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# ***FGFR2* amplification has prognostic significance in gastric cancer: results from a large international multicentre study**

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**Background:** In preclinical gastric cancer (GC) models, *FGFR2* amplification was associated with increased tumour cell proliferation and survival, and drugs targeting this pathway are now in clinical trials.

**Methods:** *FGFR2* FISH was performed on 961 GCs from the United Kingdom, China and Korea, and the relationship with clinicopathological data and overlap with *HER2* amplification were analysed.

**Results:** The prevalence of *FGFR2* amplification was similar between the three cohorts (UK 7.4%, China 4.6% and Korea 4.2%), and intratumoral heterogeneity was observed in 24% of *FGFR2* amplified cases. *FGFR2* amplification was associated with lymph node metastases ( $P < 0.0001$ ). *FGFR2* amplification and polysomy were associated with poor overall survival (OS) in the Korean (OS: 1.83 vs 6.17 years,  $P = 0.0073$ ) and UK (OS: 0.45 vs 1.9 years,  $P < 0.0001$ ) cohorts, and *FGFR2* amplification was an independent marker of poor survival in the UK cohort ( $P = 0.0002$ ). Co-amplification of *FGFR2* and *HER2* was rare, and when high-level amplifications did co-occur these were detected in distinct areas of the tumour.

**Conclusion:** A similar incidence of *FGFR2* amplification was found in Asian and UK GCs and was associated with lymphatic invasion and poor prognosis. This study also shows that *HER2* and *FGFR2* amplifications are mostly exclusive.

Despite a steady decline in incidence, gastric cancer (GC) is the second most common cause of cancer-related deaths worldwide (Jemal *et al*, 2011; GLOBOCAN statistics 2009). Most GC patients in the East are diagnosed with early-stage disease (Naylor *et al*, 2006; Jemal *et al*, 2011; 2008), whereas GC patients in the West present with locally advanced (inoperable), metastatic or recurrent disease and are treated by cytotoxic combination chemotherapy (Cunningham and Oliveira, 2008; Kang and Kauh, 2011; Bang, 2012). Median overall survival (OS) of patients treated with

palliative chemotherapy is 10–12 months (Cunningham and Oliveira, 2008). Targeted therapy has been investigated in this patient group, and the combination of trastuzumab with chemotherapy demonstrated a modest OS benefit in patients with *HER2*-positive advanced GC (Bang *et al*, 2010). However, no survival benefit was seen when bevacizumab, an antibody against VEGF (Van Cutsem *et al*, 2012a), everolimus, a drug targeting mTOR (Van Cutsem *et al*, 2012b), or the EGFR antibodies panitumumab or cetuximab (Lordick *et al*, 2013; Waddell *et al*,

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2012) were trialed in non-selected GC patients. Because of the poor prognosis of GC patients, there is a need to identify new potential targets and develop diagnostic tests to identify patients most likely to benefit from targeted therapies.

Fibroblast growth factor receptors (FGFR1–4) are transmembrane tyrosine kinase receptors (Eswarakumar *et al*, 2005; Turner and Grose, 2010; Brooks *et al*, 2012; Waddell *et al*, 2012). FGF binding to the monomeric receptor triggers dimerisation and transphosphorylation of tyrosine residues in the kinase domain (Eswarakumar *et al*, 2005; Katoh and Katoh, 2006; Turner and Grose, 2010; Brooks *et al*, 2012). This pathway regulates a variety of cellular functions including cell proliferation, migration and differentiation, which are fundamental to embryonic development, angiogenesis and wound healing (Eswarakumar *et al*, 2005; Fukumoto, 2008; Turner and Grose, 2010; Brooks *et al*, 2012).

Dysregulation of the FGFR signalling pathway due to receptor overexpression, gene amplification, mutation or aberrant transcriptional regulation is associated with cancer development and progression in multiple myeloma and cancers of the breast, bladder, lung, endometrium and prostate (Jang *et al*, 2001; Davies *et al*, 2005; Grose and Dickson, 2005; Stephens *et al*, 2005; Katoh, 2010; Turner and Grose, 2010; Brooks *et al*, 2012).

