Her2Neu FISH: ervaringen uit de praktijk

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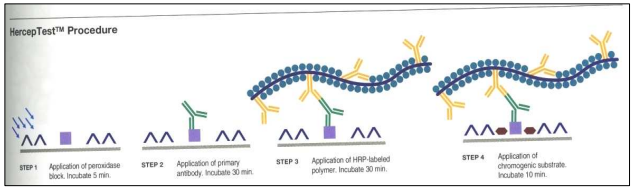
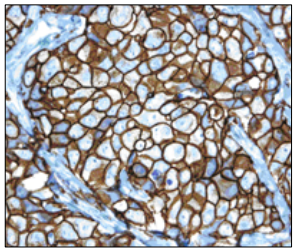
afdeling Pathologie
Radboud University Nijmegen Medical Centre

Moleculaire dag 29 januari 2010

Tot 2005: Herceptest®

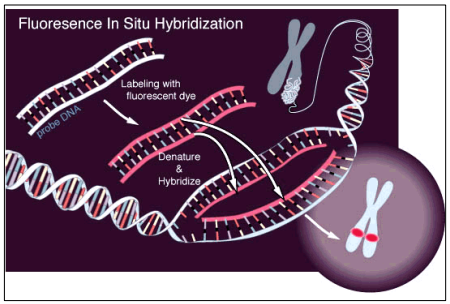


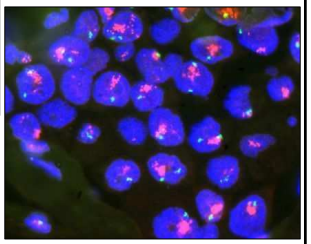
Score 2+ = 40%



3+ positieve tumor

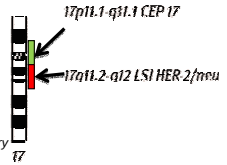
Vanaf 2005: FISH





Geamplificeerde tumor

FISH?



- Her2Neu FISH probe dual color probe

Referenties:
Pauletti et al: Assessment of methods for tissue-based detection of the Her-2/neu alteration in Human Breast Cancer: A direct comparison of fluorescence in situ hybridization an immunohistochemistry. J Clin Oncol 18:3651-3664, 2000

Hammock et al: Strong Her-2/neu protein overexpression by immunohistochemistry Often does not predict oncogen amplification by fluorescence in situ hybridization. Human Pathology 34: 1043-1047, 2003

Handleiding PathVision®; Vysis

Dal Lago et al: Correction for chromosome-17 is critical for the determination of true Her-2/neu gene amplification status in breast cancer. Mol Cancer Ther. 5(10): 2572-2581, 2006

- Bestaand FISH protocol

- Probe direct gelabeld met fluorochromen

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Kostensparend

Samenwerking met IHC

GESTANDAARDISEERD PROTOCOL

Snel en simpel

Robuust

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SCOREN (1)

Werklijst voor Her2Neu/Cerb-b2

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PA-nummer: Extern nummer:

Datum:

Aanvrager:

Analist:

Coupes beoordeeld door:

Scoringstabel:

Kern	CerbB-2	Cep 17	Kern	CerbB-2	Cep 17	Kern	CerbB-2	Cep 17
1			21			41		
2			22			42		
3			23			43		
4			24			44		
5			25			45		
6			26			46		
7			27			47		
8			28			48		
9			29			49		
10			30			50		
11			31			51		
12			32			52		
13			33			53		
14			34			54		
15			35			55		
16			36			56		
17			37			57		
18			38			58		
19			39			59		
20			40			60		
Subtotaal (1-20)			Subtotaal (21-40)			Subtotaal (41-60)		

	CerbB-2	Cep 17	CerbB-2:Cep 17 ratio
Totaal (1 t/m 60)			

Uitslag: □ negatief (CerbB-2:Cep 17 ratio: < 2)
□ positief (CerbB-2:Cep 17 ratio: ≥ 2)

opmerkingen:

aantal plaasjes:

Foto:

Behorend bij Kwint:
Autorisatiedatum: 04-03-2008
Autorisator: ML
Beheerder: PvC
Archiveringsdatum:

- standaard 20 kernen tellen
  Referentie:
  handleiding Her2 FISH pharmdX™; Dako
- scoren door patholoog + analist
- screening hele coupe
- ratio Her2Neu:cep17 ≥ 2= amplificatie
- digitale opslag

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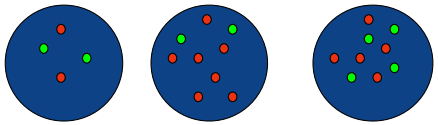
SCOREN (2)

Ratio Her2Neu/cep 17 ≥ 2 = amplificatie



normaal amplificatie polysomie

Note: Effect van polysomie op expressie Her2 nog onvoldoende duidelijk, discrepantie in de literatuur. Evenmin duidelijk of deze patiënten behandeld zouden moeten worden met Herceptin.

Referenties:
J Clin Oncol. 26(30): 4869-4874, 2008
Modern Pathology 22: 1044-1048, 2009

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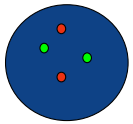

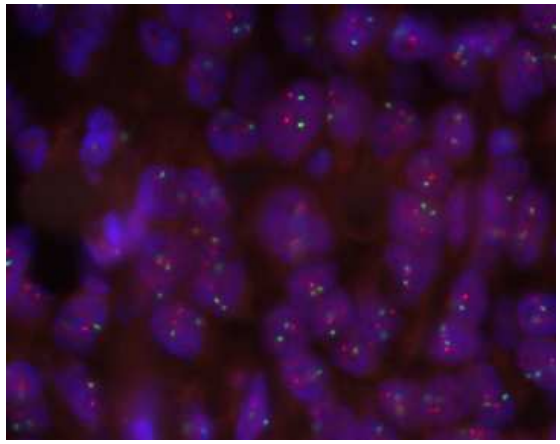

Her2Neu

geen amplificatie

4 µm coupe

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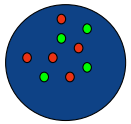
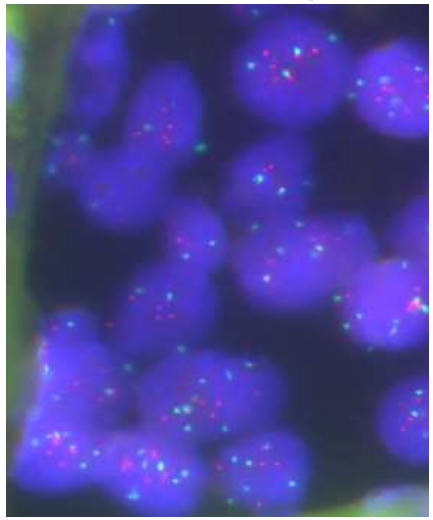


Her2Neu
geen amplificatie,
polysomie
4 µm coupe



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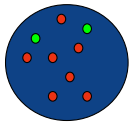
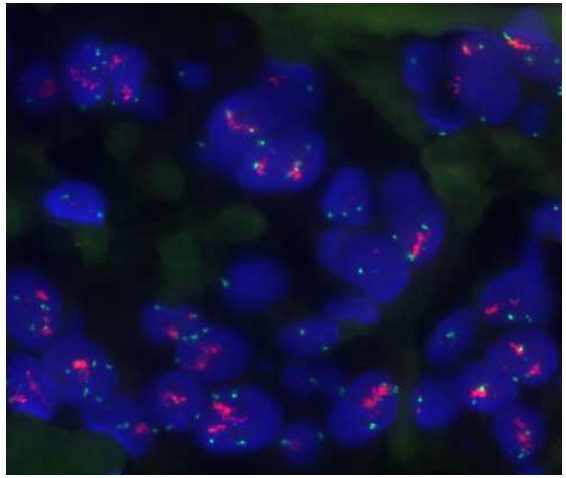


Her2Neu
amplificatie
4 µm coupe



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RESULTATEN (1)

