For Clinicians

Clinical question 1: What is the optimal testing algorithm for the assessment of HER2 status in patients with gastroesophageal adenocarcinoma?

KQ1: Should HER2 testing be requested for every patient diagnosed with gastroesophageal adenocarcinoma?

Table 1: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age		Gender				Specimen Type	WHO/Lauren/Both	Tumor Stage	Dx Addressed
							Median	Range	N Male	% male	N female	% female				
953	Van Cutsem	2015	RCT	Multiple countries	3280	NR	NR	NR	NR	NR	NR	NR	Biopsy from pry tumor, Resection	Intestinal, diffuse, mixed	NR	Primary
17	Wang	2015	Prospective cohort	Asia	277	NR	NR	NR	NR	NR	NR	NR	Resection	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Mixed carcinoma	Stage II - IV	Primary
301	Aizawa	2014	Prospective - Retrospective	Asia	1006	NR	64	18-92	677	67	329	33	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Signet-ring cell carcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	Stage I - IV	Primary, Recurrent or persistent disease
973	Wang	2011	Meta-analysis	Asia	4342	NR	NR	NR	NR	NR	NR	NR	Resection	NR	Stage I - IV	Primary
974	Xie	2009	Prospective - Retrospective	Asia	218		56.8	26-81	153	70	65	30	Resection	NR	Stage I - IV	Primary

Table 2: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
953	Van Cutsem	2015	IHC/ISH/FISH	1909	610	388	373	756	2524	Hofmann	NR	NR
17	Wang	2015	IHC/ISH/FISH	163	49	37	28	47	NR	NR	NR	HER2 detection in mixed gastric carcinoma displayed high heterogeneity.

301	Aizawa	2014	IHC/ISH/SISH	455	360	94	97	118	NR	Toga, Hofmann	NR	NR
973	Wang	2011	IHC/ISH/FISH/ Southern Blot	NR	NR	NR	NR	NR	NR	NR	NR	NR
974	Xie	2009	IHC	177	NR	NR	41	NR	NR	NR	Specimens with scores of 0 and 1+ were regarded as being HER 2/neu-negative, while scores of 2+ and 3+ indicated positive expression of HER 2/neu.	NR

Table 3: Outcomes

Refid	Bibliography	Year	Length of f/u (months)		N of pts lost to follow-up	Comparisons/Interventions	HR (CI)	Median OS (Her2+)	Median OS (Her2-)	p value for OS	HR for PFS (CI)	Median PFS (Her2+)	Median PFS (Her2-)	p value for PFS	Quality	xtra info
			Mean/ median	Range												
953	Van Cutsem	2015	NR/NA	NR/NA	NR/NA	Lower Her2 expression Vs. Higher Her2 expression	1.07 (0.70–1.62) 0.65 (0.51–0.83)	8.7months (C); 10months (T +C) 11.8 months (C); 16 months (T + C)	NR/NA	NR/NA	1.00 (0.69 – 1.45) 0.64 (0.51 – 0.79)	4.8 months (C); 5.3 month (T +C) 5.5 months (C); 7.6 months (T + C)	NR/NA	NR/NA	NR/NA	The HER2 gene copy number (<6 or >_6) did not affect overall survival in the subgroups of patients with IHC 0/1+ or IHC 2+ scores, either by stratified or unstratified analyses (P[0.05 in all cases).
17	Wang	2015	NR/NA	NR/NA	NR/NA	Chemotherapy + trastuzumab Vs. Chemotherapy alone	NR/NA	16.7mo 12.1mo	NR/NA	NR/NA	NR/NA	9.1mo 5.6mo	NR/NA	<0.05	NR/NA	NR/NA
301	Aizawa	2014	60.9	1-105.5	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	Two individuals, who were blind to the clinical data, reviewed the digital images.	HER2 overexpression was correlated with age, gender, grade of differentiation, expanding growth pattern, and nodal status. In the survival analysis, HER2 overexpression was not found to be correlated with either disease-specific survival or recurrence-free survival
973	Wang	2011	Min 5yrs	NR	NR	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	Pooled data of 15 studies using univariate analysis showed

																worse survival of patient with her-2/neu (+) (pooled HR=1.59, 95%CI: 1.20-2.12), which maintained in 7 studies on multivariate analysis (pooled HR=1.58, 95% CI: 1.18-2.12).
974	Xie	2009	51.1	6-127	0	NA	6.923	34.3%	70.6%	<0.001	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA

KQ2: Which of the following tissue specimen is the most appropriate to obtain for HER2 testing

a. Biopsy specimen from primary tumor.
b. Resection specimen
c. Tissue from metastatic site
d. FNA or cytology specimen from primary or metastatic tumor

Table 4: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/Both	Tumor Stage	Dx Addressed
							Mean /Median	Std. dev	Range	N Male	% male	N female	% female				
952	Peng	2015	Meta-analysis	Asia	1867	NR	NR	NR	NR	NR	NR	NR	NR	Biopsy, Resection, Tissue from metastatic site	NR	NR	Primary, Metastasis
20	Gumusay	2015	Prospective cohort	Europe	74	74	61	NR	27-80	47	63.5	27	36.5	Biopsy from primary tumor, Resection	NA/NR	Stage I - IV	Primary, Metastasis
23	Qiu	2015	Prospective cohort	Asia	100	NR	Grp 1: 60.8 Grp 2: 62.4	Grp1: 10.7 Grp 2: 11.8	NR	76	76	24	24	Resection, Tissue from metastatic site	Intestinal, Diffuse	Stage I - IV	Primary, Metastasis
26	Stahl	2015	Retrospective cohort	Europe	109	1248	62	NR	35-89	NR	NR	NR	NR	Resection, Tissue from metastatic site	NA/NR	NR	Primary
29	van Hagen	2015	Retrospective cohort	Europe	75	NR	63	11	NR	56	75	19	25	Biopsy from primary tumor, Resection	NA/NR	Stage II - III	Primary
53	Selcukbiricik	2014	Prospective cohort	Asia	81	NR	58	NR	29-87	50	62	31	38	Resection, Tissue from metastatic site	Intestinal, Diffuse	Stage II - III	Primary, Metastasis
61	Wong	2015	Prospective cohort	United States	43	46	61	NR	30-81	23	53	20	47	Malignant effusions	Intestinal, Diffuse	NR	Metastasis
66	Ieni	2014	Retrospective cohort	Europe	108	108	68.06	NR	39-95	67	62	41	38	Resection, Tissue from metastatic site	Papillary adenocarcinoma Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed, Intestinal, Diffuse	Stage I - IV	Primary, Metastasis
119	Grillo	2013	Prospective-Retrospective	Europe	103	302	69.5	NR	37-90	75	73	28	27	Biopsy from primary tumor, Resection	Intestinal, Diffuse	Stage I - IV	Primary