In preclinical models of GC, *FGFR2* amplification was associated with increased tumour cell proliferation and survival, and conferred sensitivity to drugs targeting this pathway, such as the FGFR selective small molecule inhibitors AZD4547 and BGJ398, and anti-FGFR2 antibodies (Bai *et al*, 2010; Zhao *et al*, 2010; Gavine *et al*, 2012; Guagnano *et al*, 2012; Zhang *et al*, 2012; Xie *et al*, 2013). Studies have reported *FGFR2* amplification in up to 10% of Asian GC patients (Deng *et al*, 2012; Jung *et al*, 2012; Matsumoto *et al*, 2012), and *FGFR2* amplification was recently described in Western GC cohorts (Deng *et al*, 2012; Dulak *et al*, 2012; Nadauld *et al*, 2012). Each of these studies employed a different platform to assess gene amplification, including RT-PCR, fluorescence *in situ* hybridisation (FISH) and SNP arrays.

This study used FISH to compare the frequency of *FGFR2* amplification in large series of GCs from UK, Chinese and Korean patients, the overlap of *FGFR2* and *HER2* amplification, and the association of *FGFR2* amplification with clinicopathological variables and OS.

## MATERIAL AND METHODS

**Patient cohorts.** The UK, Chinese and Korean GC cohorts consisted of 408, 197 and 356 patients, respectively, with sporadic gastric adenocarcinoma who underwent surgical resection at Leeds General Infirmary, the United Kingdom (1970–2004), Shanghai Renji Hospital, China (2007–2010) and Seoul National University Hospital, South Korea (1996), respectively (Table 1). Clinical outcome was determined from date of surgery until last seen or mortality status obtained in 2009, 2011 and 2003, for the UK, Chinese and Korean cohorts, respectively. At the end of the study period, 73% and 33% of UK and Chinese patients had died. Median (range) follow-up time was 1.7 years (0–20.5 years), 2.4 years (1 month–4.6 years) and 5.5 years (2 months–8 years) for UK, Chinese and Korean cohorts, respectively.

**Tissue microarray construction.** Haematoxylin/eosin-stained sections of resected specimens were reviewed, and blocks with the highest tumour cell density selected for tissue microarray (TMA) construction. TMAs were constructed by random sampling of 3–6, 0.6 mm cores from each tumour and three cores from matched normal mucosa (UK cohort), one 1-mm core from each tumour (Korean cohort), two to four 0.6-mm cores from each tumour and two from matched normal mucosa (Chinese cohort). Four (Korean/Chinese) or 5  $\mu$ m (UK) sections were cut from each

TMA for gene copy-number analysis. Full sections were cut from 26 UK *FGFR2*-amplified GC specimens to assess amplification heterogeneity within individual tumours. TMA and full sections were quality controlled by an experienced histopathologist.

**FGFR2 FISH.** The *FGFR2* FISH probe was generated in house by AstraZeneca by directly labelling BAC RP11-62L18 (Invitrogen, Grand Island, New York, USA) DNA with Spectrum Red (ENZO, Exeter, UK, 02N34-050) using a nick translation-based method (Abbott Park, IL, USA, 07J00-001) according to the manufacturer's instructions. Pericentromeric Spectrum Green labelled chromosome 10 probe (CEP10, Vysis, 32-132010) was used as an internal control. FISH was performed as described previously (Xie *et al*, 2013). Sections were deparaffinized and pretreated using the SpotLight Tissue Kit (Invitrogen, 00-8401) according to the manufacturer's instructions. Sections and *FGFR2*/CEP10 probes were co-denatured at 80 °C for 5 min and hybridised at 37 °C for 48 h. Excess probe was removed with post-hybridisation wash buffer (0.3% NP40/1  $\times$  SSC) at 75.5 °C for 5 min, then 2  $\times$  SSC at room temperature for 2 min. Sections were counterstained with 0.3  $\mu$ g ml<sup>-1</sup> DAPI (Vector, H-1200) and coverslipped. *FGFR2* and CEP10 signals were scored under a fluorescence microscope (Olympus, Center Valley, PA, USA, BX61). Scoring was adopted from (Varela-Garcia, 2006). Fifty nuclei were evaluated/case. *FGFR2* gene copy-number and *FGFR2*/CEP10 ratio was classified as follows: *FGFR2* amplification (score 6): *FGFR2*/CEP10 ratio  $\geq 2$  or *FGFR2* gene clusters in  $\geq 10\%$  tumour cells; high polysomy (score 5): *FGFR2*/CEP10 ratio  $< 2$  and  $\geq 4$  copies of *FGFR2* in  $\geq 40\%$  tumour cells; low polysomy (score 4): *FGFR2*/CEP10 ratio  $< 2$  and  $\geq 4$  copies of *FGFR2* in 10–39% tumour cells; high trisomy (score 3): *FGFR2*/CEP10 ratio  $< 2$  and 3 copies of *FGFR2* in  $\geq 40\%$  tumour cells and  $< 10\%$  tumour cells having  $\geq 4$  copies of *FGFR2*; low trisomy (score 2): *FGFR2*/CEP10 ratio  $< 2$  and 3 copies of *FGFR2* in 10–39% tumour cells and  $< 10\%$  tumour cells having  $\geq 4$  copies of *FGFR2*; disomy (score 1): two copies of *FGFR2* in 90% of tumour cells. Scoring was performed independently by two observers at AstraZeneca.