RESULTATEN JAN-NOV 2009: 193 tumoren

Her2Neu positief (geamplificeerd) 30 = 15,5%

Her2Neu negatief 163 = 84,5%

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RESULTATEN (2)

POSITIEF:
- hoge amplificatie= 90%
- lage amplificatie (o.a. monosomie) = 10%

NEGATIEF:
- monosomie= 8,0%
- diploïd= 63,2%
- polysomie=28,8%

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PITFALLS

- Tumor niet/moeilijk te herkennen
  - Scoren met de patholoog
  - HE coupe
- Ratio Her2Neu/cep 17 ligt tussen 1.8-2.2
  - Handhaven standaard ≥ 2
  - Extra kernen tellen
- Extreme polysomie
  - Ondervangen door controle probe cep 17
- Monosomie met lage amplificatie
  - Ondervangen door controle probe cep 17
- Heterogeniteit
  - Screenen hele coupe
- Geen aankleuring
  - Kleuring herhalen met evt. aangepast protocol

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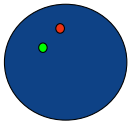
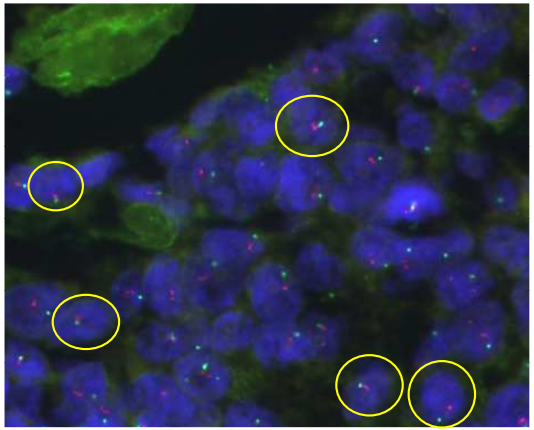
BIJZONDERE GEVALLEN



Her2Neu
monosomie
4 µm coupe



Conclusie: niet geamplificeerd

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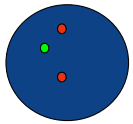
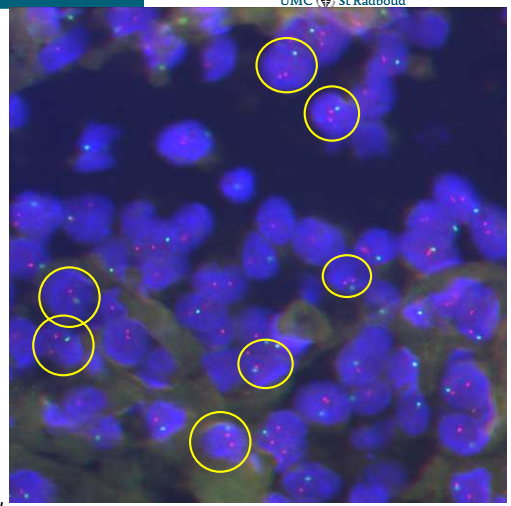


Her2Neu
monosomie
lage amplificatie
4 µm coupe



Conclusie: geamplificeerd

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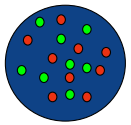
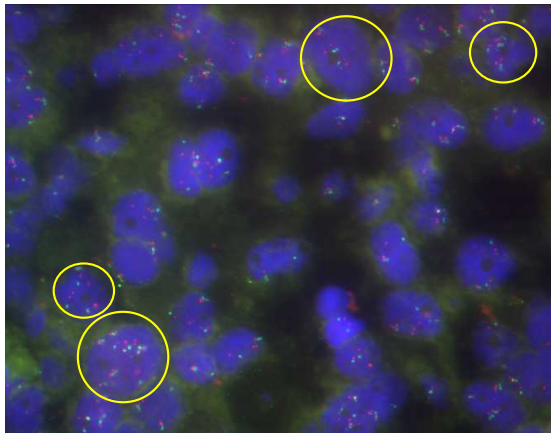
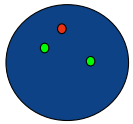
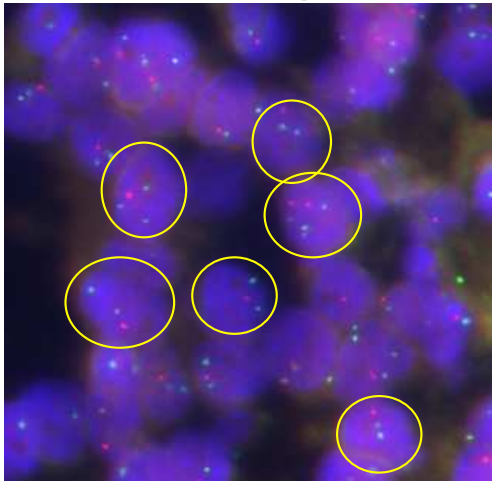


