Table 1

Summary of histological, immunohistochemical and chromogenic *in situ* hybridization findings

Case	Histological type	HER2 (Herceptest®)	HER2 (CB11)	EGFR (IHC)	*EGFR* (CISH)
1	Carcinoma with squamous metaplasia	-	-	3+	No amp
2	Carcinoma with squamous metaplasia	-	-	3+	No amp
3	Carcinoma with squamous metaplasia	-	-	3+	No amp
4	Carcinoma with squamous metaplasia	-	-	3+	No amp
5	Carcinoma with squamous metaplasia	-	-	3+	Amp
6	Carcinoma with squamous metaplasia	-	-	3+	Amp
7	Carcinoma with squamous metaplasia	1+	-	3+	No amp
8	Carcinoma with squamous metaplasia	-	-	3+	Amp
9	Carcinoma with squamous metaplasia	-	-	1+	No amp
10	Spindle cell carcinoma	-	-	3+	No amp
11	Spindle cell carcinoma	-	-	3+	No amp
12	Spindle cell carcinoma	-	-	1+	No amp
13	Spindle cell carcinoma	-	-	3+	Amp
14	Spindle cell carcinoma	-	-	-	No amp
15	Spindle cell carcinoma	-	-	3+	Amp
16	Spindle cell carcinoma	-	-	-	No amp
17	Spindle cell carcinoma	-	-	3+	Amp
18	Spindle cell carcinoma	-	-	3+	No amp
19	Spindle cell carcinoma	2+[a]	1+[a]	3+	Amp
20	Carcinoma with heterologous elements	-	-	3+	No amp
21	Carcinoma with heterologous elements	-	-	3+	No amp
22	Matrix producing carcinoma	-	-	1+	No amp
23	Matrix producing carcinoma	-	-	-	No amp
24	Matrix producing carcinoma	-	-	3+	No amp
25	Matrix producing carcinoma	-	-	3+	No amp

[a]No *HER2* gene amplification was detected by CISH. Amp, amplification; CISH, chromogenic *in situ* hybridization; EGFR, epidermal growth factor receptor; HER, epidermal growth factor receptor; IHC, immunohistochemistry.

Immunohistochemical and chromogenic *in situ* hybridization analysis

Immunohistochemistry was performed with antibodies raised against HER2 (Herceptest®(Dako, Glosatrup, Denmark), polyclonal, 1/10 epitope retrieval solution (Dako) at 98°C, prediluted; and CB11 (Novocastra, Newcastle-upon-Tyne, UK), 2 min in a pressure cooker, 1:100) and EGFR (31G7, 1:50, Zymed (South San Francisco, CA, USA)), as previously described [34]. For Her2/neu and EGFR, the Herceptest® scoring system was applied: negative = no membrane staining or <10% of cells stained; 1+ = incomplete membrane staining in >10% of cells; 2+ = >10% of cells with weak to moderate complete membrane staining; and 3+ = strong and complete membrane staining in >10% of cells.

CISH was performed using Spot-Light amplification probes for *EGFR* (Zymed) and *HER2* (Zymed), in accordance with the manufacturer protocol and as previously described [34]. Because the interpretation guidelines for Spot-Light *EGFR* and *HER2* amplification probes have previously been validated [8,34,35], we did not use α-satellite probes for chromosomes 7 and 17, respectively. All cases were subjected to CISH for *EGFR*, and only those with HER2 grade 2+ or 3+ positivity were subjected to CISH for *HER2*[36]. Appropriate gene amplified breast tumour controls were included in each run. Each section was analyzed by two of the authors (FM and SC) on a multiheaded microscope. Only unequivocal signals were counted. Signals were evaluated at 400× and 630×, and at least 60 cells were evaluated for the presence of the *EGFR* probe. A given tumour was considered to be amplified for *EGFR* or *HER2* when more than 50% of the neoplastic cells