**Assessment of FGFR2 amplification heterogeneity.** Intratumoral *FGFR2* amplification heterogeneity was assessed in TMA and full sections from 26 UK cases with *FGFR2* amplification, and was defined as the presence of areas with different FISH scores within the same tumour in full sections and presence of different FISH scores in cores from the same tumour in TMA sections. Scoring was performed independently by two observers.

**HER2 FISH.** *HER2*/CEP17 probe (Vysis, 30-161060) was used according to the manufacturer's instructions. Fifty tumour nuclei were scored/case. Tumours with an average *HER2* gene copy number  $> 6$  or a *HER2*/CEP17 ratio  $\geq 2$  were defined as *HER2* amplified. Scoring was performed independently by two observers.

**Combined FGFR2 and HER2 FISH.** To detect *FGFR2* and *HER2* copy number simultaneously, a four-colour FISH probe was generated. The above *FGFR2*/CEP10 probes were combined with a *HER2* probe generated by labelling BAC RP11-94L15 DNA (Invitrogen) with Spectrum Gold (ENZO, ENZ-42843) and a CEP17 Spectrum Aqua probe (Vysis, 32-111017) as internal control using experimental conditions described for *FGFR2* FISH. This analysis was performed only in cases identified as *FGFR2* and *HER2* amplified in the TMA. Scoring was performed independently by two observers.

**Statistical analysis.** Data from each cohort were analysed individually. The following variables were used for statistical analysis: tumour histology type (Laurén classification, (Lauren, 1965)) tumour grade of differentiation (WHO classification, (Hamilton and Aaltonen, 2000)) depth of invasion (pT), lymph node status (pN), distant metastasis status (pM), resection margin

Table 1. Comparison of the clinicopathological characteristics between UK, Chinese and Korean gastric cancer cohorts

Characteristic	UK cohort (n = 408)		Chinese cohort (n = 197)		Korean cohort (n = 356)	
	n	%	n	%	n	%
<b>Age (years)</b>						
Median	70		62		59	
Range	13–96		18–87		28–82	
<b>Gender</b>						
Male	255	62.5	133	67.5	247	69.4
Female	153	37.5	64	32.5	109	30.6
<b>Grade of differentiation</b>						
G1	45	11.0	3	1.5	20	5.6
G2	103	25.2	40	20.3	173	48.6
G3	250	61.3	133	67.5	161	45.2
G4	1	0.2	21	10.7	2	0.6
Unknown	9	2.2	0	0.0	0	0.0
<b>Laurén subtype</b>						
Intestinal	244	59.8	66	33.5	170	47.8
Diffuse	96	23.5	87	44.2	172	48.3
Mixed	64	15.7	44	22.3	14	3.9
Unknown	4	1.0	0	0.0	0	0.0
<b>Stage</b>						
I	115	28.2	15	7.6	121	34.0
II	81	19.9	45	22.8	83	23.3
III	151	37.0	100	50.8	89	25.0
IV	60	14.7	37	18.8	63	17.7
unknown	1	0.2	0	0.0	0	0.0
<b>Depth of invasion (pT)</b>						
T1	56	13.7	7	3.6	67	18.8
T2	140	34.3	20	10.2	167	46.9
T3	201	49.3	157	79.7	114	32.0
T4	11	2.7	13	6.6	8	2.2
<b>Lymph node status (pN)</b>						
N0	136	33.3	51	25.9	121	34.0
N1	163	40.0	88	44.7	127	35.7
N2	68	16.7	34	17.3	58	16.3
N3	40	9.8	24	12.2	50	14.0
<b>Distant metastasis (pM)</b>						
M0	391	95.8	182	92.4	334	93.8
M1	17	4.2	15	7.6	22	6.2
<b>Adjuvant chemotherapy</b>						
No	408	100.0	36	18.3	146	41.0
Yes	0	0.0	129	65.5	188	52.8
Unknown	0	0.0	32	16.2	22	6.2

status (R) and stage (TNM classification sixth edition) (Sobin and Wittekind, 2002).