226	Yoshida	2014	Prospective-Retrospective	Asia	207	365	68	NR	31-89	166	80	41	20	Biopsy from primary tumor, Resection	Papillary adenocarcinoma, Tubular adenocarcinoma	NR	Primary
308	Kochi	2013	Prospective cohort	Asia	102	NR	68	NR	29-86	82	80.4	20	19.6	Resection, Tissue from metastatic site	Intestinal, Diffuse	Stage I - IV	Primary, Metastasis
387	Selcukbiricik	2013	Prospective-Retrospective	Europe	74	NR	58	NR	29-87	47	64	27	36	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse	Stage II - III	Primary, Metastasis
400	Fusco	2013	Prospective-Retrospective	Europe	292	NR	NR	NR	NR	NR	NR	NR	NR	Resection, Tissue from metastatic site	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Signet-ring cell carcinoma, Mixed, Intestinal	Stage I - IV	Primary, Metastasis
416	Cho	2013	Prospective-Retrospective	Asia	498	NR	Grp 1: 58.97 Grp 2: 53.53	Grp 1: 11.03 Grp 2: 11.87	NR	328	66	170	34	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage I - IV	Primary, Metastasis
422	Okines	2013	Prospective-Retrospective	Europe	402	NR	62	NR	23-85	Biopsy:194 ; Resection: 258	Biopsy:80 ; Resection: 78	Biopsy: 50; Resection: 74	Biopsy:20 ; Resection: 22	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
449	Pirrelli	2013	Prospective-Retrospective	Europe	61	NR	67	NR	37-92	45	74	16	26	Biopsy from primary tumor, Resection	NA/NR	NR	Primary
460	Pagni	2013	Prospective-Retrospective	Europe	34	NR	68.5	NR	NR	16	47	18	53	Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage I - IV	Primary, Metastasis
494	Yoon	2012	Prospective-Retrospective	United States	675	NR	64.8	NR	NR	NR	NR	NR	NR	Resection	Signet-ring cell carcinoma	Stage I - IV	Primary
503	Asioli	2012	Prospective-Retrospective	Europe	148	NR	69	NR	34-89	89	60	59	40	Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
510	Janjigian	2012	Prospective cohort	Multiple countries	381	NR	NR	NR	NR	256	67	125	33	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage IV	Primary, Recurrent or persistent disease, Metastasis
529	Fassan	2012	Prospective-Retrospective	Europe	47	188	67.9	9.1	49-87	38	81	9	19	Resection, Tissue from metastatic site	Intestinal	Stage I - III	Primary, Metastasis

543	Jeung	2012	Prospective-Retrospective	United States	116	116	NR	NR	NR	NR	NR	NR	NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
587	Tsapralis	2012	Prospective-Retrospective	Europe	120	NR	69.6	NR	27-96	84	70	36	30	TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
613	Lee	2011	Prospective-Retrospective	Australia	178	232	NR	NR	NR	NR	NR	NR	NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	NR
614	Kim	2011	Prospective-Retrospective	Asia	575	1475	NR	NR	NR	NR	NR	NR	NR	Resection, Tissue from metastatic site	NA/NR	NR	Primary, Metastasis
630	Yan	2011	Prospective-Retrospective	Europe		119	NR	NR	NR	NR	NR	NR	NR	Biopsy from primary tumor, Resection	NA/NR	NR	NR
638	Schoppmann	2011	Prospective cohort	Europe	97	NR	63.9	10.4	NR	NR	NR	NR	NR	Resection, Tissue from metastatic site	NA/NR	Stage I - IV	Primary, Recurrent or persistent disease, Metastasis
683	Bozzetti	2011	Prospective-Retrospective	Europe	72	18 biopsies and 54 resection specimens) and their corresponding metastatic lesions (33 FNAB samples, 9 core tissue biopsies and 30 surgical resections)		NR	49-88	50	69	22	31	Biopsy from primary tumor, Resection, Tissue from metastatic site, Fine needle aspiration (FNA) or cytology sample	Intestinal, Diffuse, Indeterminate, Mixed	NR	Primary, Metastasis
772	Grabsch	2010	Retrospective cohort	Europe	Series A: 418; Series B: 506	NR	Grp 1: 64.9 Grp 2: 71	NR	Grp1: 23 – 90 Grp 2: 24-96	A: 255; B: 312	A: 60; B: 62	A: 163; B:194	A: 40; B: 38	Resection	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Signet-ring cell carcinoma, Mixed, Intestinal, Diffuse	Stage I - IV	Primary, Metastasis

Table 5: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status IHC				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
952	Peng	2015	IHC/ISH/FISH/CISH/SISH	NR	NR	NR	NR	NR	NR	NR	NR	NR

23	Gumusay	2015	IHC	67	NR	19	14	NR	NR	Hofmann	NR	NR
26	Qiu	2015	IHC/ISH/FISH	NR	NR	NR	16	19	NR	Hofmann	NR	A tumor was considered homogeneous for a given marker (i.e., amplification of HER2, CCND1, EGFR, cMYC and expression of HER2) if all analyzable tissue spots showed an identical result. All other tumors were considered heterogeneous. Intra-tumor heterogeneity was found in the primary tumors of 9 of 19 (47.3%) cancers with HER2, 8 of 17 (47.0%) cancers with CCND1, 5 of 7 (71.4%) cancers with EGFR, and 23 of 27 (85.2%) cancers with MYC amplification. Amplification heterogeneity was particularly frequent in case of low-level amplification (<10 gene copies). While the amplification status was often different between metastases, unequivocal intra-tumor heterogeneity was not found in individual metastases.
29	Stahl	2015	IHC/ISH/DISH	Biopsy, 52; Resection, 53	Biopsy, 52; Resection, 53	Biopsy, 3; Resection, 3	Biopsy, 16; Resection, 13	Biopsy, 2; Resection, 2		Hofmann	NR	NR
53	van Hagen	2015	IHC/ISH/SISH	Primary, 44; LN, 44	Primary, 18; LN, 10	Primary, 8; LN, 4	Primary, 11; LN, 23	Primary, 15; LN, 18	Primary, 59; LN, 56		NR	NR
61	Selcukbiricik	2014	IHC/ISH/SISH	33	6	3	4	3	43	Hofmann	NR	NR
66	Wong	2015	IHC/ISH/FISH	Prim: 75; Met:82	Prim: 8; Met:5	Prim: 7; Met:4	Prim: 18; Met:16	Prim: 18; Met: 0	Prim: 90; Met: 0	Dako for IHC HER2 FISH PharmDx kit for FISH	NR	NR
119	Ieni	2014	IHC/ISH/FISH	75	NR	NR	28	4	75	Hofmann	NR	Of the 54 cases that showed immunostaining (IHC score 1+, 2+, or 3+), only 13 (24%) showed homogenous expression and all of these scored 3+. Given the known intratumor heterogeneity , differences in two separate surgical paraffin blocks were also checked. Complete correspondence between blocks was seen in 87 cases (84.5%) of which 66 were negative (scores 0 or 1+), 12 were highly positive (score 3+), and 9 scored 2+. In the remaining 16 cases (15.5%), comparison

												between blocks provided different results. Particularly, 11 (10.7%) cases resulted positive (scores 2+ or 3+) in one block and negative (scores 0 or 1+) in the other.
226	Grillo	2013	IHC/ISH/FISH	Surg,117; Biop,65	Surg, 30; Biop, 19	Surg, 25; Biop, 48	Surg, 35; Biop, 26	Surg,61; Biop,47	Surg,139; Biop,100	Hofmann	NR	NR
308	Yoshida	2014	IHC/ISH/FISH	Pry: 70; Met: 68		Pry: 7; Met: 12	Pry: 25; Met: 22	Pry: 27; Met: 29	Pry: 75; Met: 73	Hofmann	NR	NR
387	Kochi	2013	HIS/SISH	NR	NR	NR	NR	Pry: 13; Met: 21	Pry: 2; Met: 8		NR	NR
400	Selcukbiricik	2013	IHC/ISH/FISH	212	29	24	27	3	14	Hofmann & Ruschoff	NR	NR
416	Fusco	2013	IHC/ISH	435	NR	NR	63	62	436	Bang, Hofmann & ruschoff	NR	Intratumoral heterogeneity was defined as different results between tissue microarray cores. In cases with heterogeneity, a representative whole paraffin tumor block was tested to confirm the results.Intratumoral heterogeneity of ERBB2 overexpression in primary gastric carcinoma was observed in 21/63 (33%) gastric carcinomas, and heterogeneous ERBB2 gene amplification was observed in 14/62 (23%) gastric carcinoma cases
422	Cho	2013	IHC/ISH/BDISH	Biopsy: 194 ; Resection: 264	Biopsy:7 ; Resection: 22	Biopsy:4 ; Resection:16	Biopsy:12 ; Resection: 27	Biopsy:12 ; Resection: 19	Biopsy:146 ; Resection: 158	Hofmann & Ruschoff	For biopsies, HER2 positive was defined by IHC3+ or IHC2+ and BDISH positive (EMEA definition for approval of trastuzumab). For resections, two definitions of HER2 positive were analysed; (i) IHC3+ or IHC2+ and BDISH positive (EMEA) or (ii), IHC3+ or BDISH positive with any IHC result, (US FDA definition for trastuzumab approval), as all samples had been subjected to both IHC and BDISH	NR

