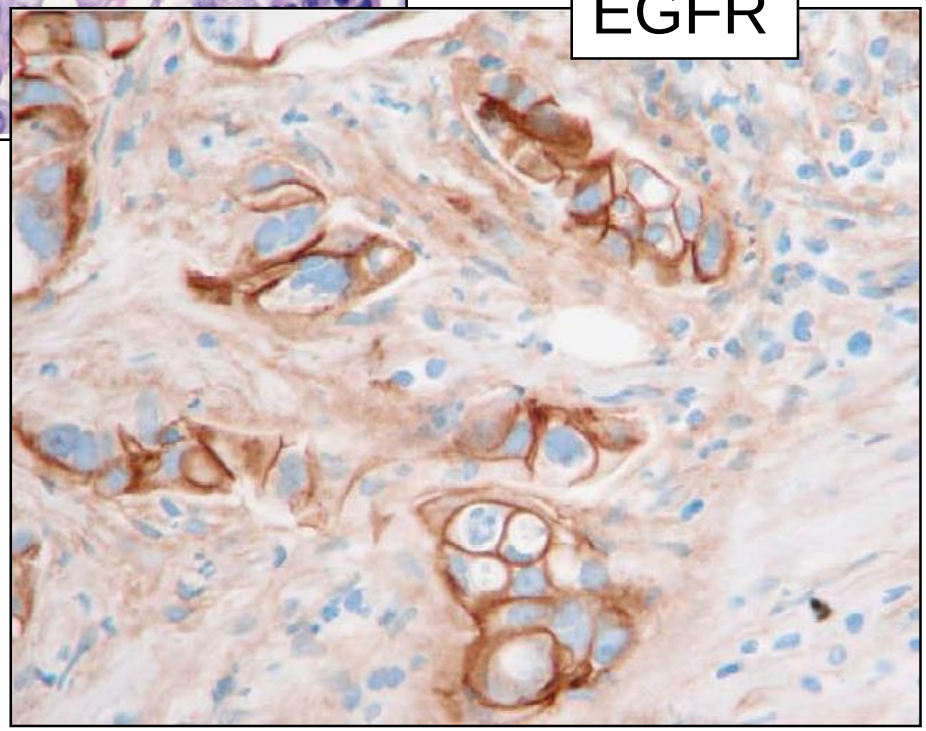
Bazal benzeri alt tip



CK5/6



EGFR



ER (-), PR (-), c-erbB-2 (-) meme Ca'larda

Bazal benzeri alt tip ve EGFR ekspresyonu ↑

«Üçlü (-)» meme Ca'larda
CK5/6 ile EGFR ekspresyonu
rutin değerlendirilmeli.

Siziopikou et al. Breast. 2007 Feb;16(1):104-7

Bazal benzeri alt tip - İmmünfenotip

ER,PR,Her-2, EGFR ve CK5/6 paneli

Bazal benzeri meme kanseri fenotipini tanımlamada,

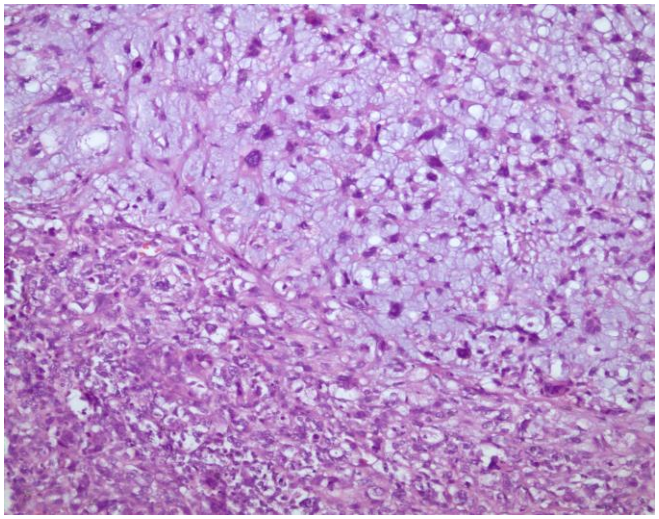
Spesifite: %100

Sensitivite: %76

Cheang MCU et al Clin Cance Res 14,1368-76, 2008

Bazal Benzeri Meme Ca'lar

- Medüller benzeri özellikler gösteren Ca
- Adenoid kistik Ca
- BRCA-1 ilişkili Ca'lar
- ~~Metaplastik Ca~~



➢ **Metaplastik Ca**'nın, moleküler olarak bazal-benzeri Ca'lardan farklı olduğu belirlenmiştir.