Chi-square tests were used to compare clinicopathological characteristics between cohorts. For association of *FGFR2* amplification with clinicopathological characteristics, logistic regression models were fitted in both univariate and multivariate analysis, and *P*-values were computed by log-likelihood Chi-square test. In multivariate models, age, gender and factors that showed

significant association in univariate analysis (pN and grade of differentiation) were included.

For OS, patients were categorised by *FGFR2* status into amplified (FISH score 6) and non-amplified (FISH score 1–5). Data were analysed using Kaplan–Meier (Kaplan and Meier, 1958) and log-rank statistics. Univariate and multivariate Cox proportional hazard models were fitted to evaluate *FGFR2* status including variables for age, gender, grade of differentiation and stage (Sobin and Wittekind,

2002). Statistical tests were two-sided,  $P < 0.05$  was considered significant. Analyses were carried out using R (version 2.10.1).

All studies were performed with the approval of Local Research Ethics committees and were conducted in accordance with the Declaration of Helsinki.

## RESULTS

Comparison of patient characteristics and clinicopathological variables between GC cohorts are detailed in Table 1. UK patients were significantly older at the time of diagnosis compared with Asian cohorts (UK/Chinese  $P < 0.0001$ , UK/Korean  $P = 0.0003$ ). The frequency of intestinal type GC was significantly higher in the UK cohort compared with Asian cohorts (UK/Chinese  $P < 0.0001$ , UK/Korean  $P < 0.0001$ ). The frequency of well, moderately and poorly differentiated GC was different between all cohorts (UK/Chinese  $P < 0.0001$ , UK/Korean  $P < 0.0001$ , Chinese/Korean  $P < 0.0001$ ). There was a significant difference in disease stage distribution between cohorts, with stage III disease more common in Chinese patients (UK/Chinese  $P < 0.0001$ , Chinese/Korean  $P < 0.0001$ ). All UK patients were treated by surgery alone, while 66% of Chinese and 53% of Korean patients received adjuvant chemotherapy. No patients received neoadjuvant chemotherapy or radiotherapy.

**FGFR2 copy number.** Results were obtained from a total of 961 cases (Table 2). *FGFR2* amplification frequency was 7.4%, 4.6% and 4.2% in the UK, Chinese and Korean cohorts, respectively, and did not differ significantly (UK/Chinese  $P = 0.258$ , UK/Korean  $P = 0.092$ , Chinese/Korean  $P = 0.983$ , UK/Chinese/Korean  $P = 0.586$ ). *FGFR2* polysomy was observed in 35.1%, 44.2% and 21.3% of UK, Chinese and Korean cohorts, respectively, and was significantly lower in the Korean cohort (UK/Korean  $P < 0.0001$ , Korean/Chinese  $P < 0.0001$ , UK/Chinese/Korean  $P < 0.0001$ ). Correspondingly, there was a significantly higher frequency of *FGFR2* disomy in the Korean cohort (UK/Korean  $P < 0.0001$ , Korean/Chinese  $P < 0.001$ , UK/Chinese/Korean  $P < 0.001$ ).

**Association of *FGFR2* copy number with clinicopathological parameters.** The relationship between *FGFR2* amplification and

clinicopathological parameters was analogous between the cohorts; hence, a combined analysis of all three cohorts is presented. Univariate and multivariate analyses of all 961 patients showed that *FGFR2* amplification was significantly more common in patients with higher pN category ( $P < 0.0001$ ). For the analysis of grade of tumour differentiation, the small number of patients with grade 4 tumours ( $n = 24$ ) were grouped together with grade 3 tumours. Statistical analysis showed that the prevalence of *FGFR2* amplification was significantly lower in grade 2 (moderately differentiated) tumours compared with grade 1 (well differentiated) or grade 3 and 4 (poorly differentiated/undifferentiated) tumours ( $P < 0.01$ ). There was no association of *FGFR2* amplification with age, gender, histological subtype (Table 3) or tumour location ( $P = 0.716$ , data not shown).