449	Okines	2013	IHC/ISH/CISH	47		7	7	7	54	Hofmann	For the overall evaluation of the HER2 status, the cases with IHC score 3+ (regardless of amplification status) and 2+ (amplified only) were considered as HER2 positive. When a case showed discordant HER2 status between biopsy and the matching surgical sample, the result of the surgical one was considered as gold standard for statistical purposes.	The surgical cases showing a mixture of <30 % positive areas (IHC 3+ or 2+) and areas with any other different score of HER2 expression, were evaluated as heterogeneous. For the biopsy material, heterogeneous expression was considered when clones of 3+ or 2+ cells were recognized only in some of the biopsy specimens. Five (36 %) out of 14 IHC 3+/2+ surgical specimens showed a heterogeneous pattern versus 5 (31 %) out of 16 IHC 3+/2+ biopsy specimens
460	Pirrelli	2013	IHC/ISH/FISH	Biopsy: 8; Resect: 9	Biopsy: 14; Resect: 14	Biopsy: 4; Resect: 3	Biopsy: 8; Resect: 8	NR	NR	Toga. Ruschoff	NR	NR
494	Pagni	2013	IHC/ISH/FISH	NR	NR	NR	NR	117	558		NR	FollowingCAP breast cancer guidelines, a sample was considered to have heterogeneous HER2 amplification if there were more than 5% but less than 50% infiltrating tumor cells with an HER2/ CEP17 ratio greater than 2.2 (results were the same for a 2.0 cut point).
503	Yoon	2012	IHC/ISH/FISH	126		2	20	NR	NR	Hofmann	NR	NR
510	Asioli	2012	IHC/ISH/DISH	NR	NR	NR	78	78	NR	Hofmann &ruschoff	Tumors showing 3+ protein expression or gene amplification were considered HER2 positive.	NR
529	Janjigian	2012	IHC/CISH	100	36	24	28	28	145	Hofmann	NR	Among the 9 cases with HER2 score 3, 3 revealed huge intratumor heterogeneity. Similar variability was also showed in their paired nodal metastases.
543	Fassan	2012	IHC	Biop: 16; Resect: 18	Biop: 2; Resect: 4	Biop: 2; Resect: 2	Biop: 7; Resect: 3	NR	NR	Hofmann & ruschoff	NR	NR
587	Jeung	2012	IHC/ISH/CISH	96	4	6	14	19	101	Hofmann	NR	NR

613	Tsapralis	2012	IHC/ISH/SISH	119	15	13	31	38	59	Hofmann	NR	HER2 heterogeneity was defined as <66% of the tumour composed of HER2-positive clones. HER2 immunostaining was variable between cases with respect to both distribution and intensity. Significant intratumoural heterogeneity was demonstrated in 50% (n = 6) of assessed cases. The proportion of IHC 2+ / 3+ positive clones in gastrectomies varied from <33% (n = 4) to 33–66% (n = 2) to >66% (n = 6). However, there was homogeneity of immunostaining with the HER2-positive areas
614	Lee	2011	IHC/ISH/FISH	606	474	289	106	160	1315	Hofmann	Scores of 0 and 1+ were considered negative and scores of 2+ and 3+ were considered positive	In the FISH analysis of 325 primary GCs, eight cases (2.5%) showed amplification with a heterogeneous pattern, whereas 27 cases (8.3%) showed amplification with a homogeneous pattern
630	Kim	2011	IHC/ISH/FISH/ dc-CISH	113	2	1	12	15	104	Hofmann	NR	NR
638	Yan	2011	IHC/ISH/CISH	NR	NR	NR	14	NR	NR	Hofmann	All tumours showing HER-2+++, or HER-2++ on IHC in combination with amplification of the HER-2 gene on CISH, were considered as positive with regard to HER-2 status	NR
683	Schoppman n	2011	IHC/ISH/FISH	45	7	9	7	10	58	Hofmann	NR	NR
772	Bozzetti	2011	IHC	870	18	5	31	NR	NR	DAKO Score	NR	Prominent heterogeneity of HER2 staining was noted within most of the tumours in the full section IHC (series A) as well as between cores taken randomly from different locations within the same tumour (series B). This heterogeneity is reflected in the distribution of cases with HER2 positive tumour cells in the different percentage categories throughout all categories

Table 6: Outcomes 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
952	Peng	2015	NR	NR	NR	Pry/mets	NR	NR	NR	NR	NR	NR	NR	NR
20	Gumusay	2015	NA/NR	NA/NR	NA/NR	IHC/SISH for primary tumor	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	89.2%	NA/NR

						Vs. IHC/SISH for metastatic tumor							77.1%	
23	Qiu	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
26	Stahl	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	
29	van Hagen	2015	NA/NR	NA/NR	NA/NR	Biopsy specimen Vs. Resection specimen	93	94	82	98	NA/NR	NA/NR	78% 78%	IHC: 83%, ISH: 55% (Interobserver agreement) IHC: 85%, ISH: 47% (Interobserver agreement)
53	Selcukbiricik	2014	16mo	6-30mo		SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	92.5 for primary & LN mets	NA/NR
61	Wong	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
66	Ieni	2014	NA/NR	NA/NR	NA/NR	IHC/FISH Primary tumor/Mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	90.74% K=0.651	NA/NR
119	Grillo	2013	NA/NR	NA/NR	NA/NR	IHC V s. FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	80% on biopsy and surgical samples (60 IHC 0/1+ cases and 22 IHC 2/3+ cases); 95% on biopsy and surgical samples (71.5% not amplified and 23.5% amplified)	NA/NR
226	Yoshida	2014	NA/NR	NA/NR	NA/NR	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	57.0 % (κ=0.224) btw surg and biop spec; 72.7 % (κ=0.313) btw surg and biop spec	NA/NR
308	Kochi	2013	NA/NR	NA/NR	NA/NR	IHC/FISH Pry/Met	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	90.2% k=0.754	NA/NR
387	Selcukbiricik	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
400	Fusco	2013	40.6 mo	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
416	Cho	2013	NA/NR	NA/NR	NA/NR	IHC/SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	95%	NA/NR
422	Okines	2013	NA/NR	NA/NR	NA/NR	IHC/BDISH Biopsy/resection	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	96% 92.9%	NA/NR
449	Pirrelli	2013	NA/NR	NA/NR	NA/NR	Resection Vs.	62.5	96.2	71.4	94.4			87%	NA/NR

						Biopsy							84%	
460	Pagni	2013	NA/NR	NA/NR	NA/NR	IHC Biopsy/resection							71.4 % (κ=0.45) 87.5%	NA/NR
494	Yoon	2012	12.6	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
503	Asioli	2012	29mo	1-64mo	NA/NR	Between the 3 antibodies	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	94.5%	NA/NR
510	Janjigian	2012	18.2	3.3-44.1	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
529	Fassan	2012	NA/NR	NA/NR	NA/NR	IHC/CISH Pry vs mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Agreement, 95.2%; κ = 0.84; P < .001 IHC (Cohen Φ, ρ = 0.89, P b .001) and CISH (Cohen Φ, ρ = 1.00, P b .001)	NA/NR
543	Jeung	2012	NA/NR	NA/NR	NA/NR	Manual vs automated IHC in Gastric cancer/ manual vs Automated IHC in GEJ cancer	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy: 70%; resect: 85%; All: 78%; Biopsy: 67%; resect: 100%; All: 84%	NA/NR
587	Tsapralis	2012	NA/NR	NA/NR	NA/NR	IHC+/CISH+	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	95%	NA/NR
613	Lee	2011	NA/NR	NA/NR	NA/NR	IHC/SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	96.2%	NA/NR
614	Kim	2011	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
630	Yan	2011	NA/NR	NA/NR	NA/NR	FISH/dc-CISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	100% (Pearson correlation coefficient 0.987, p<0.001)	(Pearson correlation coefficient 0.89, p=0.01)
638	Schoppmann	2011	NA/NR	57mo	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR		
683	Bozzetti	2011	NA/NR		NA/NR	IHC (pry & met) V FISH (pry &met)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	94.9% 98.5%	
772	Grabsch	2010	A: 1.49; B: 1.82	A: 0.09 - 9.09; B0.09 - 20.56	15	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR

