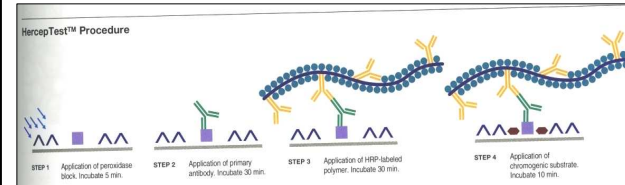


Her2Neu FISH: ervaringen uit de praktijk

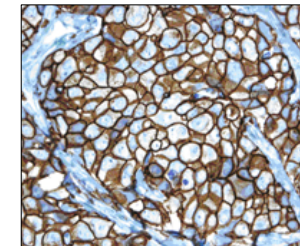
Patricia van Cleef
afdeling Pathologie
Radboud University Nijmegen Medical Centre

Moleculaire dag 29 januari 2010

Tot 2005: Herceptest®

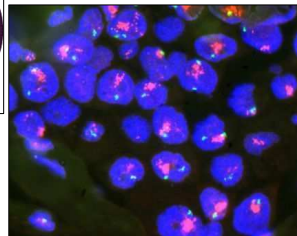
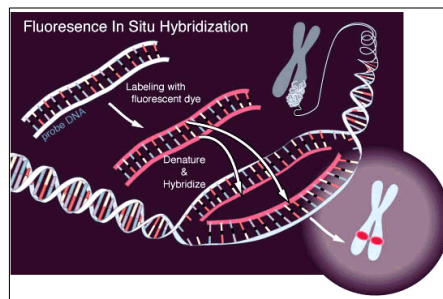


Score 2+ = 40%



3+ positieve tumor

Vanaf 2005: FISH



Geamplificeerde tumor

FISH?

- Her2Neu FISH probe dual color probe

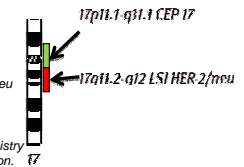
Referenties:

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

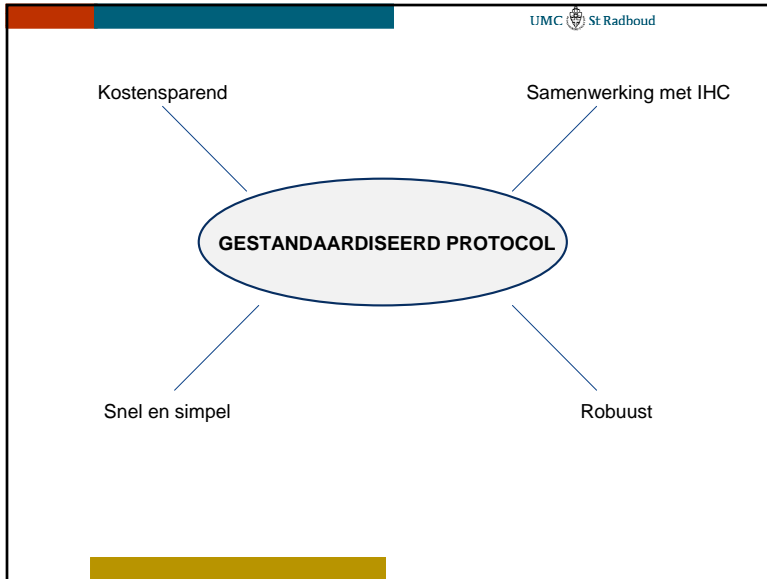
Handleiding PathVision®, Vysis

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



- Bestand FISH protocol

- Probe direct gelabeld met fluorochromen



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SCOREN (1)

