HER2/neu testing in primary colorectal carcinoma

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Background: Anti-HER2/neu therapy is well-established in breast and gastric carcinoma. The increased understanding of this pathway led to the identification of new promising drugs in addition to trastuzumab, offering further perspectives. The role of HER2/neu in colorectal carcinoma is controversially discussed, as discrepant data has been reported.

Methods: Here, we retrospectively assessed the prevalence of HER2/neu positivity in a large series of colorectal carcinoma, testing HER2/neu status according to current recommendations. We correlated the results to clinico-pathological data and patient survival.

Results: Overall, in 1645 primary colorectal carcinoma cases, 1.6% of the cases were HER2/neu positive. HER2/neu positivity significantly correlated with higher UICC stages ($P = 0.017$) and lymph node metastases ($P = 0.029$). In the subgroup of sigmoideal and rectal carcinomas, positive HER2/neu status was associated with T-category ($P = 0.041$) and higher UICC stages ($P = 0.022$). Although statistically not significant, HER2/neu-positive colorectal carcinomas displayed a tendency to poorer overall survival.

Conclusions: These results illustrate the importance of testing HER2/neu by approved diagnostic techniques and scoring systems. We assume that although the prevalence of HER2/neu positivity in colorectal carcinoma is low, HER2/neu testing in advanced, nodal-positive colorectal carcinoma is reasonable, offering a potential target in high risk colorectal carcinoma.

Colorectal carcinoma (CRC) is one of the most frequent cancers and still a major cause of cancer mortality, although the treatment regime has continuously improved (Ramsoekh *et al*, 2007). About 30–50% of all patients with CRC will develop metastases, which are the major culprit of CRC that lowers the 5-year survival to < 10% (Parker *et al*, 1997). In the era of personalised medicine with targeted therapy, monoclonal antibodies have become key molecules in the treatment of metastatic CRC (mCRC). Since 2004, the US Food and Drug Administration (FDA) has approved cetuximab, panitumumab and bevacizumab for the treatment of mCRC, which are humanised monoclonal antibodies directed against the epidermal growth factor receptor (EGFR; cetuximab and panitumumab) or the vascular endothelial growth factor (bevacizumab) (McIntire and Redston, 2012). With regard to cetuximab and panitumumab, treatment response largely depends on the absence of oncogenic mutations in key signalling molecules,

for example, *KRAS*, *NRAS* and *BRAF* (Custodio and Feliu, 2013), and the drug can only be administered after molecular tests exclude the presence of somatic *RAS*-mutations in mCRC (companion diagnostics). Trastuzumab is another member of this group of monoclonal antibodies, targeting the HER2/neu protein (human EGFR2). Different from cetuximab and panitumumab, treatment response to trastuzumab highly depends on the HER2/neu protein overexpression and gene amplification, which is detectable in about 15–25% of breast carcinomas and is associated with a more aggressive phenotype and poor prognosis (Ross *et al*, 2009). The HER2/neu status is assessed immunhistochemically by surgical pathologists in combination with *in situ* hybridisation using tumour tissue obtained by biopsy or resection. A validated breast cancer scoring system has to be applied prior to administration of trastuzumab on breast cancer patients. Trastuzumab has also been approved for the treatment of gastric cancer,

Table 1. Patient characteristics

	n valid	% valid	Median	Range
Whole cohort				
Number of patients	1645			
Gender				
Valid/missing	1645/0			
Male	839	51.0		
Female	806	49.0		
Age at diagnosis				
Valid/missing	1645/0		71.0	16–98
<71 years	810	49.2		
≥71 years	835	50.8		
Survival data				
Valid/missing	1645/0			
Alive	856	52.0		
Died	789	48.0		
Localisation				
Valid/missing	1561/84			
Rectum	366	23.4		
Sigmoid colon/rectum	75	4.8		
Sigmoid colon	430	27.5		
Descending colon	45	2.9		
Left flexure	38	2.4		
Transverse colon	72	4.6		
Right flexure	14	0.9		
Ascending colon	251	16.1		
Caecum	270	17.3		
Localisation (grouped)				
Valid/missing	1561/84			
Sigmoid colon-rectum	871	55.8		
Caecum-descending colon	690	44.2		
Stage				
Valid/missing	1645/0			
I	346	21.0		
IIA	477	29.0		
IIB	41	2.5		
IIC	28	1.7		
IIIA	46	2.8		
IIIB	332	20.2		
IIIC	189	11.5		
IVA	121	7.4		
IVB	65	4.0		
Stage (four groups)				
Valid/missing	1645/0			
I	346	21.0		
II	546	33.2		
III	567	34.5		
IV	186	11.3		
Stage (two groups)				
Valid/missing	1645/0			
I/II	892	54.2		
III/IV	753	45.8		
T				
Valid/missing	1645/0			
T1	78	4.7		
T2	346	21.0		
T3	948	57.6		
T4a	189	11.5		
T4b	84	5.1		