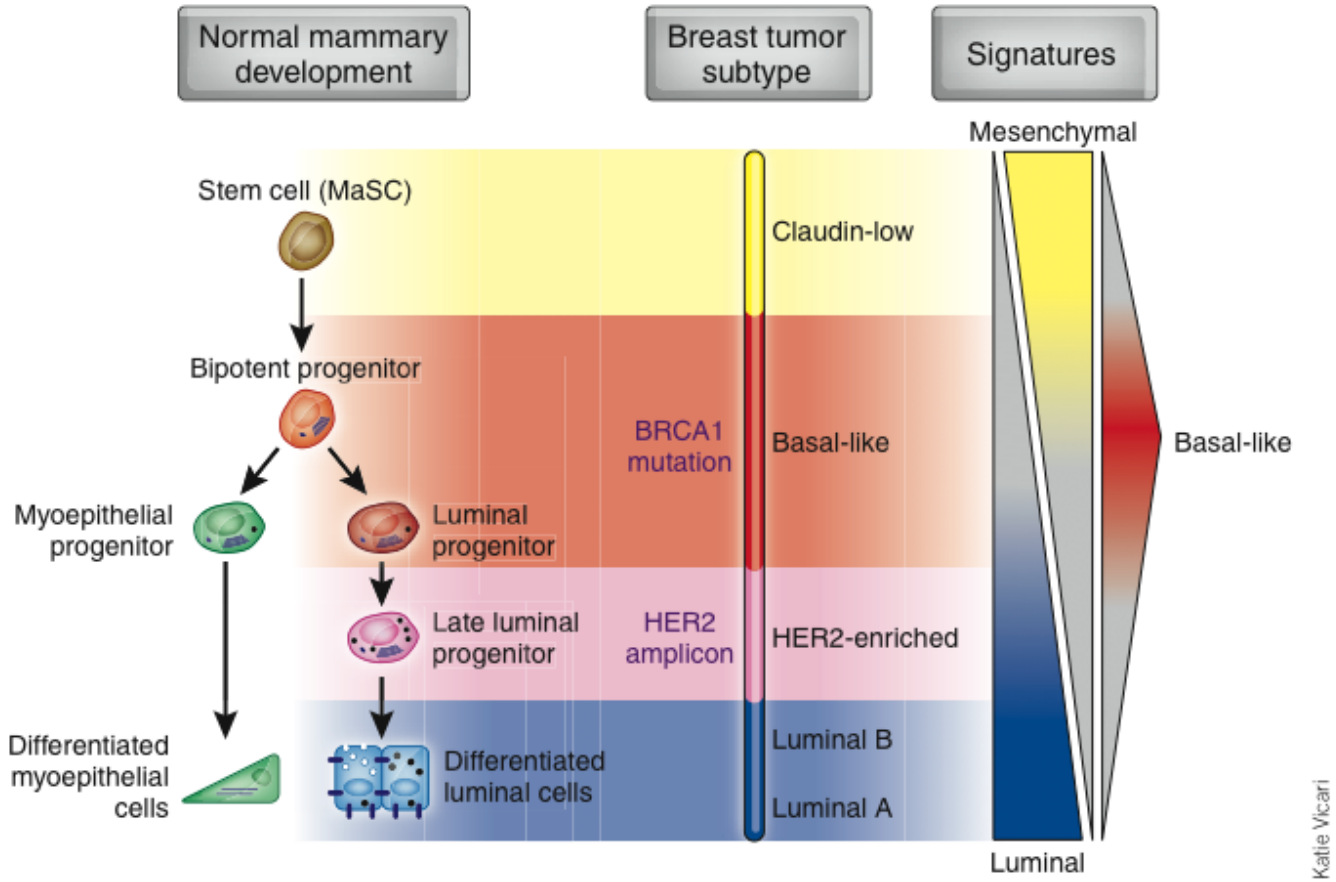
Metaplastik ve «Claudin low» Ca'lar

- ✓Benzer gen ekspresyon patterni gösterirler.
- ✓Epitelyal-mezenkimal geçiş (EMT) belirteçlerini yüksek oranda eksprese ederler.
- ✓CD44/24 ve CD29/CD24 oranları (kök hücre özelliği) yüksektir.

