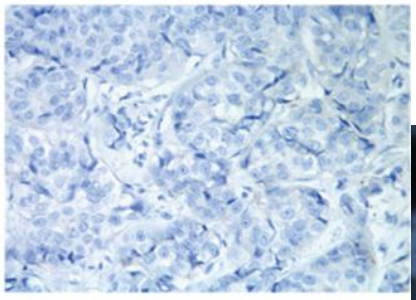
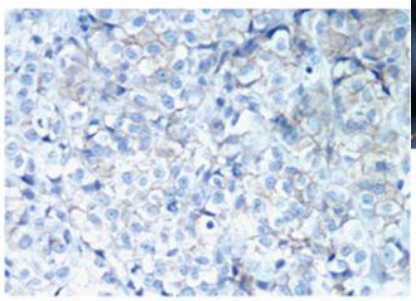
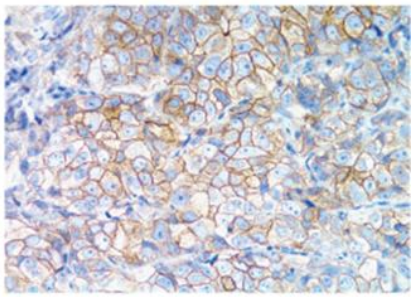
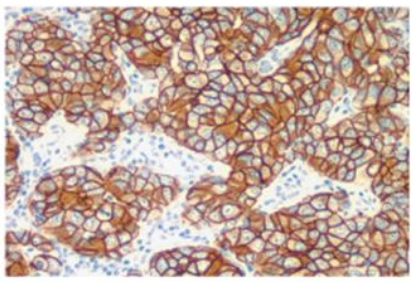
A)

	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

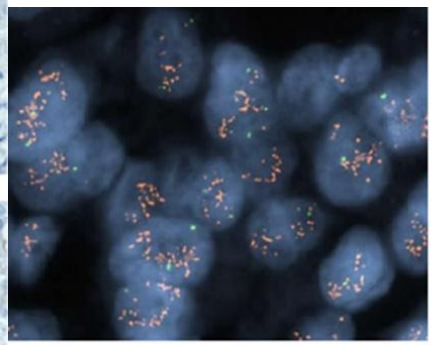
HER2=human epidermal growth factor receptor 2 (also known as ERBB2).

Table 1: **Immunohistochemistry scoring for HER2 in gastric and gastro-oesophageal junction cancer, by type of diagnostic specimen**

B)