Table 1. (Continued)

	n valid	% valid	Median	Range
N				
Valid/missing	1626/19			
N0	912	56.1		
N1a	158	9.7		
N1b	180	11.1		
N1c	3	0.2		
N2a	166	10.2		
N2b	207	12.7		
N (two groups)				
Valid/missing	1626/19			
N0	912	56.1		
N+	714	43.9		
M				
Valid/missing	187/1458			
M1	187	11.4		
MX	1458	88.6		
Grading				
Valid/missing	1597/48			
G1	46	2.9		
G2	1253	78.5		
G3	298	18.7		
Lymphatic invasion				
Valid/missing	1645/0			
L0	1237	75.2		
L1	408	24.8		
Vessel invasion				
Valid/missing	1645/0			
V0	1529	92.9		
V1	116	7.1		

further necessitating testing of HER2/neu status. Interestingly, the scoring system of HER2/neu in gastric cancer is different from the breast cancer scoring system, illustrating the need to validate companion diagnostics for each specific tumour type.

HER2/neu positivity rates vary between different carcinoma subtypes and show different expression patterns. In lung carcinomas (adeno- and squamous cell carcinomas), HER2/neu positivity is found in 2.5–43% (Grob et al, 2012). In gastric cancer, 7–34% of the tumours show overexpression or amplification of HER2/neu, and addition of trastuzumab to chemotherapy improved survival in patients with advanced gastric cancer compared with chemotherapy alone (Bang et al, 2010). The role of HER2/neu overexpression/amplification in CRC is less clear and controversial expression rates, ranging from 2.7 (Marx et al, 2010) to 47.7% (Park et al, 2007), have been published. This discrepancy may be due to several factors including small study populations, evaluation of distinct subgroups and the use of different scoring systems.

Here, we retrospectively assessed the prevalence and putative clinico-pathological significance of HER2/neu overexpression/amplification in a large series of 1645 CRC-samples. The HER2/neu status was evaluated according to the current recommendations of HER2/neu testing (Hofmann et al, 2008; Ruschoff et al, 2012; Hanna et al, 2014).

MATERIALS AND METHODS

Study population. Tissue samples of primary CRC cases were obtained from 1645 patients who had undergone elective surgery

for CRC at the University Hospital Kiel (1995–2009). The clinico-pathological characteristics of the patients are summarised in Table 1. Briefly, the cohort consisted of 839 men and 806 women. The median age of the patients at the time of diagnosis was 71 years (range 16–98). Survival data was available from 1603 patients. Out of 1645 patients, 789 died during follow-up. Median follow-up for those patients still alive at the endpoint of analysis was 134.9 months. Only patients with histologically confirmed CRC and those in whom adequate tissue was available were included.

Furthermore, in 29 cases lymph node metastases were available for further analysis.

Histology and TNM classification. For histology, tissue samples were fixed in 10% neutralised formalin and embedded in paraffin. Deparaffinized sections were stained using haematoxylin and eosin. Tumours were classified according to the WHO classification. The pTNM stage was determined according to the seventh edition of the UICC guidelines.

Tissue microarray (TMA) construction. Formalin-fixed and paraffin-embedded tissue samples were used to generate TMAs as described previously (Kononen et al, 1998). Briefly, three separated, morphologically representative regions of the paraffin 'donor' block were chosen (triplets). Tissue cylinders of 1 mm diameter were punched from these areas and precisely arrayed into a new 'recipient' paraffin block using a customer built instrument (Beecher Instruments, Silver Spring, MD, USA). After completing the block construction, 4 μm sections of the resulting tumour TMA block were cut for further analysis.

HER2/neu status. The HER2/neu status was assessed by immu-nohistochemistry (IHC) using a monoclonal anti-HER2/neu-antibody (clone SP3, Thermo Fisher Scientific, Fremont, CA, USA) and the autostainer Bond Max System (Leica-Menarini, Berlin, Germany). Analysis of HER2/neu gene amplification was performed by chromogenic in situ hybridisation (CISH) using the ZytoDot 2C SPEC HER2/CEN17 Probe Kit (ZytoVision GmbH, Bremerhaven, Germany). Both, IHC and CISH, were assessed by one pathologist (BIH) blinded to clinical data.