Hennessy BT et al Cancer Res 69, 4116-124,2009

«Claudin low» Ca: Claudin-3, Claudin-4, E-Kaderin, EpCAM ve MUC-1'in ekspresyon yokluğu ile karakterize Ca

Alex Prat& Charles M Perou Nature Med 2009 :15(8) 842-844



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Alex Prat& Charles M Perou Nature Med 2009 :15(8) 842-844

Moleküler Alt tipler & İmmünohistokimyasal İzdüşümleri

Luminal A	Luminal B	HER2	Bazal benzeri	Claudin-low
ER (+) HER2 (-)	ER (+) HER2 (+)	ER (-) HER2 (+)	ER (-) HER2 (-) CK5/6 (+) ve/veya EGFR (+)	ER (-) HER2 (-) Claudin 3 (-) E-Kaderin (-)

Hannemann J et al. Br J Cancer. 2006 Nov 20;95(10):1334-41.
Genome Biology 2007, 8:R76.

Prognoz

- Luminal tip A → En iyi
- Luminal tip B → Orta
- Bazal benzeri ve HER2 (+) → En kötü

Üçlü (-) meme Ca'lar:

- Viseral metastaz daha sık (kemik metastazına göre)
- Serebral metastaz insidansı yüksek
- Lokal rekürrens sık
- Daha erken yaş

Cleator S et al. Lancet Oncol. 2007;8(3):235-44.

HER2 testinde akılda tutulması gereken zorluklar…

- İHK ile yalancı negatiflik (%1- %11)
  - Bazı hastalar, bu nedenle hayat kurtarabilecek bir tedaviyi alamayabilirler.

- Hem İHK, hem de İSH ile arada kalan (equivocal, 2+) olgular

Ki67 (MIB1)

Proliferasyon belirteci

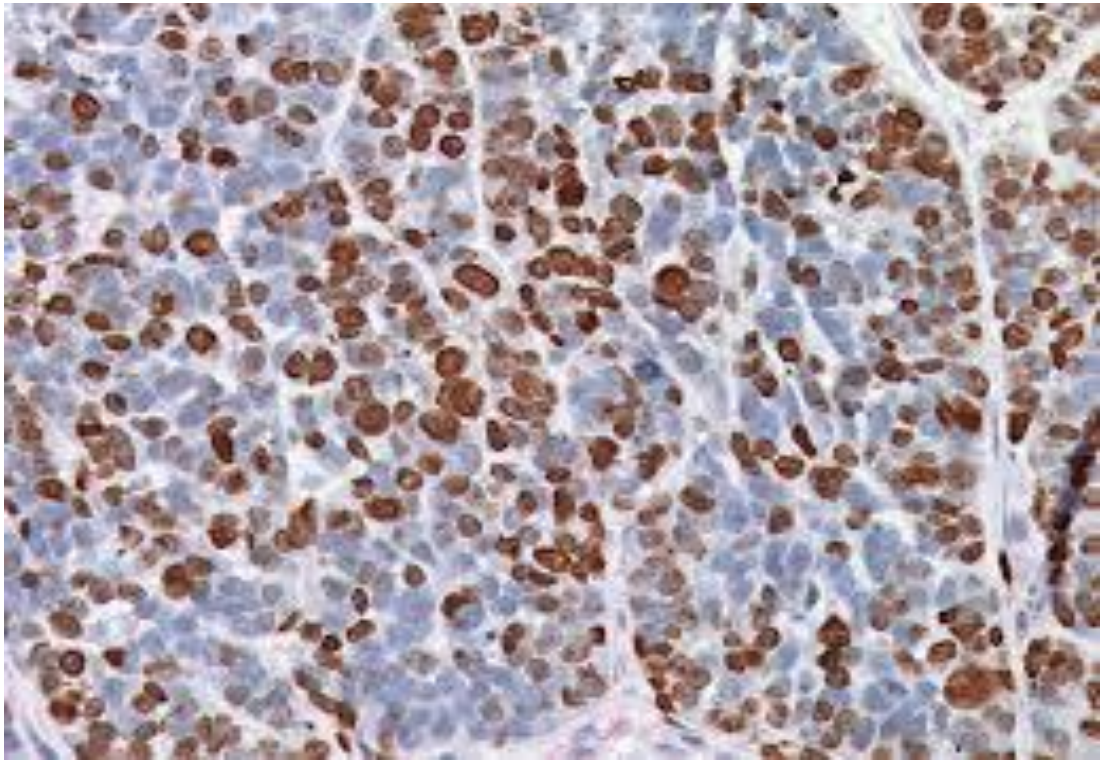

Table 4. Univariate analysis carried out with different Ki67 fraction cut-off points to find those best dividing the patients according to survival n=265