**Association of *FGFR2* copy number with overall survival.** Type of treatment and other patient characteristics differed significantly between the three cohorts. Therefore, the relationship between *FGFR2* FISH status and overall survival was analysed separately. Median OS was significantly shorter in patients with *FGFR2* amplified GC compared with patients with *FGFR2* non-amplified GC in UK ( $P < 0.0001$ ) and Korean ( $P = 0.0073$ ) cohorts by univariate analysis (Table 4). A similar trend was observed for the Chinese cohort but did not achieve significance ( $P = 0.0646$ ; Figure 1). Multivariate survival analysis from the Cox proportional hazard model adjusting for age, gender and grade of tumour differentiation confirmed *FGFR2* amplification status as an independent prognostic marker in the UK cohort ( $P = 0.0002$ ; Table 4).

To assess whether there was an effect of adjuvant chemotherapy treatment on the prognostic value of *FGFR2* amplification, a subset analysis was performed in patients treated by surgery only vs patients treated by adjuvant chemotherapy after surgery. Results from Korean and Chinese cohorts were pooled for this analysis. *FGFR2* amplification was similarly predictive for shorter OS in patients treated with adjuvant chemotherapy ( $P = 0.002$ ) and the surgery only group ( $P = 0.03$ ).

UK and Korean patients with *FGFR2* polysomy GC (score 4–5) had a significantly shorter OS when compared with others with *FGFR2* non-amplified disease (score 1–3) in univariate analysis

Table 2. *FGFR2* FISH analysis

	UK cohort (n = 408)		Chinese cohort (n = 197)		Korean cohort (n = 356)	
<b>FGFR2 FISH score</b>	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>
Disomy	142	34.8	39	19.8	190	53.4
Low trisomy	65	15.9	51	25.9	63	17.7
High trisomy	28	6.9	11	5.6	12	3.4
Low polysomy	87	21.3	51	25.9	66	18.5
High polysomy	56	13.7	36	18.3	10	2.8
Gene amplification	30	7.4	9	4.6	15	4.2
	<b>P-values<sup>a</sup> (univariate)</b>					<b>P-values<sup>b</sup> (multivariate)</b>
<b>FGFR2 FISH score</b>	<b>UK vs Chinese</b>		<b>UK vs Korean</b>		<b>Chinese vs Korean</b>	<b>UK vs Chinese vs Korean</b>
1–5 vs 6	0.2584		0.0921		0.9827	0.5855
1–3 vs 4–5	0.0664		$8.96 \times 10^{-8}$		$1.90 \times 10^{-8}$	$3.73 \times 10^{-9}$

Abbreviations: FGFR2 = fibroblast growth factor receptor 2; FISH = fluorescent *in situ* hybridisation.

<sup>a</sup>P-values contrasted for a given pair of cohorts are computed from  $\chi^2$ -test by collapsing score 1–5 subjects in 1–5 vs 6 comparison and score 1–3 and score 4–5 subjects, respectively, in 1–3 vs 4–5 comparison.

<sup>b</sup>Multivariate P-values are from a log-likelihood ratio test after adjusting for age, gender, stage, grade and Lauren subtype.

Table 3. Comparisons of clinicopathological characteristics by *FGFR2* amplification status

Characteristics	<i>FGFR2</i> non-amplified (FISH 1–5)		<i>FGFR2</i> amplified (FISH 6)		P-value (uni)	P-value (multi) <sup>a</sup>
	n	%	n	%		
<b>Age</b>						
<median age	444	95	25	5	0.1873	
≥median age	462	94	29	6		
<b>Gender</b>						
Male	602	95	33	5	0.7480	
Female	305	94	21	6		
<b>Grade</b>						
1	64	94	4	6	0.0176	0.0073
2	309	98	7	2		
3	501	92	43	8		
4	24	100	0	0		
<b>Laurén subtype</b>						
Intestinal	459	96	21	4	0.1793	0.2248
Diffuse	329	93	26	7		
Mixed	115	94	7	6		
<b>Stage</b>						
1, 2	442	96	18	4	0.1021	0.1442
3, 4	464	93	36	7		
<b>T</b>						
1	126	97	4	3	0.5671	0.4805
2	306	94	21	6		
3	444	94	28	6		
4	31	97	1	3		
<b>N</b>						
0	304	99	4	1	1.22 × 10 <sup>-5</sup>	<0.0001
1	356	94	22	6		
2	146	91	14	9		
3	100	88	14	12		
<b>M</b>						
0	856	94	51	6	0.4456	0.4281
1	51	94	3	6		

<sup>a</sup>P-value is calculated from logistic regression adjusting for age and gender.