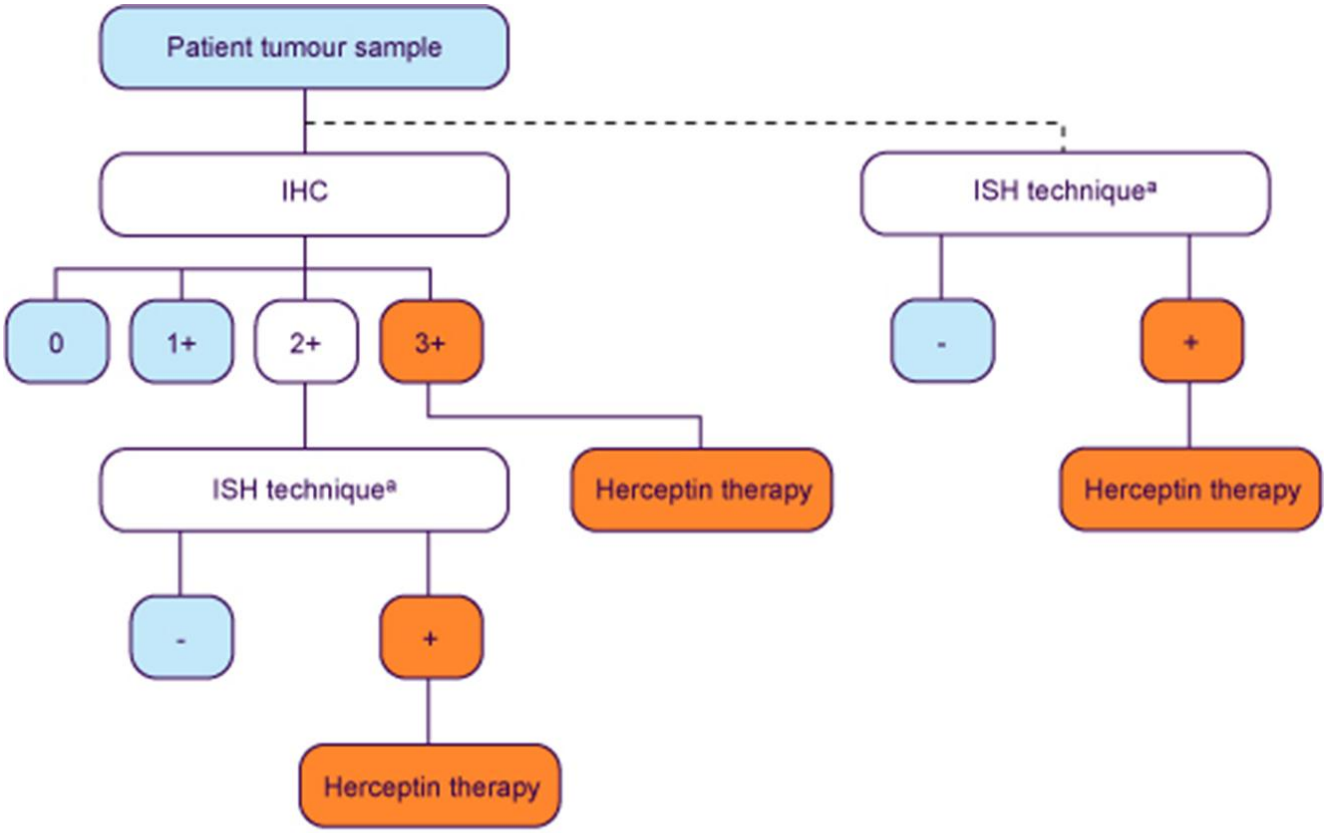
Staining characteristics	Score/ classification	Example staining patterns
No staining/ membrane staining in <10% of tumour cells	0/negative	
Faint/barely perceptible membrane staining in >10% of tumour cells; cells are only stained in part of their membrane	1+/negative	
Weak or moderate complete staining in >10% of tumour cells	2+/equivocal → Retest with ISH	
Strong complete membrane staining in >10% of tumour cells	3+/positive	

[a]Although other commercial IHC-testing kits are available, HercepTest™ is the most commonly used.
IHC, immunohistochemistry; ISH, *in situ* hibridisation



FISH+: ratio of HER2 (orange) signals to CEP17 (green) signals is ≥2 (PathVysion™ kit, PathVysion)

C)