Variable	*P*-value	Hazard ratio	95% hazard ratio confidence intervals
Continuous variable	0.003	1.03	1.01, 1.05
5% cut-off point	0.43	1.52	0.55, 4.21
10% cut-off point	0.07	1.91	0.96, 3.81
15% cut-off point	**0.01**	**2.29**	**1.24 ,4.20**
20% cut-off point	0.02	1.94	1.12, 3.38
25% cut-off point	0.02	1.97	1.13, 3.43
30% cut-off point	**0.002**	**2.48**	**1.39, 4.41**
35% cut-off point	0.01	2.47	1.27, 4.84
40% cut-off point	0.20	1.84	0.73, 4.64
45% cut-off point	0.22	2.07	0.64, 6.64
50% cut-off point	0.99	0.00	0.00, . . .

Chosen cut-off point candidates are shown in bold. The analysis on the continuous mode shows hazard ratios associated with an increment of 1 unit (%).

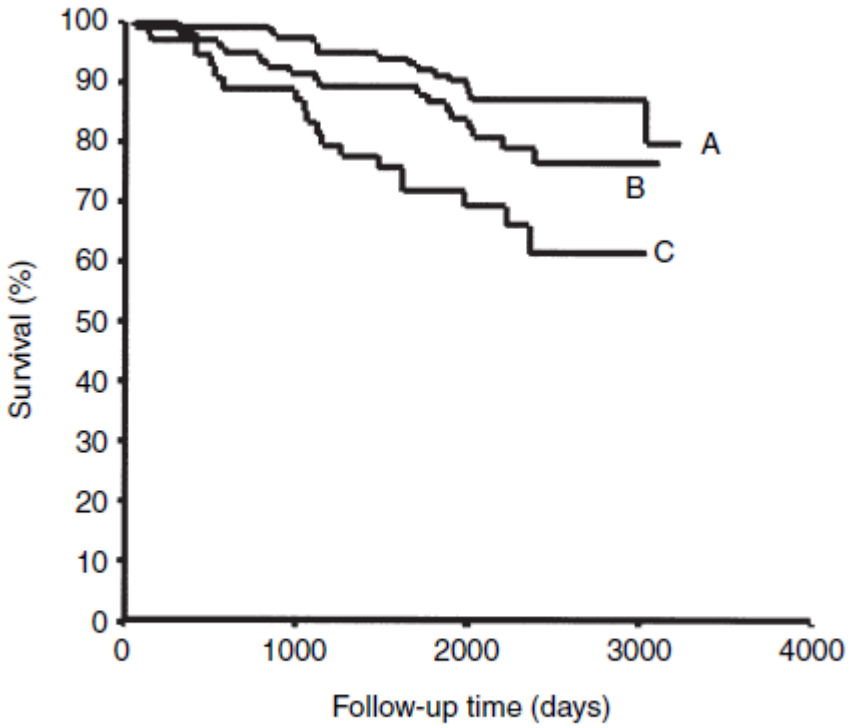

Figure 1. Survival curves for different Ki67 fraction groups: A = Ki67 ≤ 15%, B = Ki67 15–30% and C = Ki67 > 30%.

Histopathology 2006, 48, 674–682.

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Table 3. Chemoendocrine therapy in patients with ER-positive, HER2-negative disease