HER2/neu expression was determined on TMA according to the consensus panel recommendations on HER2/neu scoring (Hofmann et al, 2008; Ruschoff et al, 2012; Hanna et al, 2014): HER/2neu-immunostaining was assessed according to the gastric cancer scoring system for biopsies (TMAs). Scores 3 + were reported as positive, scores 2 + as equivocal and scores 0/1 + as negative. A randomly selected subgroup of 54, 0/1 + samples as well as all cases, that scored 2 + and 3 + were further immunohistochemically studied on whole tissue sections.
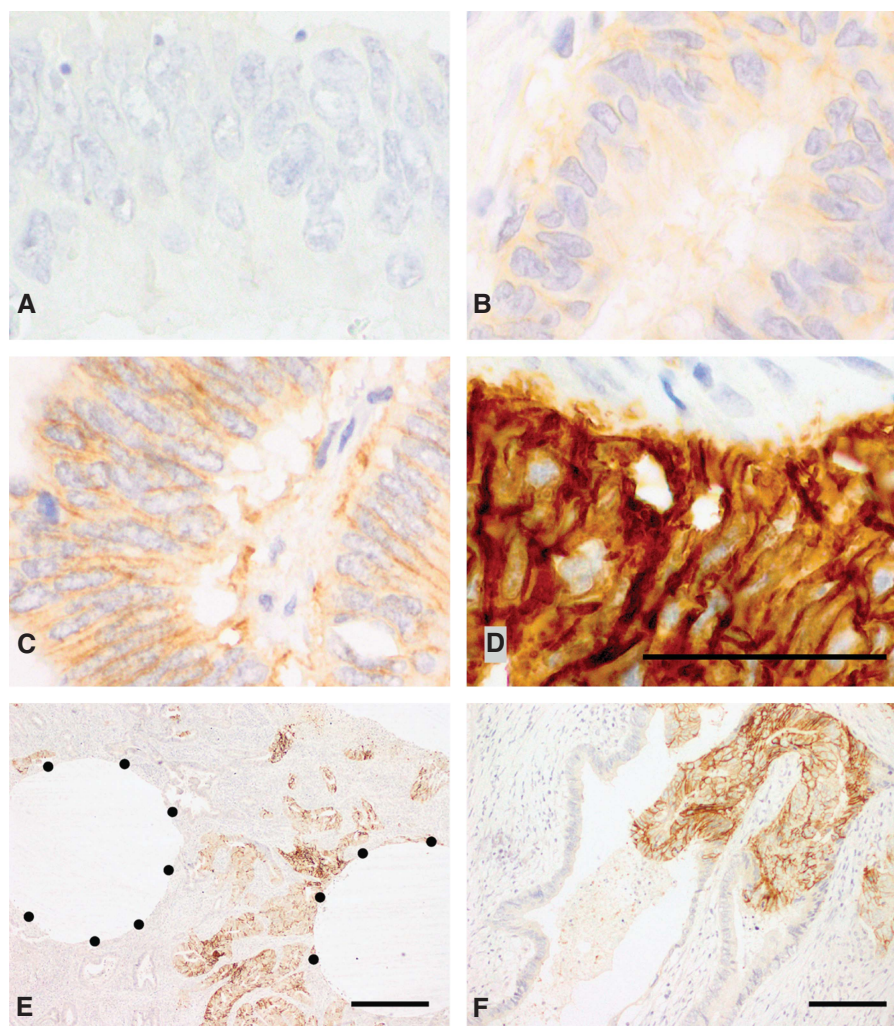

Figure 1. HER2/neu immunoreactivity. Colorectal carcinoma samples displaying absent (score 0; **A**), weak (score 1 + , **B**), moderate (score 2 + , **C**) or strong (score 3 + , **D**) HER2/neu membrane staining according to the magnification rule of current HER2/neu-testing guidelines in gastric cancer. Whole tissue section of a CRC sample showing a 'black-and-white' HER2/neu expression and corresponding TMA punch areas (**E, F**). Scale bars: **A–D**, 50 μm; **E**, 500 μm; **F**, 200 μm.

Tumours with scores 2+ were further tested by CISH on large sections. Furthermore, CISH was extended to all tumour samples scored 3+ and additionally to 10 randomly selected 0/1+ specimens. According to the gastric cancer scoring system, a minimum number of 20 tumour cells per sample were evaluated. A HER2/CEN17-ratio ⩾2 was considered positive, whereas a ratio <2 was considered negative. In case of heterogeneous gene amplification, CRC revealing >10% amplified tumour cells were counted as positive, as suggested in a review by Hanna *et al* (2014) for breast cancer.