Table 7: Outcome 2

Ref id	First Author	Year	Comparisons	HR (CI)	Median OS (Her2+)	Median OS (Her2-)	p value for OS	HR for PFS (CI)	Median PFS (Her2+)	Median PFS (Her2-)	p value for PFS	Repeat test	Quality	Algorithm	xtra info
952	Peng	2015	Pry/Mets	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Pooled proportions of tumors shifting from positive to negative and from negative to positive were 17% (95% CI: 7-29%) and 4% (95% CI: 2-6%) respectively.
20	Gumusay	2015	IHC/SISH for primary tumor Vs. IHC/SISH for metastatic tumor	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	The assessment of Her2 status with the IHC staining method and SISH revealed a discordance rate of 9.5 and 16.2%, respectively. However, this discordance was clinically meaningful in only one patient leading to a change in treatment decision.
23	Qiu	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Her2 overexpression in lymph node mets were regarded if Her2 staining scored 2+ or 3+ in any of the metastatic lymph nodes. her2 overexpression was found in 39.4% of the corresponding metastatic lymph nodes. discordance btw the primary tumors and the corresponding metastases was observed in 31 paired samples. concordance among lymph node metastasis was observed in 59 cases. discordance among the mets lymph node in the same pt was observed in 20 cases.
26	Stahl	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Amplification was found in 17.4% of 109 interpretable cases for HER2, 6.4% for EGFR, 17.4% for CCND1, and 24.8% for MYC. HER2 amplification was strongly linked to protein overexpression by IHC in a spot-by-spot analysis ($p < 0.0001$).

29	van Hagen	2015	Biopsy specimen Vs. Resection specimen	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2 expression and amplification was independently assessed by three experienced pathologists from three institutions. The pathologists were blinded for the patients. Biopsies and resection specimens were rotating among the centers in three batches making sure that matching biopsies and resection specimens were never present at the same location simultaneously.	In case of no or weak HER2 expression (1+), the tumor is scored as HER2-negative, and in case of strong expression (3+), the tumor is scored as HER2-positive. In case of moderate expression (2+), the results of ISH are evaluated, and the HER2 status is determined based on the absence (negative) or presence (positive) of HER2 amplification	NA/NR
53	Selcukbiricik	2014	SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	In total, six patients demonstrated discordance between the primary tumor and lymph node metastases. The prevalence of HER2 discordance was significantly higher for patients in the pN2 and N3 stages (p=0.007). Although discordant patients had worse survival rates than concordant patients, the differences were not significant (p>0.05)
61	Wong	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Cell blocks showed a poorly cohesive pattern in 30 (65%), aggregates in 7 (15%), and mixed pattern in 9 (20%). Three (7%) showed HER2 amplification on SISH. In 18 cases (39%), HER2 status was compared with histological specimens, showing 100% concordance. Difficulties were encountered in distinguishing malignant cells from reactive mesothelial cells in 12 (26%).

66	Ieni	2014	IHC/FISH Primary tumor/ Mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	In order to avoid a significant inter-observer variability in HER2 IHC scoring, after a first assessment in each unit of pathology, all the immunostained slides were reassessed by two observers, who were blinded to the previous paired data, and in a random order. In case of disagreement, cases were jointly discussed by using a double-headed microscope, until agreement was reached.	NA/NR	86 cases were concordantly not amplified for HER2 in primary GC and corresponding nodal metastases, while 12 cases were HER2 amplified in both primitive and metastatic tumours. Changes in HER2 status between primary GC and matched synchronous metastases were evidenced in 10 (9.26%) cases. 6 cases were HER2 amplified in the primary GC and not amplified in the metastases (negative conversion), while 4 of the discordant cases were HER2 not amplified in the primitive tumour and amplified in the lymph node metastases (positive conversion). No significant differences in the various clinico-pathological parameters were evidenced between discordant and concordant GC On the other hand, HER2 amplification was significantly more frequent in the intestinal-type GC compared to diffuse-type (p = 0.0496).
119	Grillo	2013	IHC V s. FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	IHC was jointly evaluated by four expert gastrointestinal pathologists , and any discrepancies were resolved by consensus. All cases were evaluated in a blinded fashion for FISH	NA/NR	Comparison between Different Anti-HER2 Antibodies (4B5 and CB11): Excellent agreement between the PATHWAY HER2/neu (4B5) and the Oracle HER2 Bond IHC system (CB11) methods was observed both in biopsies (agreement 90.3%; k = 0.766; P < .001) and in surgical samples (agreement 90.3%; k = 0.815; P < .001). The concordance rate between IHC and FISH was 99% in surgical samples and 89.9% in biopsies after exclusion of the IHC score 2+ group, which is known to be “equivocal” . No significant difference was noted in HER2 overexpression or amplification when compared to tumor site, mean tumor size, differentiation, growth pattern according to Ming, or stage. Statistically significant differences were observed only for Laurén classification.

226	Yoshida	2014	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Same as the one used in the Toga trial	HER2 gene amplification was detected in 31 % (61/200) of surgically resected tumors and 32 % (47/147) of biopsy specimens. Except for immunohistochemistry (IHC) equivocal (2+) cases, the concordance rates between IHC and FISH was 90.9 % in surgically resected tumors and 90.2 % in biopsy specimens. In IHC 2+ cases, the rate of HER2 gene amplification was 56 and 38 % in surgically resected tumors and biopsy specimens, respectively. IHC-FISH discordance was mainly due to intratumoral heterogeneity and low-level gene amplification. Polysomy 17 was detected in 5.5 and 7.5 % of surgically resected tumors and biopsy specimens and significantly correlated with IHC score, but polysomy 17 could explain one IHC score 3+ and FISH-negative tumor only.
308	Kochi	2013	IHC/FISH Pry/Met	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	No statistically significant difference was observed in any clinical feature between males and females apart from in number of metastatic sites.IHC 0 and 1+ combined as "negative". primary goal is to compare lymph node metastases with primary gastric cancer
387	Selcukbiricik	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	H. pylori was positive in 40 cases (54%), and negative in 34 (46%).Initial CEA values were high in 18 cases (78%) with positive H. pylori and in 5 cases (22%) with negative H. pylori (P=0.009). While SISH data of patients were negative in 59 cases (80%) and positive in 15 cases (20%) in primary tissues, they were negative in 56 cases (75%) and positive in 18 cases (25%) in lymph nodes. Discrepancy between primary tissue and lymph node results was detected in 3 cases, in which SISH was negative in the primary tissue and HER-2 expression was positive in the lymph nodes.