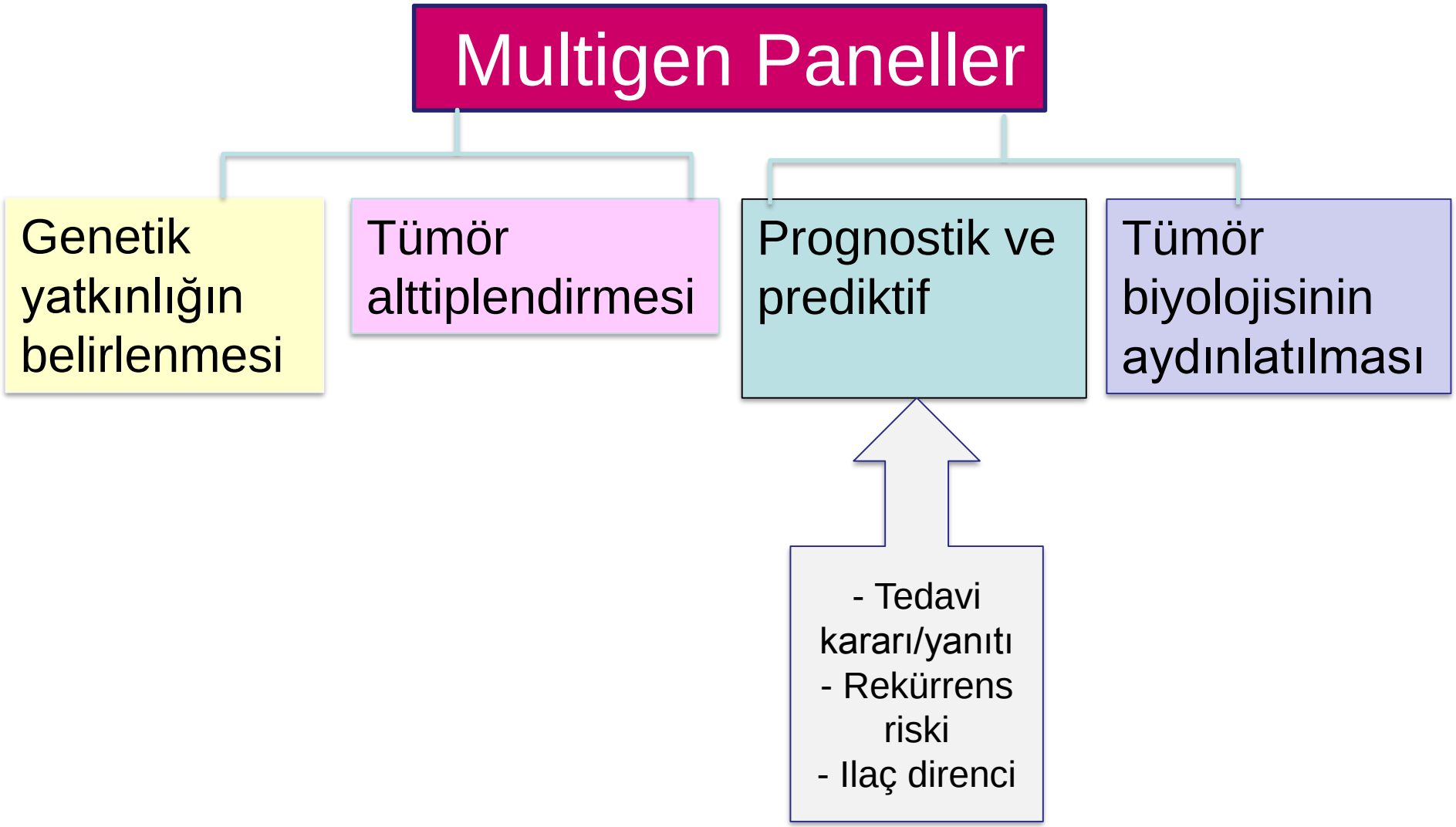
	Relative indications for chemoendocrine therapy	Factors not useful for decision	Relative indications for endocrine therapy alone
Clinicopathological features			
ER and PgR	Lower ER and PgR level		Higher ER and PgR level
Histological grade	Grade 3	Grade 2	Grade 1
Proliferation	High[a] Ki67 > %30	Intermediate[a] Ki67: %16-30	Low[a] Ki67≤ %15
Nodes	Node positive (four or more involved nodes)	Node positive (one to three involved nodes)	Node negative
PVI	Presence of extensive PVI		Absence of extensive PVI
pT size	>5 cm	2.1–5 cm	≤2 cm
Patient preference	Use all available treatments		Avoid chemotherapy-related side-effects
Multigene assays			
Gene signature[b]	High score	Intermediate score	Low score

[a]Conventional measures of proliferation include assessment of Ki67-labelling index (e.g. low, ≤15%; intermediate, 16%–30%; high, >30%) [77] and pathological description of the frequency of mitoses. The reliability of these measures will vary in different geographic settings. First-generation genetic signatures contain genes sampling the ER, HER2, and proliferative pathways [78, 79]. Meta-analysis indicates that much of the prognostic information in these signatures resides in their sampling of proliferative genes [80], but their respective total scores may be the only form in which information is provided at present and could be used in this component of assessment of relative indications for chemotherapy.