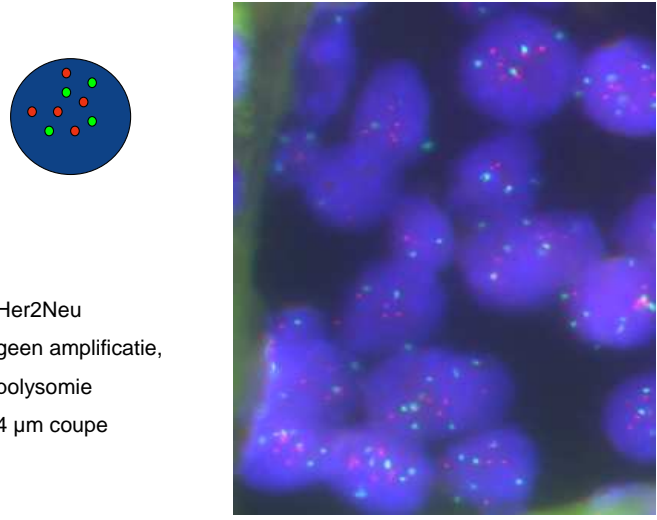
Werklijst voor Her2Neu/Cerb-b2

Klantnaam: Extra nummer:
 Datum:
 Aanspreek:
 Afdeling:
 Casus besloten door:

Scoretabel:

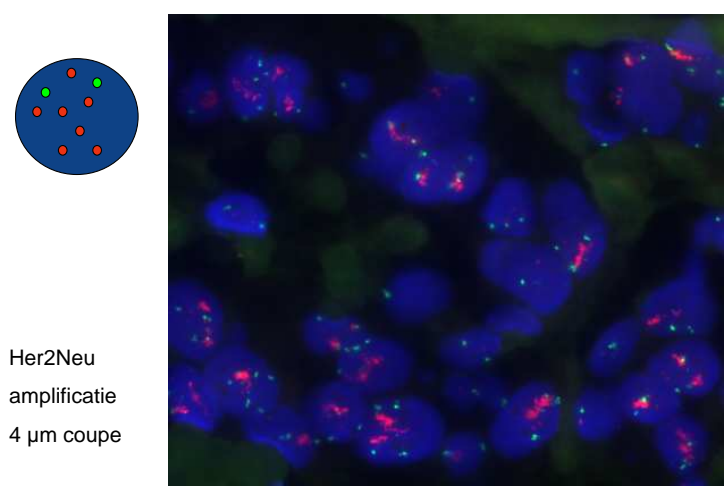
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167	167	167	168	167	168	169	167	169
168	168	168	169	168</				

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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%

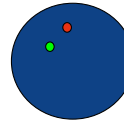
NEGATIEF:

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

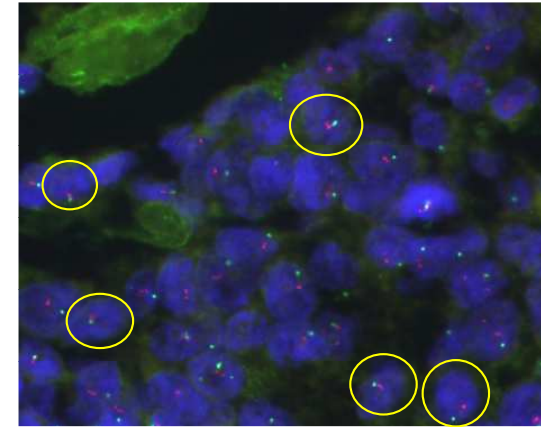
PITFALLS

- 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

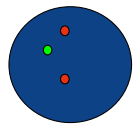
BIJZONDERE GEVALLEN



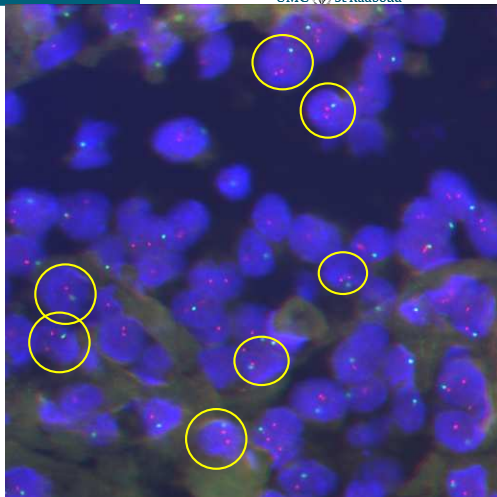
Her2Neu
monosomie
4 μ m coupe



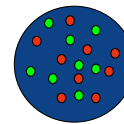
Conclusie: niet geamplificeerd



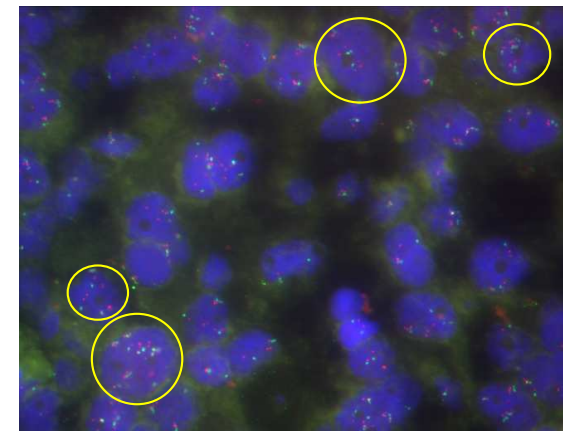
Her2Neu
monosomie
lage amplificatie
4 μ m coupe



Conclusie: geamplificeerd

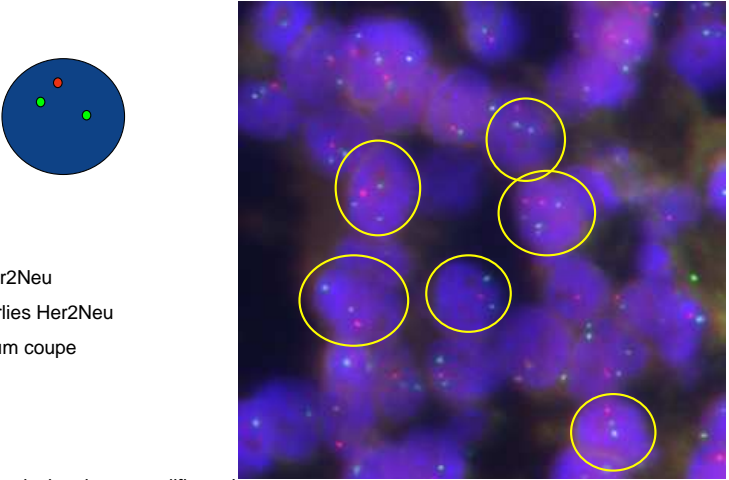


Her2Neu
"extreme"
polysomie
4 μ m coupe



Conclusie: niet geamplificeerd

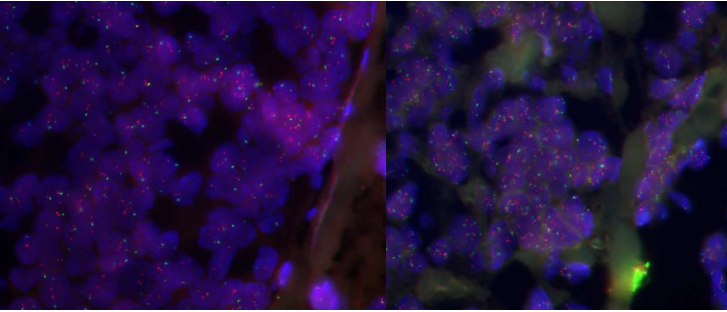
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Her2Neu
verlies Her2Neu
4 µm coupe

Conclusie: niet geamplificeerd

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Her2Neu geen amplificatie ratio Her2/cep17= 1,83 4 µm coupe <i>Conclusie: niet geamplificeerd</i>	Her2Neu amplificatie ratio Her2/cep17= 2,10 4 µm coupe <i>Conclusie: geamplificeerd</i>
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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%)

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CONCLUSIE

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

VRAGEN?

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, Durbeca V, Desmedt C, Salgado R, Verjat 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 Her2>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; Her2>6, but not Her2>4, could be used as an alternative.

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 investigate whether polysomy 17 tumors benefit from HER-2-targeted therapy.

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

[Uma Krishnamurti¹, Jennifer L Hammers², Folefac D Atem³, Patrick D Storto¹ 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.**