Assessment of HER2/neu status was extended to all available lymph node metastases of 2+ and 3+ scored CRC ($n=12$) as well as randomly selected 0/1+ specimens ($n=17$).

External quality assurance. HER2/neu-testing methods, IHC as well as CISH, were certified successfully in 2013 by the quality assurance programme of the German Society of Pathology and the Bundesverband Deutscher Pathologen e.V. Furthermore, HER2/neu positivity rate of the year 2013 was monitored for breast and gastric cancer and results lay within the 99.5% confidence interval of expected results (Choritz *et al*, 2011).

Statistical analyses. All statistical analyses were carried out using SPSS version 20 (IBM Corp., Armonk, NY, USA). To determine the significance of correlation between HER2/neu status and clinico-pathological parameters, we used Fisher's exact test except for variables of ordinal scale (T, N, UICC stage and grading) where we used Kendall's τ-test. We applied the Kaplan–Meier method to calculate median survival with 95% confidence intervals. The significance of differences of median survival was calculated using the log rank test. P-values ⩽0.05 were considered to be statistically significant.

RESULTS

HER2/neu status in CRC. Immunohistochemical HER2/neu expression was available of all 1645 CRC cases. Thousand six hundred and one cases (97.3%) were HER2/neu negative (1548 samples scored 0, 53 samples scored 1+, Figure 1A and B). Thirty-five cases showed an equivocal result (2+; 2.1%; Figure 1C) and nine cases were HER2/neu positive (3+; 0.5%; Figure 1D). All cases scored 2+ and 3+, and 54 randomly selected 0/1+ cases were additionally tested on whole tissue sections. No significant change in scoring, that is, from 0/1+ to 2+ or 3+, was detected. However, four cases showed a heterogeneous HER2/neu expression ('black-and-white'-staining pattern; Figure 1E and F), revealing HER2/neu-positive areas next to areas completely devoid of HER2/neu expression.

All tumours with equivocal or positive HER2/neu IHC results underwent HER2/neu CISH. As a control, 10 randomly selected 0/1+ cases were also evaluated by CISH. Overall, 26 of 45 (57.8%) were reported positive, that is, all nine 3+ cases and 17 of 35 equivocal tumours (2+) showed HER2/neu amplification (Figure 2A and B). The four immunohistochemically heterogeneous cases showed a heterogeneous gene amplification, ranging from 30% ($n=3$) to 50% of the tumour cells ($n=1$; Figure 2C and D), and were therefore counted as HER2/neu positive. In summary, 1.6% of the whole CRC cohort showed a positive HER2/neu status (26 of 1645; Table 2).

HER2/neu status in corresponding lymph node metastases. Of 29 primary CRC cases, we evaluated the HER2/neu status of corresponding lymph node metastases in order to see if the results of the metastases were concordant with the primary tumour. This included all available lymph node metastases of HER2/neu-positive CRC ($n=12$) as well as 17 HER2/neu-negative cases. All lymph node metastases evaluated showed an identical HER2/neu profile to that of primary tumour (12 positive, 17 negative; Figure 3A–D).

Correlation of HER2/neu status with clinico-pathological data. HER2/neu positivity was significantly correlated with higher UICC stages ($P=0.020$; four groups I/II/III/IV: $P=0.017$; two groups I/II *vs* III/IV: $P=0.017$) and lymph node metastases ($P=0.033$; N0 *vs* N+: $P=0.029$; Table 3). Although statistically not significant, HER2/neu-positive tumours tended to be more frequent in colon
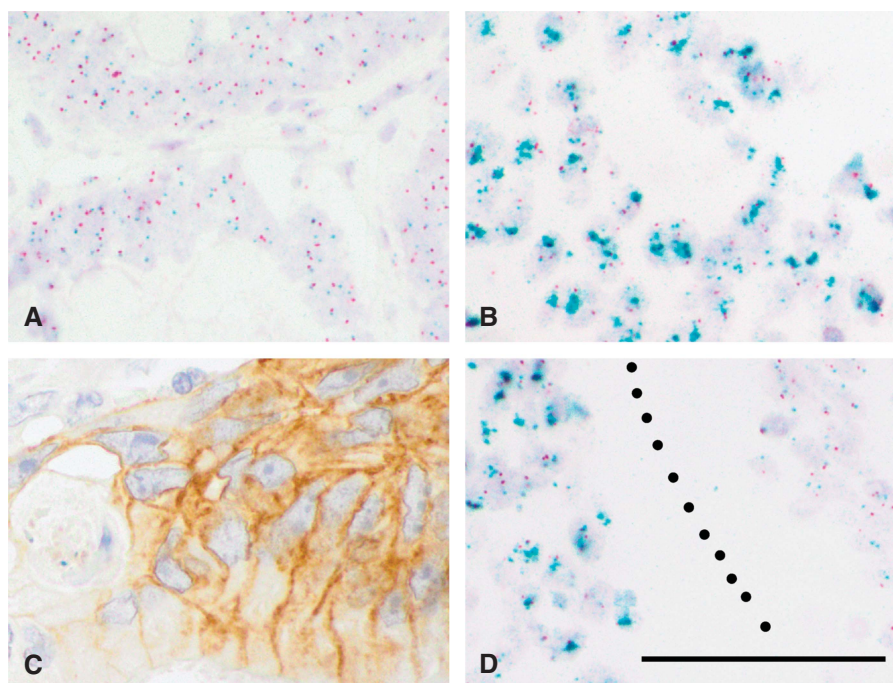