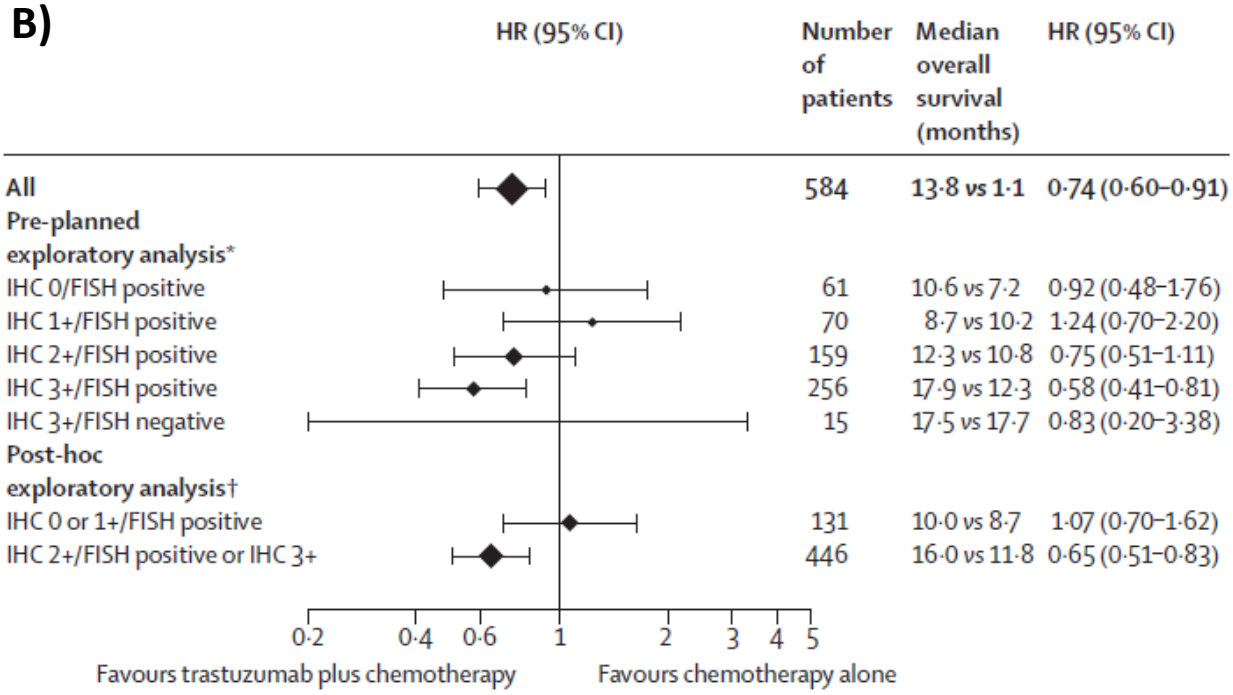
[a]ISH techniques used should have been validated and may include fluorescence, chromogenic or silver-enhanced ISH
IHC, immunohistochemistry; ISH, *in situ* hybridisation

Supplementary Figure 1: Immunohistochemistry (IHC) and Flourescence in situ hybridization (FISH) scoring for gastroesophageal adenocarcinoma. **(A)** Detailed IHC scoring criteria. **(B)** IHC imaging examples (left) and FISH imaging example (right). **(C)** Current Her2 status testing recommendation for identifying GEC patients eligible for trastuzumab (Herceptin) therapy. Taken from Bang *et al* Lancet 2010;376(9742):687-97 and Hoffmann *et al* Histopathology 2008;52(7):797-805.