400	Fusco	2013	NA/NR	NA/NR	14.1	40.8	0.006	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2-positive cases were 13% and heterogeneous HER2 expression was observed in 71% of positive samples. Analysis of HER2 status in tumor and tumor invasive front demonstrate concordance in 177 cases (88%). Comparison of HER2 expression in primary cancer and synchronous lymph node metastasis exhibited discordant status in 14% of cases. Dysplastic epithelium surrounding the tumor showed immunohistochemical score 2 or 3 in 19% of high-grade and in 9% of low-grade dysplastic samples. HER2 status was significantly associated with intestinal-type carcinomas (P = 0.018) and prognosis since patients with primary HER2-positive tumor showed decreased overall survival (P = 0.006)
416	Cho	2013	IHC/SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Repeated immunohistochemistry and silver in situ hybridization in representative paraffin tumor blocks confirmed focal ERBB2 overexpression and ERBB2 gene amplification and did not change the final results	NA/NR	NA/NR	ERBB2-positivity was observed in 52 gastric carcinomas (10%) and was not associated with recurrence of disease or survival of patients. In ERBB2-positive primary gastric carcinomas, heterogeneous ERBB2 overexpression was observed in 21/63 (33%) gastric carcinomas and heterogeneous ERBB2 gene amplification in 14/62 (23%) cases.Discrepancies in ERBB2 results between primary and paired metastatic lymph nodes were observed in 11% of cases by immunohistochemistry and 7% by silver in situ hybridization. Out of the 41 paired primary and distant metastases, 5 (12%) cases were ERBB2-positive, and discrepancy was observed in one case.
422	Okines	2013	IHC/BDISH Biopsy/resection	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2 positive rate (IHC3+, or IHC2+/BDISH positive) was 10.9% in the whole cohort and 10.4% in resection specimens. A further 4.0% of resections were IHC negative/BDISH positive. HER2 status was neither prognostic, nor (in pre-treatment biopsies) predicted enhanced benefit from chemotherapy [HER2 positive HR 0.74 (0.14–3.77); HER2 negative HR 0.58 (0.41–0.82), interaction P = 0.7]. However, the power of the predictive analysis was limited by the small number of HER2 positive pre-treatment biopsies
449	Pirrelli	2013	Resection Vs.	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Slides were scored by a pathologist blinded to CISH results	NA/NR	NA/NR

			Biopsy												
460	Pagni	2013	IHC Biopsy/resection	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Three independent observers scored HER2 IHC status on each slide and, in case of disagreement, the case was jointly discussed (Cohen's κ test was used for statistical analysis). All the observers were same-experienced in reading HER2 IHC slides and, purposely, no one was gastrointestinal or breast specialized pathologist	NA/NR	Comparative analysis between different scoring methods (biopsy vs resection) applied in lymph nodes revealed two discordant cases (#4 and #10).
494	Yoon	2012	NA/NR	2.04	NA/NR	NA/NR	p=.025	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Overall, 117 EACs (17%) demonstrated HER2 amplification, of which 20 (17%) showed HER2 heterogeneity. All HER2-heterogeneous tumors were amplified. Among HER2-amplified tumors, heterogeneous tumors had significantly higher frequency of poor histologic grade and polysomy 17. In multivariable models that included number of metastatic lymph nodes, grade, tumor stage, and polysomy 17, only HER2 heterogeneity and node number were prognostic among HER2-amplified tumors, with heterogeneity showing worse DSS (hazard ratio, 2.04; 95% CI, 1.09 to 3.79; P □ .025) and OS (P □ .026). Among HER2-nonamplified EACs, polysomy 17 was independently associated with worse DSS (P □ .012) and OS (P □ .023). All-cause and disease-specific deaths within 5 years of surgery were experienced by 492 patients (72.9%) and 458 patients (67.9%), respectively.

503	Asioli	2012	Between the 3 antibodies		NA/NR	NA/NR		NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	One tissue block for each of 88 primary tumors and 60 paired primary tumors and metastases was examined for human epidermal growth factor receptor 2 status by IHC using 3 different antibodies (HercepTest, CB11, and 4B5) and by FISH. Two additional tissue blocks of the primary tumor were tested by IHC if the results were negative on the first tissue block. Two cases showed a clinically significant discrepancy between primary tumor (score 0) and lymph nodes metastases (score 3+). Additional block analysis increased both the sensitivity (from 63% to 83%) and the accuracy (from 91% to 94%) of immunohistochemistry as compared with FISH
510	Janjigian	2012	NA/NR	NA/NR	13.9mo	11.4mo	0.047	NA/NR	6.5mo	6.4mo	0.889	NA/NR	NA/NR	NA/NR	In the multivariate logistic model, there were significantly higher rates of HER2 positivity in patients with liver metastasis (liver metastasis 31%; no liver metastasis 11%; P = 0.025) and intestinal histology (intestinal 33%; diffuse/mixed 8%; P = 0.001). No significant differences in HER2 positivity were found between resections and biopsies or primaries and metastases. multivariate analysis indicated that HER2 status was not an independent prognostic factor (hazard ratio 0.79; 0.44–1.14; P = 0.194).
529	Fassan	2012	IHC/CISH Pry vs mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	All sections were jointly assessed by 2 pathologists blinded to any clinicopathologic information. Discordant cases were reconsidered by a third gastrointestinal pathologist, and a final consensus was reached	NA/NR	Her 2 gene amplification (25.5%) was associated with more differentiated phenotype (Fisher exact test, P = .039) and advanced tumor stage (Fisher exact test, P = .015). Both IHC and CISH documented an excellent intratumor agreement in human epithelial growth factor receptor 2 status (kappa = 0.75, P < .001; kappa = 0.88, P < .001, respectively).
543	Jeung	2012	Manual vs automated IHC in Gastric cancer/	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR

			manual vs Automated IHC in GEJ cancer												
587	Tsapralis	2012	IHC+/CISH+	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Amplification was associated with intestinal phenotype (P < 0.05). No association with grading, staging or survival was found
613	Lee	2011	IHC/SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	SISH amplification was seen in 35.3% of IHC 2+ and in a case with previously unrecognized staining pattern. Concordance of IHC HER2 status on biopsies and gastrectomies was seen in 74.1%. False negative IHC results on either the biopsy or gastrectomy were seen in 19.4% of HER2 amplified cases
614	Kim	2011	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	In the FISH analysis of 325 primary GCs, the discordant:concordant FISH ratio based on examination of three different areas in each primary lesion was 0.30:1. FISH testing using 250 paired primary and metastatic lesions revealed seven cases (2.8%) with discordant amplification. In metastatic disease positive conversion occurred in six cases (2.4%), whereas negative conversion happened in one case (0.4%). The discordant:concordant ratio of primary versus secondary lesions was 0.23:1. When the seven discordant cases were re-evaluated using whole sections of primary GCs, six showed a heterogeneous pattern of amplification.
630	Yan	2011	FISH/dc-CISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Five of the 11 high-HER2 expressors (defined as IHC 3+ or IHC 2+/FISH-amplified according to Trastuzumab for Gastric Cancer trial criteria) showed an IHC 3+ score on matched biopsies (concordance rate 45.5%). Nine of these 11 cases showed HER2 amplification on matched biopsies (concordance rate 81.8%). There was no significant correlation of the HER2-status between biopsy and surgical resection specimens (p=0.1)
638	Schoppmann	2011	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	The HER-2 status was identical in the primary tumour and lymph node metastases in 95 per cent of ACs. Nineteen of 22 distant metastases from AC had identical HER-2 status to the primary tumour. In two of 22 patients with AC the primary tumour was classed as negative but

															distant metastases were HER-2-positive.
683	Bozzetti	2011	IHC (pry & met) V FISH (pry &met)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Only one case showed discordance between primary tumour and metastasis, being negative by both IHC and FISH in the primary and showing HER2 overexpression and amplification in the corresponding pancreatic lymph node metastasis.
772	Grabsch	2010	NA/NR	NA/NR	NA/NR	NA/NR	(series A: p = 0.074 (5% cut off) and p = 0.277 (DAKO-Score); series B: p = 0.347 (5% cut off) and p = 0.167 (DAKO-Score)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	All sections were scored by an experienced histopathologist blinded to any of the clinicopathological parameters including patient outcome.	NA/NR	In Series A, good agreement (κ: 0.8; p < 0.001) was seen between the two different scoring systems with 406 (97%) GC being classified identically using either score and only 12 (3%) GC were classified “positive” using the 5% cut off but “negative” using the DAKO-Score. In series B, good agreement (κ: 0.7; p < 0.001).no association of HER2 status with survival in large cohort examined by full tissue sections (418 cases) and TMAs (506 cases). 3 tumor cores per block. Lower rate of positivity in TMA cases, due to sampling error. was seen between the two different scoring systems with 496 (98%) GC being classified identically using either score and only 10 (2%) GC were classified “positive” using the 5% cut off but “negative” using the DAKO-Score.