Figure 2. HER2/neu *in situ* hybridisation. Analysis of *HER2/neu* amplification by dual-color chromogenic *in situ* hybridisation (CISH) using *HER2/neu* (green) and chromosome 17 enumeration probes (red) revealing colorectal carcinoma negative (**A**) and positive (**B**) for HER2/neu amplification. Genetically heterogeneous sample examined by IHC (**C**) and CISH (**D**). Scale bar: **A**–**D**, 50 μm.

sigmoideum/rectum than in ascending/descending colon (18 *vs* 6; $P = 0.063$). Therefore we decided to re-evaluate the clinico-pathological correlations in the subgroup of colon sigmoideum/rectum tumours separately. In this subgroup, HER2/neu positivity was associated with local tumour growth (T-category; $P = 0.041$) as well as higher UICC stages ($P = 0.014$; four groups I/II/III/IV: $P = 0.022$). No further correlations of HER2/neu positivity and clinico-pathological parameters were detected.

Survival analysis. Univariate analysis revealed that HER2/neu status had no significant impact on patient overall survival ($P = 0.208$), but HER2/neu-positive CRCs tended to have a worse survival compared with -negative CRCs (Figure 4). However, patient prognosis was highly significantly associated with T-category, N-category, M-category, grading, lymph and venous vessel invasion as well as UICC stage.

Table 2. HER2/neu status in primary colorectal carcinoma			
HER2/neu (IHC)	Valid/missing	1645/0	
	0	1548	94.1
	1+	53	3.2
	2+	35	2.1
	3+	9	0.5
HER2/neu (CISH)	Valid/missing	45/1600	
	Not amplified	19	42.2
	Amplified	26	57.8
HER2/neu status	Valid/missing	1645/0	
	Negative	1619	98.4
	Positive	26	1.6

Abbreviations: CISH = chromogenic *in situ* hybridisation; IHC = immunohistochemistry.

DISCUSSION

In this study we investigated the prevalence of HER2/neu overexpression and amplification in a cohort of 1645 primary CRC. We found a positive HER2/neu status in 1.6% of all cases, with a significant association with higher UICC stages and positive nodal status. In the subgroup of colon sigmoideum and rectum, we detected that HER2/neu positivity was significantly correlated with local tumour growth (T-category) as well as higher UICC stages. Although statistically not significant, HER2/neu-positive CRCs showed a tendency to poorer overall survival. To our knowledge, this is the first study evaluating a large series of primary CRC with validated methods and a scoring system recommended by an expert panel (Hofmann *et al*, 2008; Ruschoff *et al*, 2012; Wolff *et al*, 2013; Hanna *et al*, 2014).