(UK cohort *P* = 0.0427; Korean cohort *P* = 0.0434) that was not significant in multivariate analysis adjusting for age, gender, grade of differentiation and stage (Table 4). No relationship was seen in the Chinese cohort.

**FGFR2 amplification heterogeneity.** Seven of 29 UK *FGFR2* amplified GCs (24.1%) displayed intratumoral heterogeneity within TMAs. Tissue sections were unavailable for 3/29 GCs. Analysis of full sections confirmed *FGFR2* amplification in 23/26 cases and intratumoral heterogeneity in six cases, which also showed heterogeneity in the TMA study. Three cases found to be *FGFR2* amplified in the TMA study showed no evidence of *FGFR2* amplification in full sections, most likely related to *FGFR2* amplification heterogeneity.

**FGFR2 and HER2 amplification are predominantly exclusive.** *HER2* FISH results were available from 204 and 338 of Chinese and Korean GCs; amplification was present in 14.7% and 8% of GCs,

respectively. Co-amplification of *HER2* and *FGFR2* was present in one Chinese GC but not detected in any GCs from the Korean cohort. In the UK cohort, the frequency of *HER2* and *FGFR2* co-amplification was investigated in 26 full sections of GCs found to be *FGFR2* amplified in the TMA study. Two of the 26 UK GC cases showed *FGFR2* and *HER2* co-amplification. In order to assess whether co-amplification was present in the same cell, *FGFR2/HER2* four-colour FISH was performed. High-level amplifications of *HER2* and *FGFR2* (*FGFR2* average copy number > 100 and *HER2/CEP17* ratio > 10) were found in both UK GCs, and *HER2* amplification was seen in tumour cells that were *FGFR2* non-amplified and vice versa (Figure 2D). In contrast, *HER2* and *FGFR2* amplifications occurred in the same tumour cell in the single Chinese GC, but amplifications were of low level and only just satisfied the minimal amplification criteria (*FGFR2* average copy number 5.52 and *HER2/CEP17* ratio 2.26) (Supplementary Figure 1).



Table 4. Analysis of prognostic value of FGFR2 amplification (FISH 6) or polysomy (FISH 4-5) status for overall survival (Cox proportional hazard model)

FGFR2 FISH status	UK cohort						Chinese cohort						Korean cohort					
	Median (95% CI)		Univariate		Multivariate		Median (95% CI)		Univariate		Multivariate		Median (95% CI)		Univariate		Multivariate	
	(year)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	(year)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	(year)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	
Amp vs non amp	Non amp	1.9 (1.5~2.8)	2.54 (1.72~3.74)	<0.0001	2.33 (1.56~3.46)	0.0002*	3.92 (2.42~NA)	2.26 (1.04~4.91)	0.0646	1.98 (0.9~4.35)	0.1176*	(6.17~NA)	2.49 (1.38~4.48)	0.0073	1.87 (1.02~3.42)	0.0612 <sup>b</sup>		
	Amp	0.45 (0.4~1.0)				1.5 (0.92~NA)						1.83 (0.92~NA)						
1-3 vs 4-5	1-3	2.1 (1.6~3.4)	1.29 (1.01~1.64)	0.0427	1.09 (0.85~1.41)	0.4954*	(2.08~NA)	1.18 (0.76~1.85)	0.4605	1.19 (0.75~1.89)	0.4498 <sup>b</sup>	(7.5~NA)	1.44 (1.02~2.04)	0.0434	1.3 (0.92~1.85)	0.1450 <sup>b</sup>		
	4-5	1.7 (1.1~2.8)				3.33 (2.08~NA)						3.75 (3.0~NA)						

Abbreviation: NA = not available.  
<sup>a</sup>Adjusted for age, gender, grade and stage.  
<sup>b</sup>Adjusted for age, gender and stage.

DISCUSSION

FGFR amplification has been reported in various cancers, including FGFR1 amplification in ER + breast cancer and squamous cell lung cancer, and FGFR2 amplification in triple negative breast cancer and GCs (Andre *et al*, 2009; Turner *et al*, 2010a, b; Weiss *et al*, 2010; Deng *et al*, 2012; Dulak *et al*, 2012; Jung *et al*, 2012; Matsumoto *et al*, 2012; Nadauld *et al*, 2012). Hence, there is significant interest in FGFR2 as a therapeutic target for FGFR2-amplified GCs, and clinical trials of FGFR inhibitors are ongoing.