KQ3: In patients with HER2 positive results, under what clinical scenario should HER2 targeted therapy be initiated?

Table 8: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/ Median	Std dev	Range	N Male	% male	N female	% female				
419	He	2013	Prospective cohort	Asia	197	NR	62	NR	22-88	132	67	65	33	Resection	Intestinal, Diffuse	Stage I - IV	Primary

Table 9: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status IHC				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
419	He	2013	IHC/ISH/FISH	125	28	25	19	31	166	Hofmann	NR	NR

Table 10: Outcomes 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
419	He	2013	NA/NR	NA/NR	26	IHC Vs. FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	88.83 88.83	NA/NR

Table 11: Outcomes 2

Refid	Bibliography	Year	Comparisons/Interventions	HR (CI)	Median OS (Her2+)	Median OS (Her2-)	p value for OS	HR for PFS (CI)	Median PFS (Her2+)	Median PFS (Her2-)	p value for PFS	Quality	xtra info
419	He	2013	IHC Vs. FISH	NR/NA	NR/NA	NR/NA	p=0.006	NR/NA	NR/NA	NR/NA	NR/NA	NR/NA	The positivity rates in intestinal type and well-differentiated gastric cancer were higher than those in diffuse/mixed type and poorly-differentiated gastric cancer respectively (28.57% vs 13.43%, P = 0.0103; 37.25% vs 11.64%, P < 0.0001), but were not correlated with gender, age, tumor location or TNM stage, depth of invasion, lymph node metastases and distant metastasis. In poorly-differentiated gastric cancer patients, those without lymph node metastasis

													showed a higher HER2 positivity rate than those with lymph node metastasis (26.47% vs 7.14%, P = 0.0021). This association was not present in those patients with well-differentiated gastric cancer (28.57% vs 43.33%, P = 0.2832). in patients presenting well-differentiated tumors, the overall survival of the HER2-positive group was significantly worse than that of the HER2-negative group (P = 0.0123)

KQ4: Should HER2 directed therapy be delayed if HER2 status cannot be confirmed as positive or negative (i.e. if an equivocal result is found with IHC)?

Table 12: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/ Median	Std dev	Range	N Male	% male	N female	% female				
739	Bang	2010	Randomized control trial	Multiple countries	584	NR	Grp1: 59.4 Grp2: 58.5	Grp1: 10.8 Grp2: 11.2	NR	444	76	140	24	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage IV	Primary, Recurrent or persistent disease, Metastasis

Table 13: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status IHC				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
739	Bang	2010	IHC/ISH/FISH	61	70	159	287	553	15	Hofmann	NR	NR

Table 14: Outcomes 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
739	Bang	2010	18·6 months (IQR 11–25) in the trastuzumab plus chemotherapy group and 17·1 months (9–25) in the chemotherapy alone group		NA/NR	Trastuzumab + chemotherapy Vs. Chemotherapy alone	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR

Table 15: Outcomes 2

Refid	Bibliog	Year	Comparisons/Inte	HR (CI)	Median	Median	p value	HR for	Median PFS	Median	p value	Quality	xtra info

	raphy		rventions		OS (Her2+)	OS (Her2-)	for OS	PFS (CI)	(Her2+)	PFS (Her2-)	for PFS		
739	Bang	2010	Trastuzumab + chemotherapy Vs. Chemotherapy alone	NR 0·74; 95% CI 0·60–0·91)	13·8 months (95% CI 12–16) 11·1 months (10–13)	NR/NA	p=0·0046	NR/NA	6.7 months 5.5 months	NR/NA	NR/NA	NR/NA	The most common adverse events in both groups were nausea (trastuzumab plus chemotherapy, 197 [67%] vs chemotherapy alone, 184 [63%]), vomiting (147 [50%] vs 134 [46%]), and neutropenia (157 [53%] vs 165 [57%]). Rates of overall grade 3 or 4 adverse events (201 [68%] vs 198 [68%]) and cardiac adverse events (17 [6%] vs 18 [6%]) did not diff er between groups. ToGA trial report. 20 had locally advanced disease, 564 metastatic. 106 were GEJ, 478 stomach.

KQ5: Under what circumstances should patient samples be retested?

a. Biopsy (primary tumor) vs resection
b. Biopsy (primary tumor) vs resection vs mets
c. Concurrent vs later mets
d. Institutional variations?
e. Inadequate/poor tissue sample

Table 16: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/ Median	Std dev	Range	N Male	% male	N female	% female				
23	Qiu	2015	Prospective cohort	Asia	100	NR	Grp1: 60.8; Grp2: 62.4	Grp1: 10.7, Grp2: 11.8	NR	76	76	24	24	Resection, Tissue from metastatic site	Intestinal, Diffuse	Stage I - IV	Primary, Metastasis
29	van Hagen	2015	Retrospective cohort	Europe	75	NR	63	11	NR	56	75	19	25	Biopsy from primary tumor, Resection	NA/NR	Stage II - III	Primary
66	Ieni	2014	Retrospective cohort	Europe	108	108	68.06	NR	39-95	67	62	41	38	Resection, Tissue from metastatic site	Papillary adenocarcinoma Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed, Intestinal, Diffuse	Stage I - IV	Primary, Metastasis
119	Grillo	2013	Prospective-Restrospective	Europe	103	302	69.5	NR	37-90	75	73	28	27	Biopsy from primary tumor, Resection	Intestinal, Diffuse	Stage I - IV	Primary
226	Yoshida	2014	Prospective-Restrospective	Asia	207	365	68	NR	31-89	166	80	41	20	Biopsy from primary tumor, Resection	Papillary adenocarcinoma, Tubular adenocarcinoma	NR	Primary
529	Fassan	2012	Prospective-Restrospecti	Europe	47	188	67.9	9.1	49-87	38	81	9	19	Resection, Tissue from	Intestinal	Stage I - III	Primary, Metastasis

			ve											metastatic site			
614	Kim	2011	Prospective-Restrospective	Asia	575	1475	NR	NR	NR	NR	NR	NR	NR	Resection, Tissue from metastatic site	NA/NR	NR	Primary, Metastasis
630	Yan	2011	Prospective-Restrospective	Europe		119	NR	NR	NR	NR	NR	NR	NR	Biopsy from primary tumor, Resection	NA/NR	NR	
683	Bozzetti	2011	Prospective-Restrospective	Europe	72	18 biopsies and 54 resection specimens) and their corresponding metastatic lesions (33 FNAB samples, 9 core tissue biopsies and 30 surgical resections)	NR	NR	49-88	50	69	22	31	Biopsy from primary tumor, Resection, Tissue from metastatic site, Fine needle aspiration (FNA) or cytology sample	Intestinal, Diffuse, Indeterminate, Mixed	NR	Primary, Metastasis