In CRC, studies on HER2/neu status revealed controversial results regarding frequency of HER2/neu positivity and its association with clinico-pathological data. HER2/neu positivity rates ranged from 2.7% (Marx *et al*, 2010) to 47.7% (Park *et al*, 2007). There are several explanations, for example different scoring systems as counting the percentages of positive tumour cells (Kluftinger *et al*, 1992) or applying a five-graded scoring system (Drebber *et al*, 2011). In studies with high HER2/neu positivity rates, tumours scored as 2+ (>10% of tumour cells showing moderate immunoreactivity) were counted as positive cases disregarding the *HER2/CEP17* ratio or *HER2* copy number in ISH (Kavanagh *et al*, 2009; Marx *et al*, 2010). A further difficulty in comparing immunohistochemical studies is the multiplicity of available antibodies. Five different antibodies were used in six different studies, that is, p185 (Kluftinger *et al*, 1992), 4B5 (Kavanagh *et al*, 2009; Conradi *et al*, 2013), HercepTest (Marx *et al*, 2010; Drebber *et al*, 2011) and Z4881 (Park *et al*, 2007). In addition, different preanalytical methods, variable laboratory-based procedures as fixation methods, antigen retrieval and incubation time influences testing results essentially (Blok *et al*, 2013). Taken all together, this emphasises the importance of external quality
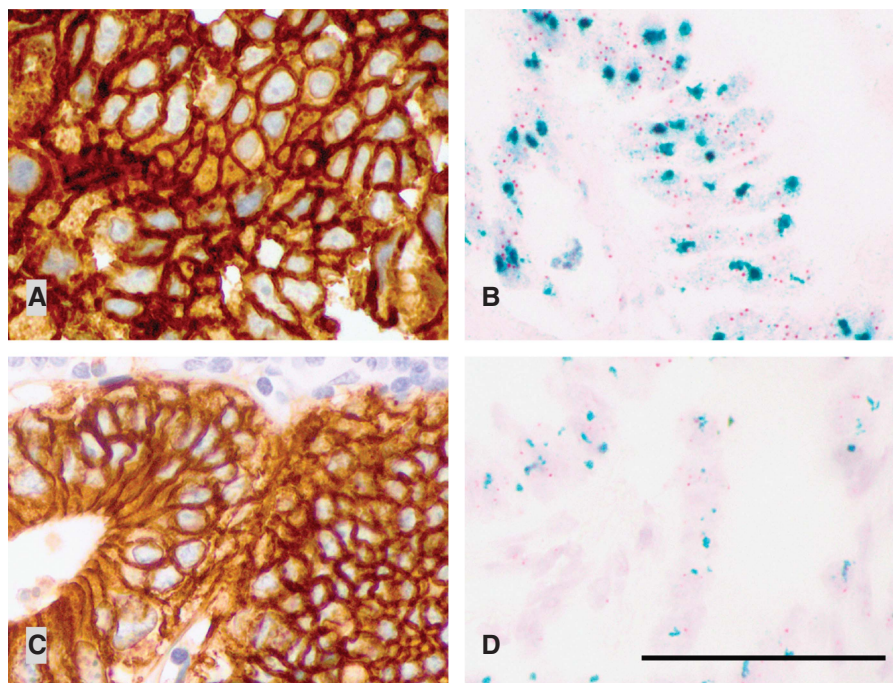

Figure 3. Primary colorectal carcinoma (A, B) and corresponding lymph node metastasis (C, D) showing the identical HER2/neu status in IHC (A, C) as well as in CISH (B, D). Scale bar: **A–D**, 50 μm.