Her2Neu
“extreme”
polysomie
4 µm coupe



Conclusie: niet geamplificeerd



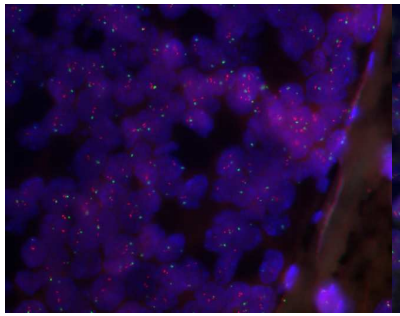


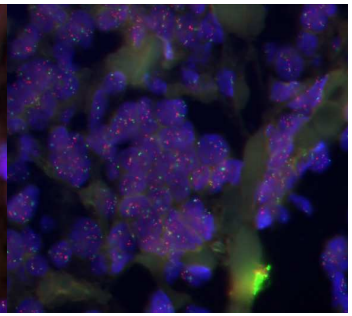
Her2Neu

verlies Her2Neu

4 µm coupe

Conclusie: niet geamplificeerd





Her2Neu

geen amplificatie

ratio Her2/cep17= 1,83

4 µm coupe

Conclusie: niet geamplificeerd

Her2Neu

amplificatie

ratio Her2/cep17= 2,10

4 µm coupe

Conclusie: geamplificeerd

SAMENVATTEND

- 193 tumoren: 193x uitslag (100%)
- 12x > 20 kernen geteld (6%)
- 4x ratio 1.8-2.2 (2%)
- 1x extreme polysomie (0,5%)
- 1x monosomie met amplificatie (0,5%)
- 3x heterogeniteit (1,5%)

CONCLUSIE

- Geen technische valkuilen die niet opgelost kunnen worden (tot nu toe)
- Tevreden over Her2Neu bepaling m.b.v. FISH
- Openstaan voor nieuwe ontwikkelingen

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VRAGEN?

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Correction for chromosome-17 is critical for the determination of true Her-2/neu gene amplification status in breast cancer.

Mol Cancer Ther. 2006 Oct;5(10):2572-9.

Dal Lago L, Durbecq V, Desmedt C, Salgado R, Veriat T, Lespagnard L, Ma Y, Veys I, Di Leo A, Sotiriou C, Piccart M, Larsimont D.
Translational Research Unit, Bordet Institute, Brussels, Belgium.

PURPOSE: Trastuzumab is the cornerstone for treatment of women with HER2-overexpressing breast cancer, both in the adjuvant and in the metastatic settings.

METHODS: FISH assay was done on 893 samples of breast cancer. Three scoring methods were evaluated: Her2/CEP17> or =2, Her2>4, or Her2>6. Protein and gene expression were evaluated by *immunohistochemistry (n = 584) and mRNA/assay/nucleic acid sequence-based amplification (NASBA; n = 90).*