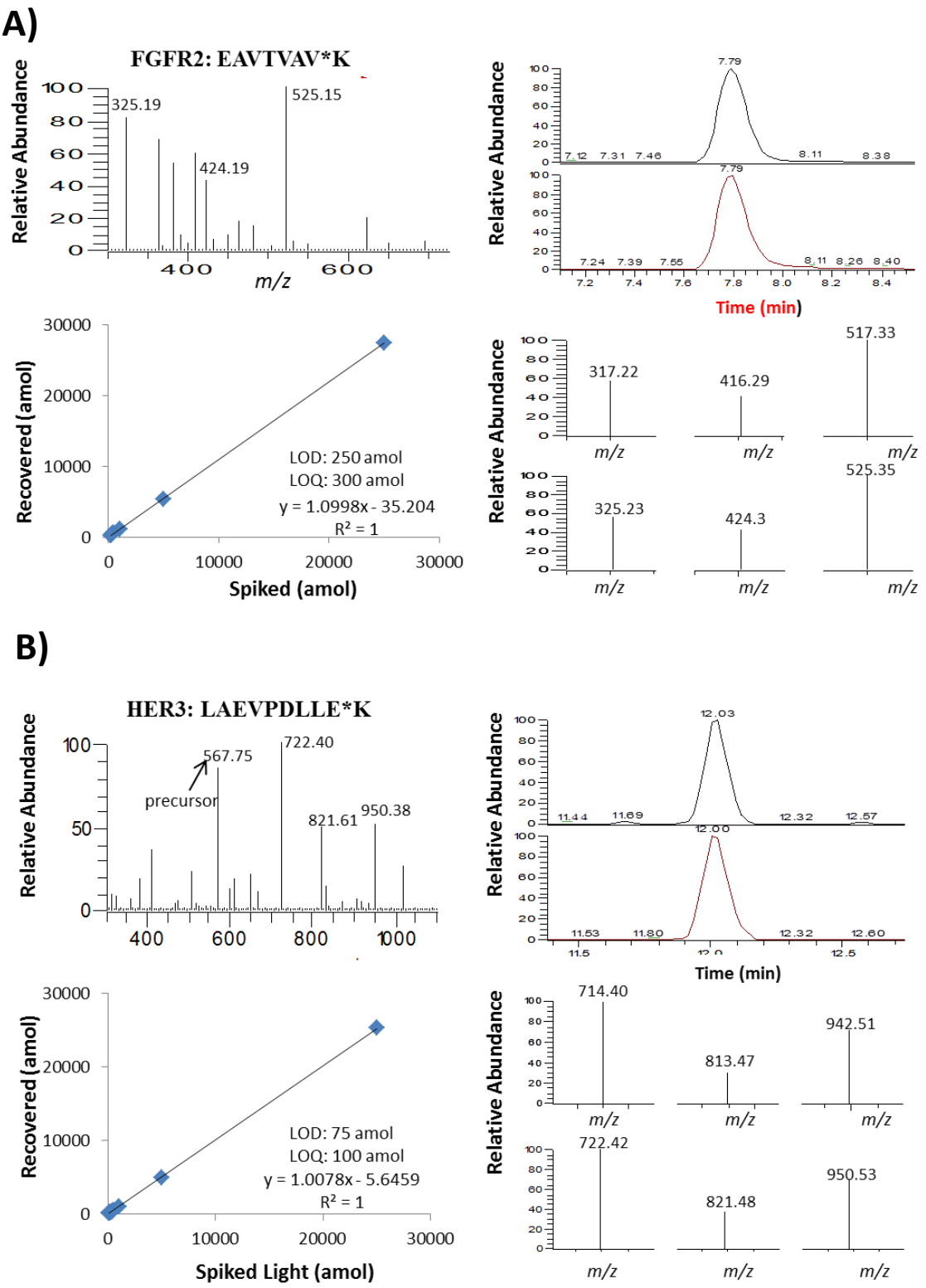
A)

HER2 status		
FISH positive/IHC 0	23 (8%)	38 (13%)
FISH positive/IHC 1+	38 (13%)	32 (11%)
FISH positive/IHC 2+	80 (27%)	79 (27%)
FISH positive/IHC 3+	131 (45%)	125 (43%)
FISH negative/IHC 3+	9 (3%)	6 (2%)
FISH positive/IHC no result	5 (2%)	2 (1%)
FISH no result/IHC 3+	8 (3%)	8 (3%)