Table 3. HER2/neu status correlated to patient characteristics				
	Correlation with HER2/neu status		Correlation with HER2/neu status	
	All cases		Rectosigmoidal	
	Negative n (%)	P-value Positive n (%)	Negative n (%)	P-value Positive n (%)
Gender		NS		NS
Male	824 (98.2)	15 (1.8)	450 (97.8)	10 (2.2)
Female	795 (98.6)	11 (1.4)	403 (98.1)	8 (1.9)
Age at diagnosis		NS		NS
<71 years	795 (98.1)	15 (1.9)	463 (97.3)	13 (2.7)
⩾71 years	824 (98.7)	11 (1.3)	390 (98.7)	5 (1.3)
Localisation		NS		NS
Rectum	358 (97.8)	8 (2.2)	358 (97.8)	8 (2.2)
Sigmoid colon/rectum	73 (97.3)	2 (2.7)	73 (97.3)	2 (2.7)
Sigmoid colon	422 (98.1)	8 (1.9)	422 (98.1)	8 (1.9)
Descending colon	45 (100.0)	0 (0.0)		
Left flexure	38 (100.0)	0 (0.0)		
Transverse colon	71 (98.6)	1 (1.4)		
Right flexure	13 (92.9)	1 (7.1)		
Ascending colon	250 (99.6)	1 (0.4)		
Caecum	267 (98.9)	3 (1.1)		
Localisation (grouped)		NS		
Sigmoid colon-rectum	853 (97.9)	18 (2.1)	853 (97.9)	18 (2.1)
Caecum-descending colon	684 (99.1)	6 (0.9)		
Stage		0.020		0.014
I	364 (99.7)	1 (0.3)	228 (100.0)	0 (0.0)
IIA	486 (98.8)	6 (1.2)	219 (97.3)	6 (2.7)
IIB	45 (100.0)	0 (0.0)	19 (100.0)	0 (0.0)
IIC	32 (100.0)	0 (0.0)	15 (100.0)	0 (0.0)
IIIA	47 (95.9)	2 (4.1)	26 (96.3)	1 (3.7)
IIIB	333 (97.9)	7 (2.1)	165 (98.2)	3 (1.8)
IIIC	187 (96.9)	6 (3.1)	92 (93.9)	6 (6.1)
IVA	122 (99.2)	1 (0.8)	61 (96.8)	2 (3.2)
IVB	69 (100.0)	0 (0.0)	28 (100.0)	0 (0.0)
Stage (four groups)		0.017		0.022
I	346 (100.0)	0 (0.0)	228 (100.0)	0 (0.0)
II	538 (98.5)	8 (1.5)	253 (97.7)	6 (2.3)
III	551 (97.2)	16 (2.8)	283 (96.6)	10 (3.4)
IV	184 (98.9)	2 (1.1)	89 (97.8)	2 (2.2)
Stage (two groups)		0.017		NS
I/II	884 (99.1)	8 (0.9)	481 (98.8)	6 (1.2)
III/IV	735 (97.6)	18 (2.4)	372 (96.9)	12 (3.1)
T		NS		0.041
T1	78 (100.0)	0 (0.0)	49 (100.0)	0 (0.0)
T2	343 (99.1)	3 (0.9)	222 (99.6)	1 (0.4)
T3	930 (98.1)	18 (1.9)	468 (97.1)	14 (2.9)
T4a	185 (97.9)	4 (2.1)	69 (97.2)	2 (2.8)
T4b	83 (98.8)	1 (1.2)	45 (97.8)	1 (2.2)
N		0.033		NS
N0	903 (99.0)	9 (1.0)	485 (98.6)	7 (1.4)
N1a	154 (97.5)	4 (2.5)	82 (96.5)	3 (3.5)
N1b	176 (97.8)	4 (2.2)	92 (100.0)	0 (0.0)
N1c	3 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
N2a	162 (97.6)	4 (2.4)	81 (96.4)	3 (3.6)
N2b	202 (97.6)	5 (2.4)	99 (95.2)	5 (4.8)
N (two groups)		0.029		NS
N0	903 (99.0)	9 (1.0)	485 (98.6)	7 (1.4)
N+	697 (97.6)	17 (2.4)	356 (97.0)	11 (3.0)

Table 3. (Continued)

	Correlation with HER2/neu status		Correlation with HER2/neu status	
	All cases		Rectosigmoidal	
	Negative n (%)	P-value Positive n (%)	Negative n (%)	P-value Positive n (%)
M		NS		NS
M1	185 (98.9)	2 (1.1)	90 (97.8)	2 (2.2)
MX	1434 (98.4)	24 (1.6)	763 (97.9)	16 (2.1)
Grading		NS		NS
G1	44 (95.7)	2 (4.3)	17 (94.4)	1 (5.6)
G2	1236 (98.6)	17 (1.4)	682 (98.3)	12 (1.7)
G3	292 (98.0)	6 (2.0)	108 (96.4)	4 (3.6)
Lymphatic invasion		NS		NS
L0	1218 (98.5)	19 (1.5)	667 (98.1)	13 (1.9)
L1	401 (98.3)	7 (1.7)	186 (97.4)	5 (2.6)
Vessel invasion		NS		NS
V0	1506 (98.5)	23 (1.5)	793 (98.1)	15 (1.9)
V1	113 (97.4)	3 (2.6)	60 (95.2)	3 (4.8)

Abbreviation: NS, not significant.

assurance programs, leading to a significant improvement of performance (Wasielewski *et al*, 2008; Choritz *et al*, 2011; Moelans *et al*, 2011). Furthermore, small study populations and cohorts of particular subgroups, for example, neoadjuvantly treated CRC (Conradi *et al*, 2013) may further contribute to the inconsistency of results.

Although we found a statistically significant association of HER2/neu positivity with higher UICC stages and nodal status, as well as T-category and nodal status in the subgroup of sigmoideal and rectal tumours, several studies failed to find an association of positive HER2-status with clinico-pathological parameters (Kountourakis *et al*, 2006; Schuell *et al*, 2006; Marx *et al*, 2010). Other authors in turn reported that HER2/neu positivity was associated with high grade tumours, higher UICC stages and positive nodal status (Kountourakis *et al*, 2006; Conradi *et al*, 2013). But again it is important to note that all of these studies used different scoring systems and/or different antibodies than what is currently recommended, which makes comparative conclusions difficult if not impossible.