Table 17: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status IHC				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
23	Qiu	2015	IHC	67	NR	19	14	NR	NR	Hofmann	NR	NR
29	van Hagen	2015	IHC/ISH/DISH	Biopsy, 52; Resection, 53	Biopsy, 52; Resection, 53	Biopsy, 3; Resection, 3	Biopsy, 16; Resection, 13	Biopsy, 2; Resection, 2	NR	Hofmann	NR	NR
66	Ieni	2014	IHC/ISH/FISH	Prim: 75; Met:82	Prim: 8; Met:5	Prim: 7; Met:4	Prim: 18; Met:16	Prim: 18; Met: 0	Prim: 90; Met: 0	Dako for IHC HER2 FISH PharmDx kit for FISH	NR	NR
119	Grillo	2013	IHC/ISH/FISH	75	NR	NR	28	4	75	Hofmann	NR	Of the 54 cases that showed immunostaining (IHC score 1+, 2+, or 3+), only 13 (24%) showed homogenous expression and all of these scored 3+. Given the known intratumor heterogeneity , differences in two

												separate surgical paraffin blocks were also checked. Complete correspondence between blocks was seen in 87 cases (84.5%) of which 66 were negative (scores 0 or 1+), 12 were highly positive (score 3+), and 9 scored 2+. In the remaining 16 cases (15.5%), comparison between blocks provided different results. Particularly, 11 (10.7%) cases resulted positive (scores 2+ or 3+) in one block and negative (scores 0 or 1+) in the other.
226	Yoshida	2014	IHC/ISH/FISH	Surg,117; Biop,65	Surg, 30; Biop, 19	Surg, 25; Biop, 48	Surg, 35; Biop, 26	Surg,61; Biop,47	Surg,139; Biop,100	hofmann	NR	NR
529	Fassan	2012	IHC/CISH	100	36	24	28	28	145	hofmann	NR	Among the 9 cases with HER2 score 3, 3 revealed huge intratumor heterogeneity. Similar variability was also showed in their paired nodal metastases.
614	Kim	2011	IHC/ISH/FISH	606	474	289	106	160	1315	hofmann	Scores of 0 and 1+ were considered negative and scores of 2+ and 3+ were considered positive	In the FISH analysis of 325 primary GCs, eight cases (2.5%) showed amplification with a heterogeneous pattern, whereas 27 cases (8.3%) showed amplification with a homogeneous pattern
630	Yan	2011	IHC/ISH/FISH/ dc-CISH	113	2	1	12	15	104	hofmann	NR	NR
683	Bozzetti	2011	IHC/ISH/FISH	45	7	9	7	10	58	hofmann	NR	NR

Table 18: Outcomes

Refid	First Author	Year	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability	Quality	Algorithm	Extra info
23	Qiu	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Her2 overexpression in lymph node mets were regarded if Her2 staining scored 2+ or 3+ in any of the metastatic lymph nodes. her2 overexpression was found in 39.4% of the corresponding metastatic lymph nodes. discordance btw the primary tumors and the corresponding metastases was

														observed in 31 paired samples. concordance among lymph node metastasis was observed in 59 cases. discordance among the mets lymph node in the same pt was observed in 20 cases.
29	van Hagen	2015	Biopsy specimen Vs. resection specimen	93	94	82	98	NA/NR	NA/NR	78% 78%	IHC: 83%, ISH: 55% (Interobserver agreement); IHC: 85%, ISH: 47% (Interobserver agreement)	HER2 expression and amplification was independently assessed by three experienced pathologists from three institutions. The pathologists were blinded for the patients. Biopsies and resection specimens were rotating among the centers in three batches making sure that matching biopsies and resection specimens were never present at the same location simultaneously.	In case of no or weak HER2 expression (1+), the tumor is scored as HER2-negative, and in case of strong expression (3+), the tumor is scored as HER2-positive. In case of moderate expression (2+), the results of ISH are evaluated, and the HER2 status is determined based on the absence (negative) or presence (positive) of HER2 amplification	NA/NR
66	Ieni	2014	IHC/FISH V primary tumor/mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	90.74%, K=0.651	NA/NR	In order to avoid a significant inter-observer variability in HER2 IHC scoring, after a first assessment in each unit of pathology, all the immunostained slides were reassessed by two observers , who were blinded to the previous paired data, and in a random order. In	NA/NR	86 cases were concordantly not amplified for HER2 in primary GC and corresponding nodal metastases, while 12 cases were HER2 amplified in both primitive and metastatic tumours. Changes in HER2 status between primary GC and matched synchronous metastases were evidenced in 10 (9.26%) cases. 6 cases

												case of disagreement, cases were jointly discussed by using a double-headed microscope, until agreement was reached.		were HER2 amplified in the primary GC and not amplified in the metastases (negative conversion), while 4 of the discordant cases were HER2 not amplified in the primitive tumour and amplified in the lymph node metastases (positive conversion). No significant differences in the various clinico-pathological parameters were evidenced between discordant and concordant GC On the other hand, HER2 amplification was significantly more frequent in the intestinal-type GC compared to diffuse-type (p = 0.0496).
119	Grillo	2013	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	80% on biopsy and surgical samples (60 IHC 0/1+ cases and 22 IHC 2/3+ cases); 95% on biopsy and surgical samples (71.5% not amplified and 23.5% amplified)	NA/NR	IHC was jointly evaluated by four expert gastrointestinal pathologists , and any discrepancies were resolved by consensus. All cases were evaluated in a blinded fashion for FISH	NA/NR	Comparison between Different Anti-HER2 Antibodies (4B5 and CB11): Excellent agreement between the PATHWAY HER2/neu (4B5) and the Oracle HER2 Bond IHC system (CB11) methods was observed both in biopsies (agreement 90.3%; k = 0.766; P < .001) and in surgical samples (agreement 90.3%; k = 0.815; P < .001). The concordance rate between IHC and FISH was 99% in surgical samples and 89.9% in biopsies after exclusion of the IHC score 2+ group, which is known to be “equivocal” . No significant difference was noted

														in HER2 overexpression or amplification when compared to tumor site, mean tumor size, differentiation, growth pattern according to Ming, or stage. Statistically significant differences were observed only for Laurén classification.
226	Yoshida	2014	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	57.0 % (κ=0.224) btw surg and biop spec; 72.7 % (κ=0.313) btw surg and biop spec	NA/NR	NA/NR	Same as the one used in the Toga trial	HER2 gene amplification was detected in 31 % (61/200) of surgically resected tumors and 32 % (47/147) of biopsy specimens. Except for immunohistochemistry (IHC) equivocal (2+) cases, the concordance rates between IHC and FISH was 90.9 % in surgically resected tumors and 90.2 % in biopsy specimens. In IHC 2+ cases, the rate of HER2 gene amplification was 56 and 38 % in surgically resected tumors and biopsy specimens, respectively. IHC-FISH discordance was mainly due to intratumoral heterogeneity and low-level gene amplification. Polysomy 17 was detected in 5.5 and 7.5 % of surgically resected tumors and biopsy specimens and significantly correlated with IHC score, but polysomy 17 could explain one IHC score 3+ and FISH-negative tumor only.

529	Fassan	2012	IHC/CISH V Pry vs mets	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	agreement, 95.2%; κ = 0.84; P < .001; IHC (Cohen Φ, ρ = 0.89, P b .001) and CISH (Cohen Φ, ρ = 1.00, P b .001)	NA/NR	All sections were jointly assessed by 2 pathologists blinded to any clinicopathologic information. Discordant cases were reconsidered by a third gastrointestinal pathologist, and a final consensus was reached	NA/NR	Her 2 gene amplification (25.5%) was associated with more differentiated phenotype (Fisher exact test, P = .039) and advanced tumor stage (Fisher exact test, P = .015). Both IHC and CISH documented an excellent intratumor agreement in human epithelial growth factor receptor 2 status (kappa = 0.75, P < .001; kappa = 0.88, P < .001, respectively).
614	Kim	2011	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	In the FISH analysis of 325 primary GCs, the discordant:concordant FISH ratio based on examination of three different areas in each primary lesion was 0.30:1. FISH testing using 250 paired primary and metastatic lesions revealed seven cases (2.8%) with discordant amplification. In metastatic disease positive conversion occurred in six cases (2.4%), whereas negative conversion happened in one case (0.4%). The discordant:concordant ratio of primary versus secondary lesions was 0.23:1. When the seven discordant cases were re-evaluated using whole sections of primary GCs, six showed a heterogeneous pattern of amplification.
630	Yan	2011	FISH/dc-CISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	100% (Pearson correlation coefficient	(Pearson correlation coefficient 0.89, p=0.01)	NA/NR	NA/NR	Five of the 11 high-HER2 expressors (defined as IHC 3+ or IHC 2+/FISH-amplified

										0.987, p<0.001)				according to Trastuzumab for Gastric Cancer trial criteria) showed an IHC 3+ score on matched biopsies (concordance rate 45.5%). Nine of these 11 cases showed HER2 amplification on matched biopsies (concordance rate 81.8%). There was no significant correlation of the HER2-status between biopsy and surgical resection specimens (p=0.1)
683	Bozzetti	2011	IHC (pry & met) V s. FISH (pry &met)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	94.9% 98.5%	NA/NR	NA/NR	NA/NR	Only one case showed discordance between primary tumour and metastasis, being negative by both IHC and FISH in the primary and showing HER2 overexpression and amplification in the corresponding pancreatic lymph node metastasis.