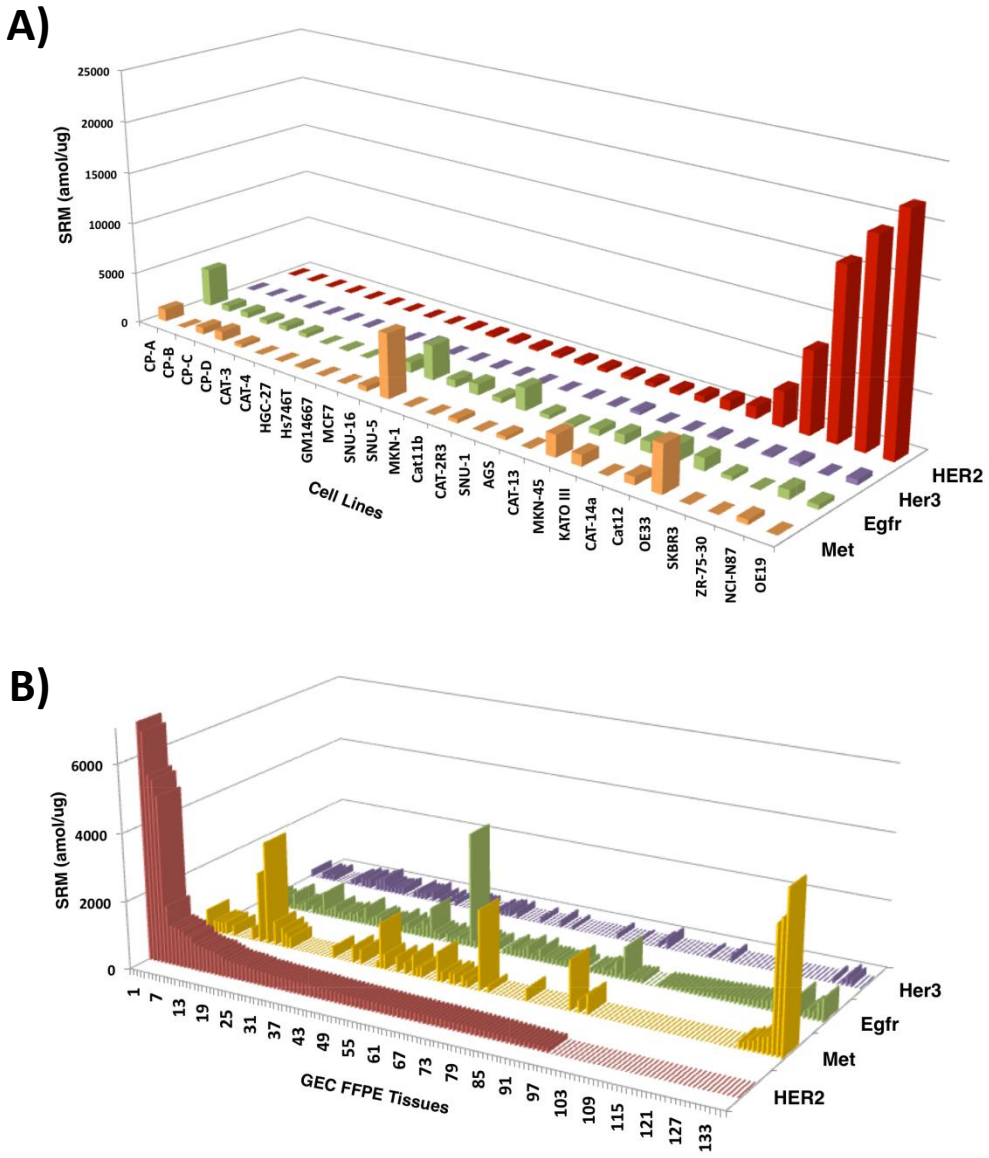
B)



Supplementary Figure 2: (A) Breakdown of FISH and IHC status in the TOGA trial. **(B)** Clinical outcome based on pre-specified subgroup analyses. As in **Supplementary Figure 1C**, 'HER2+' now includes IHC2+/FISH+ and IHC3+ groups. Taken from Bang *et al* Lancet 2010;376(9742):687-97.



Supplementary Figure 3: Development of (A) Fgfr2 and (B) Her3 SRM assays. The fragmentation spectrum for heavy peptides (top lefts) and the standard curve generated in human PC3 cell lysates (bottom lefts) The total ion chromatograms for the light and heavy isotopically labeled peptides (top rights), with the transition ions used to identify and quantitate each peptide (bottom rights).



Supplementary Figure 4: GEC cell lines (N=27) (A) and tissues (N=139) (B) multiplex expression for HER2, Met, Egfr, and Her3 within the 'GEC-Plex'. Samples sorted by HER2 expression. When adjusting for Her3-, Met-, and Egfr-SRM covariates, the correlation between HER2-SRM and *HER2* FISH significantly improved for both sample sets (see text and **Supplementary Table 5** for further details).