[b]The Panel agreed that validated multigene tests, if readily available, could assist in deciding whether to add chemotherapy in cases where its use was uncertain after consideration of conventional markers.

ER, estrogen receptor; PgR, progesterone receptor; pT, pathological tumour size (i.e. size of the invasive component); PVI, peritumoral vascular invasion.

Annals of Oncology 20: 1319–1329, 2009


Multigen Paneller
Genetik yatkınlığın belirlenmesi
Tümör alttiplendirmesi
Prognostik ve prediktif
Tümör biyolojisinin aydınlatılması
- Tedavi kararı/yanıtı
- Rekürrens riski
- Ilaç direnci

Multigen Paneller

Avantaj	Limitasyon
Tek seferde çoklu gen sekansı	Yeni tanımlanan gen mutasyonlarının yönetilmesine dair kılavuz eksikliği
Zamansal verimlilik, etkin maliyet	Orta derece (moderate/intermediate) risk genleri
Patojenik mutasyon saptamada artış	Önemi belirsiz varyantlar (VUS)
Kişisel kanser riski için daha eksiksiz değerlendirme	Kompleks data havuzu ve analiz güçlüğü
Bireysel tarama yöntemlerinin daha etkin yönetilmesi	
Nadir görülen mutasyon taşıyıcılarının tespiti	

Multigen Paneller

Özellikle erken evre, ER(+), lenf nodu(-) meme Ca'da

- rekürrens riskini belirleme;
- erken ve geç metastazı predikte etme;
- adjuvan KT yanıtını öngörme;
- genişletilmiş/uzatılmış endokrin tedavi kararını belirlemede yardımcıdır.

«Multigen paneller, kişiye özgü tedavi seçiminde klinikopatolojik paramatrelerle birlikte değerlendirilmelidir.»

Multigen Paneller

- **Onkogene DX: 16 gen**
- **EndoPredict: 8 kanser ilişkili gen, 3 referans gen**
- **PAM50: 50 gen seti**
- **Breast Cancer Index**
- MammaPrint: 70 gen
- Genomic Grade Index: 97 gen
- Prosigna: 50 gen
- MammaTyper
- BRCA1/2; BRCA plus; BRCAplus-Expanded
- BreastNext
- Invitae Breast Cancer Panel
- Myriad myRisk Hereditary Cancer test

Oncotype DX

- Kanser ilişkili 16 gen ve ek olarak 5 referans genden oluşan 21 gen paneli
- RT-PCR
- Gen ekspresyon seviyesine göre rekürrens skoru
- Erken evre, ER(+), HER2(-), Lenf nodu(-/+)
- KT'den yarar görme olasılığını tahmin etme
- 5-10 yıllık uzak metastaz riskini ön görme
- Yaygın kullanılan bir test
- DKİS'te rekürrens riskini öngörme

Oncotype Dx

PROLIFERATION	INVASION	HER2
Ki-67	STROMELYSIN 3	GRB7
STK15	CATHEPSIN L2	HER2
SURVIVIN		
CYCLIN B1		
MYBL2		

ESTROGEN	OTHER
ER2	GSTM1
PR	CD68
Bcl-2	BAG1
SCUBE2	

Recurrence Score® result = + 0.47 x HER2 group score – 0.34 x Estrogen group score + 1.04 x Proliferation group score + 0.10 x Invasion group score + 0.05 x CD68 – 0.08 x GSTM1 – 0.07 x BAG1[6]

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline

Lyndsay N. Harris, Nofisat Ismaila, Lisa M. McShane, Fabrice Andre, Deborah E. Collyar, Ana M. Gonzalez-Angulo, Elizabeth H. Hammond, Nicole M. Kuderer, Minetta C. Liu, Robert G. Mennel, Catherine Van Poznak, Robert C. Bast, and Daniel F. Hayes

Recommendations

In addition to estrogen and progesterone receptors and human epidermal growth factor receptor 2, the panel found sufficient evidence of clinical utility for the biomarker assays Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer. No biomarker except for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and patient preferences.

J Clin Oncol 34:1134-1150.


Teşekkür ederim...