There is variability in the reported incidence of FGFR2 overexpression in GC; FGFR2 protein overexpression was reported to be 30%–40% by immunohistochemistry, (Hattori *et al*, 1996) while the incidence of FGFR2 amplification varies from 3 to 10% (Mor *et al*, 1993; Hara *et al*, 1998; Deng *et al*, 2012; Dulak *et al*, 2012; Jung *et al*, 2012; Matsumoto *et al*, 2012). Gene copy-number evaluation in solid tumour cells by FISH is widely accepted as a ‘gold-standard approach’ for clinical application, and the current study is the largest to date assessing FGFR2 amplification by FISH in patients with resectable GC from three different countries, Korea, China and UK. The prevalence of FGFR2 amplification was investigated in a total of 961 GCs and was 7.4%, 4.6% and 4.2% in UK, Chinese and Korean GCs, respectively, with no significant difference of incidence between cohorts. Our results are similar to those reported previously for Korean (Jung *et al*, 2012) and Japanese cohorts (Matsumoto *et al*, 2012). In addition to FGFR2 amplification, we found a significant incidence of FGFR2 polysomy in GC, which was significantly higher in UK (35.1%) and Chinese (44.2%) GC cohorts than in the Korean cohort (21.3%). Further work is required to demonstrate whether FGFR2 polysomy is related to tumour growth, survival and sensitivity to therapeutic intervention.

The observation that FGFR2 amplification was significantly associated with lymph node disease suggests that this molecular aberration may contribute to the development of metastasis. An association between FGFR2 amplification and lymphatic invasion was recently reported (Jung *et al*, 2012).

It has been reported that FGFR2 amplification is more frequently found in diffuse type GC compared with intestinal type GC (Nakatani *et al*, 1990). In contrast, our study did not find an association of FGFR2 amplification with histological subtype according to the Laurén classification, which is in agreement with findings recently reported for another Korean GC cohort (Jung *et al*, 2012). This contrasts with HER2 gene amplification, which is associated with the intestinal subtype of gastric cancer (Bang, 2012).

The current study suggests that FGFR2 amplification is a molecular factor related to poor prognosis in patients with resectable GC, irrespective of ethnic origin and irrespective of the underlying significant differences in clinicopathological parameters, survival and treatment between cohorts from Asia and the United Kingdom. The potential usefulness of FGFR2 amplification as a predictive factor for response to FGFR2 targeting therapies remains to be evaluated in patients with GC.

Amplification of HER2 has been identified in 6–35% of patients with GC (Bang, 2012); however, the majority of recent studies have reported incidences of 6–13% (Okines *et al*, 2012; Terashima *et al*, 2012; Narita *et al*, 2013; Warneke *et al*, 2013; Aizawa *et al*, 2014). HER2 amplification has led to the successful development and approval of trastuzumab in patients with GC (Bang *et al*, 2010). In the present study, we assessed the overlap between FGFR2 and HER2 amplification. Only 3/50 FGFR2-amplified samples (3/961 of total population samples) were also HER2 amplified, confirming that HER2 and FGFR2 amplifications are usually mutually exclusive (Deng *et al*, 2012). Interestingly, low-level HER2 and FGFR2 amplifications were detected in the same tumour cells in

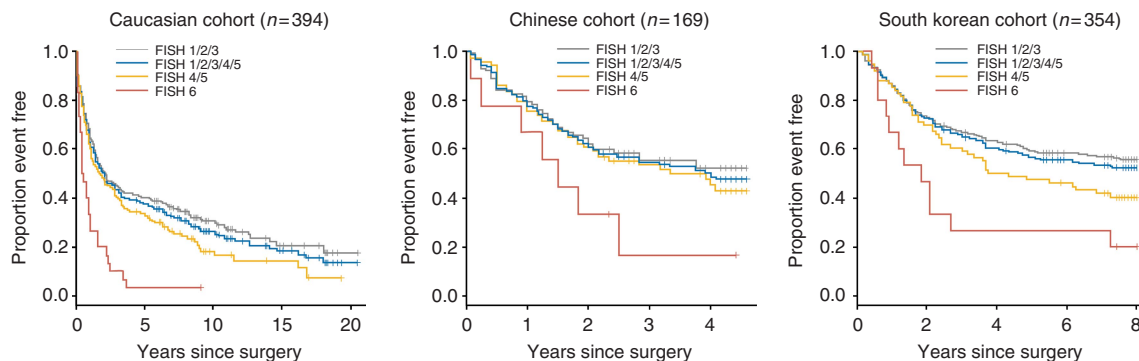


Figure 1. Kaplan–Meier OS analysis using *FGFR2* FISH score in three separate patient cohorts. Median OS and 95% CI for pairwise comparisons are provided.