In an analysis of 225 resection specimens of advanced rectal carcinomas after neoadjuvant radiochemotherapy, Conradi *et al*, (2013) reported a better cancer-specific survival (CSS; $P = 0.03$) after 5 years in HER2/neu-positive tumours than in HER2/neu-negative cases. Since in this study 5% of the rectal carcinomas, which were diagnosed as HER2/neu positive before radiochemotherapy in biopsy samples, have converted to HER2/neu-negative tumours in the resection specimen–mostly due to tumour heterogeneity–HER2/neu-positive tumour cells probably have had a better response to the neoadjuvant treatment. Therefore HER2-positive tumours may reveal a better 5-year CSS than HER2/neu-negative ones.

HER2/neu positivity had no statistically significant impact on patients' overall survival, but HER2/neu-positive tumours displayed a tendency to poorer courses, which is in line with other tumours, for example, breast cancer (Ross *et al*, 2009).

Unfortunately, we have no detailed information about the different applied therapy regimen, which may be a limitation of the presented study. But this bias is related to all subgroups, the HER2/neu-positive and the -negative ones. And the tendency of poorer overall survival of HER2/neu-positive CRC also reflects the association with advanced CRC, independent of treatment.
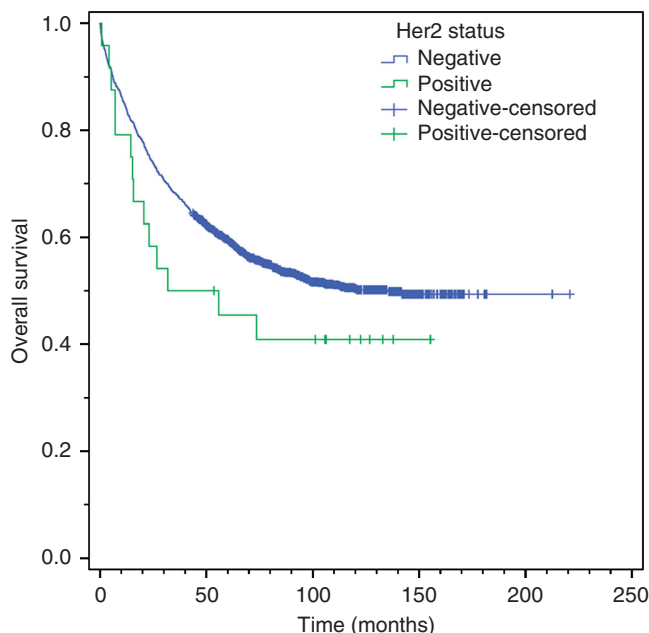

Figure 4. Overall survival. Kaplan–Meier curve depicting a tendency to poorer overall survival in HER2/neu-positive colorectal carcinoma.

In the era of personalised medicine, a multitude of new biomarkers have been published and the choice of treatment increasingly depends on the validated analysis of predictive biomarkers (companion diagnostics). Nevertheless, apparently identical tumour subtypes respond differently to the same drug, which may be–among many others–an effect of intratumoral heterogeneity. HER2/neu heterogeneity implies tumour cell subpopulations with different HER2/neu status in the same or different regions of one individual primary tumour as well as discordance between the primary tumour and its metastases. This could lead to different HER2/neu status in core biopsies compared with surgical specimens. Current guidelines for

HER2/neu assessment are different concerning the definition of HER2/neu positivity between IHC and ISH. For IHC, tumours that amounts to >10% intense membrane staining are counted as HER2/neu positive, which somewhat covers the problem of tumour heterogeneity at the level of protein expression. For ISH methods, no consistent guidelines exist (Wolff *et al*, 2013). In a review by Hanna *et al*, (2014), the authors therefore recommend that after counting 20–60 tumour cells in heterogeneous cases, an amplification in >10% of tumour cells should be considered as HER2/neu positivity. To at least minimise sampling errors using the TMA technique, we examined three core biopsies of three differently located tumour regions. In our cohort of 26 HER2/neu-amplified CRCs, four tumours showed a heterogeneous pattern with amplification in ⩾30% of tumour cells. Consequently, these samples were counted as HER2/neu positive. Tumour heterogeneity of these cases was already captured by TMA punch biopsies, but further evaluated and approved by whole tissue sections. In 29 cases, corresponding lymph node metastases were further examined, in which all cases showed identical HER2/neu status like the primary tumour. Nonetheless, further studies are needed to evaluate the potential effect of an anti-HER2/neu-therapy on tumours with heterogeneous gene expression.

In summary, in our large series of CRCs, we classified 1.6% as HER2/neu positive using current guidelines for HER2/neu testing. HER2/neu overexpression/amplification was associated with higher UICC stages and positive nodal status. Thus, future exploitations of a targeted therapy directed against HER2/neu should focus on advanced and node-positive CRCs.

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