RESULTS: Samples were divided into five groups based on FISH results: disomic amplified and nonamplified, polysomic amplified, nonamplified, and discordant (10.8% of cases, mostly positive with Her2>4 scoring, but negative with the others). Her2/CEP17> or =2 and Her2>6 scoring methods showed the best association (a) with regard to FISH scoring (kappa = 0.906, P < 10(-6)) and (b) between FISH and immunohistochemistry (3+ as positive; kappa > 0.650, P < 10(-6)) or NASBA (kappa > 0.536, P < 10(-6)). **Polysomy had an effect on Her2 copy number (P < 10(-6)), but had no effect on protein and mRNA content. Therefore, within the discordant subgroup, for which additive Her-2 gene copies are due to high polysomy, protein and mRNA levels were similar to those of the nonamplified samples.** For this subgroup, the best concordance between FISH/immunohistochemistry/NASBA was observed with the Her2/CEP17 ratio and Her-2>6 scoring (68% and 58% perfect matches, respectively). No perfect matches were observed using the Her2>4 scoring method.

CONCLUSION: Correction for chromosome-17 is the method of choice for clinical practice; Her-2>6, but not Her-2>4, could be used as an alternative.

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J Clin Oncol. 2008 Oct 20;26(30):4869-74. Epub 2008 Sep 15.

Polysomy 17 in breast cancer: clinicopathologic significance and impact on HER-2 testing.

Vanden Bempt I, Van Loo P, Drijkoningen M, Neven P, Smeets A, Christiaens MR, Paridaens R, De Wolf-Peeters C.
Department of Pathology, Katholieke Universiteit Leuven, Leuven, Belgium.

PURPOSE: Polysomy 17 is frequently found in breast cancer and may complicate the interpretation of HER-2 testing results. We investigated the impact of polysomy 17 on HER-2 testing and studied its clinicopathologic significance in relation to HER2 gene amplification.
PATIENTS AND METHODS: In 226 patients with primary invasive breast carcinoma, HER2 gene and chromosome 17 copy numbers were determined by dual-color fluorescent in situ hybridization (FISH). The interpretation of FISH results was based on either absolute HER2 gene copy number or the ratio HER2/chromosome 17. Results were correlated with HER-2 protein expression on immunohistochemistry (IHC), HER2 mRNA expression by reverse transcriptase polymerase chain reaction (RT-PCR), and with various clinicopathologic parameters.

RESULTS: All cases with an equivocal HER-2 result by FISH, either by absolute HER2 copy number (44 of 226 patients; 19.5%) or by the ratio HER2/chromosome 17 (three of 226 patients; 1.3%), displayed polysomy 17. **On its own, polysomy 17 was not associated with HER-2 overexpression on IHC or increased HER2 mRNA levels by RT-PCR. Moreover, and in contrast with HER2 gene amplification, polysomy 17 was not associated with high tumor grade, hormone receptor negativity, or reduced disease-free survival.**

CONCLUSION: Polysomy 17 affects HER-2 testing in breast cancer and is a major cause of equivocal results by FISH. **We show that tumors displaying polysomy 17 in the absence of HER2 gene amplification resemble more HER-2-negative than HER-2-positive tumors. These findings highlight the need for clinical trials to investigative whether polysomy 17 tumors benefit from HER-2-targeted therapy.**

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Poor prognostic significance of unamplified chromosome 17 polysomy in invasive breast carcinoma

Uma Krishnamurti[1], Jennifer L Hammers[2], Folefac D Atem[3], Patrick D Storto[1] and Jan F Silverman[1,2]

Modern Pathology (2009) **22,** 1044–1048; doi:10.1038/modpathol.2009.61; published online 24 April 2009

Conclusion:
We found that invasive breast carcinomas with **unamplified chromosome 17 polysomy** are associated with **several adverse prognostic indicators such as a higher nuclear grade, mitotic activity, Nottingham score, histologic grade, tumor stage, and greater estrogen receptor negativity with a trend towards the amplified group,** in contrast to patients with neither amplification or polysomy. Although most patients with unamplified 17 polysomy have a 2+ equivocal score on immunohistochemistry, a minority has a 3+ positive score. **An increased adverse role for unamplified polysomy along with 3+ protein expression in some patients supports the idea that these patients should be considered for therapy with trastuzumab and/or anthracyclines.**