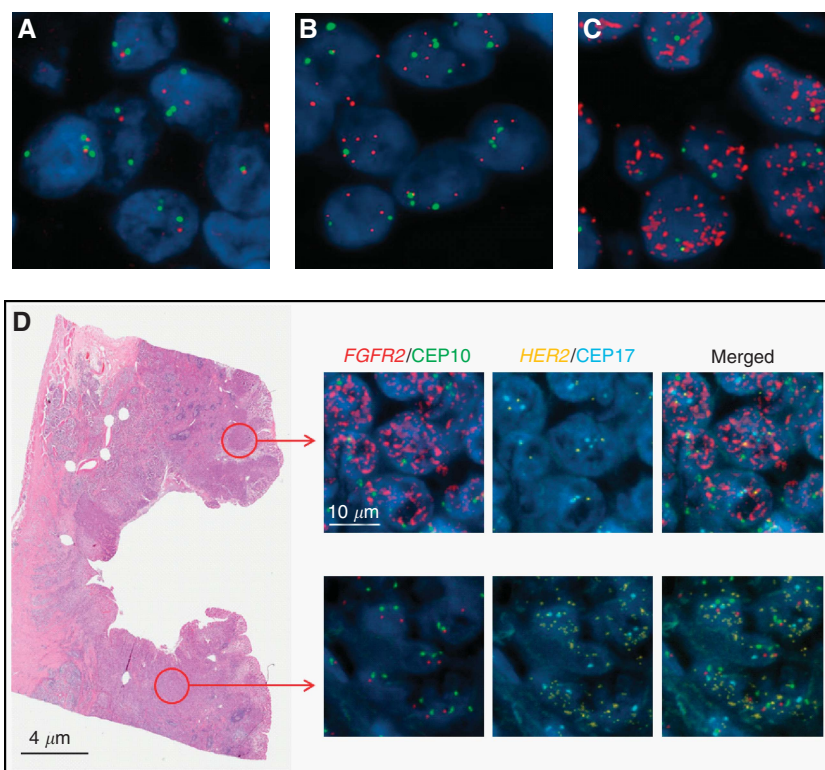


Figure 2. Dual-colour FISH shows *FGFR2* copy-number normal (A), copy-number increase (B) and amplification (C). Red and green signals highlight *FGFR2* gene and centromere 10 probes, respectively. Four-colour FISH reveals distinct tumour regions with either *FGFR2* or *HER2* amplification (D). Gold and aqua probes highlight *HER2* and centromere 17.

one Chinese GC, whereas in two UK GCs tumour cells with high-level *HER2* amplification were located in a different area to tumour cells with high-level *FGFR2* amplification. The findings in these latter two GCs suggest that *FGFR2*- and *HER2*- amplified tumour cells may have developed from different tumour cell clones with differing genetic characteristics. In these patients, combined *FGFR2* and *HER2* inhibitor therapies might be required for durable and potent antitumour responses.

We found evidence for intratumoural heterogeneity of *FGFR2* amplification in about 25% of GCs, indicating that there is a potential for missing areas of amplification especially when analysis is performed on small biopsies, rather than sections from resection specimens. Similar intratumoural heterogeneity has been reported for *HER2* amplification in GC, leading to a recommendation that multiple biopsies should be assessed to determine *HER2* status of a tumour.

In conclusion, this is the largest study of *FGFR2* FISH in GC and the first study to compare the incidence of *FGFR2* amplification in UK and Asian cohorts, demonstrating a similar incidence across cohorts. Furthermore, our data show that *FGFR2* amplification is associated with lymph node metastasis and related to poor OS. Overall, this study suggests that *FGFR2* may represent an attractive therapeutic target in a subgroup of GCs, irrespective of ethnicity, and FISH methodology could be used for patient selection.

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## CONFLICT OF INTEREST

YJB and SAI have received honoraria and research funding from, and acted within advisory roles for, AstraZeneca. WHK and HIG have received research funding from AstraZeneca. XS, PZ, PRG, SM, CW and EK are employees of and own stocks in AstraZeneca. The remaining authors declare no conflict of interest.

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