KQ6: What are the clinical performance characteristics of IHC and FISH/ISH/CISH? (PPV,NPV: gold standard, response to treatment; outcome, OS , PFS)

Table 19: Patient and disease characteristics

Refid	First Author	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/ Median	Std dev	Range	N Male	% male	N female	% female				
41	Otsu	2015	Prospective-Restrospective	Asia	360	NR	63.5	12	NR	242	67	118	33	Resection	Papillary adenocarcinoma, Mucinous adenocarcinoma, Signet-ring cell carcinoma	Stage I - IV	Primary
206	Chen	2014	Prospective-Restrospective	Asia	394	NR	NR	NR	NR	264	67.0%	130	33.0%	Resection	NA/NR	Stage I - IV	Primary
226	Yoshida	2014	Prospective-Restrospective	Asia	207	365	68	NR	31-89	166	80	41	20	Biopsy from primary tumor, Resection	Papillary adenocarcinoma, Tubular adenocarcinoma	NR	Primary
239	Geppert	2014	Prospective-Restrospective	Europe	130	NR	63.4	NR	33-83	118	90.8	12	9.2	Resection	NA/NR	Stage I - IV	Primary
303	Prins	2014	Prospective-Restrospective	Europe	154	NR	64	NR	33.8-81.3	126	81.8	28	18.2	NR	NA/NR	Stage I - III	Primary
307	Wang	2013	Prospective cohort	Asia	72	72		NR		57	79.17	15	20.83	NR	NA/NR	Stage I - III	Primary
388	Gordon	2013	Prospective-Restrospective	United States	258	258	59	NR	23-83	179	69	79	31	Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
409	Shan	2013	Prospective-Restrospective	Asia	1463	NR	58	NR	20-82	1104	75.5	359	24.5	Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
419	He	2013	Prospective cohort	Asia	197	NR	62	NR	22-88	132	67	65	33	Resection	Intestinal, Diffuse	Stage I - IV	Primary
421	Prins	2013	Prospective-Restrospective	Europe	154	NR	64	NR	33.8-81.3	126	81.8	28	18.2	Resection	NA/NR	Stage I - III	Primary, Metastasis
422	Okines	2013	Prospective-Restrospective	Europe	402	NR	62	NR	23-85	Biopsy:194 ; Resection: 258	Biopsy:80 ; Resection: 78	Biopsy: 50; Resection: 74	Biopsy: 20 ; Resection: 22	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
433	Phillips	2013	Prospective-Restrospective	United States	135	NR		NR		121	90	14	10	Biopsy from primary tumor,	NA/NR	Stage II - IV	Primary

														Resection			
434	Lee	2013	Retrospective cohort	Asia	322	NR		NR		221	69	101	31	Resection, blood samples	Intestinal, Diffuse, Mixed	Stage II - IV	Primary, Metastasis
471	Zhou	2012	Prospective-Restrospective	Asia	227	NR	60	NR	24-79	157	69	70	31	TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
494	Yoon	2012	Prospective-Restrospective	United States	675	NR	64.8	NR						Resection	Signet-ring cell carcinoma	Stage I - IV	Primary
496	Terashima	2012	Prospective-Restrospective	Asia	1059	829	63; 63	NR	27-80; 33-80	736	69	323	31	Resection	NA/NR	Stage I - IV	Primary
510	Janjigian	2012	Prospective cohort	Multiple countries	381	NR	NR	NR	NR	256	67	125	33	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage IV	Primary, Recurrent or persistent disease, Metastasis
526	Gomez-Martin	2012	Prospective-Restrospective	Europe	148	NR	67	9.6	33-83	111	75	37	25	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Indeterminate	Stage IV	Primary, Recurrent or persistent disease, Metastasis
528	Tamura	2012	Prospective cohort	Asia		73		NR						Biopsy from primary tumor, Resection	NA/NR	NR	NR
544	Halon	2012	Prospective cohort	Europe	78	NR	62	NR	37-84	54	69	24	31	NR	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
553	Chua	2012	Systematic review	Australia	11337	NR	59	NR	51-69	NR	NR	NR	NR	Biopsy from primary tumor, Resection	NA/NR	Stage I - IV	Primary
555	Kim	2012	Prospective-Restrospective	Asia	114		59	NR	24-77	82	71.9	32	28.1	Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage IV	Recurrent or persistent disease
595	Kunz	2012	Prospective-Restrospective	United States	169	647	65	NR	23-93	116	69	53	31	Resection	NA/NR	Stage I - IV	Primary

Table 20: Test characteristics

Refid	First Author	Year	HER2 Methodology	HER2 Status IHC				Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
				Neg/0	1+	2+	3+					
41	Otsu	2015	IHC	182	86	54	38	NR	NR	Hofmann	NR	NR
206	Chen	2014	IHC			16.0%	5.1%	NR	NR	NR	NR	NR
226	Yoshida	2014	IHC/ISH/FISH	Surg,117; Biop,65	Surg, 30; Biop, 19	Surg, 25; Biop, 48	Surg, 35; Biop, 26	Surg,61; Biop,47	Surg,139; Biop,100	hofmann	NR	NR
239	Geppert	2014	FISH	NR	NR	NR	NR	NR	NR	NR	NR	NR
303	Prins	2014	IHC/ISH/SISH	NR	NR	NR	NR	auto: 46.8%; Conv: 18.1%	NR	NR	NR	NR
307	Wang	2013	IHC	52	NR	NR	20 (1 of 6 adenocarcinomas)	NR	NR	hofmann	NR	NR
388	Gordon	2013	IHC/ISH/FISH	130	NR	NR	18	28	230	press 1994 & 2002 ISH- amplified if >2.0 and average gene copy number was at least 4.0; not amplified if <2.0 (no mention of 2.0)	NR	NR
409	Shan	2013	IHC/ISH/FISH	1109	NR	211	143	53	255	hofmann & ruschoff	NR	NR
419	He	2013	IHC/ISH/FISH	125	28	25	19	31	166	Hofmann	NR	NR
421	Prins	2013	IHC/ISH/FISH/SISH	89	39	6	15	FISH: 27; SISH; 25	FISH: 122; SISH; 128	Blair 2009		HER-2 heterogeneity was defined as the presence of both HER-2 protein overexpression/gene amplification in one to two tumor core(s) (not expressed as a percentage) and low HER-2/absence of gene amplification in the other core(s), observed in one tumor.HER-2 protein intratumoral heterogeneity was observed in 10 of 148 (7%) cases. HER-2

												gene copy number heterogeneity was found in 3% (5 of 153) using SISH, and was found in 6% (9 of 149) using FISH.
422	Okines	2013	IHC/ISH/BDISH	Biopsy: 194 ; Resection: 264	Biopsy:7 ; Resection: 22	Biopsy:4 ; Resection:16	Biopsy:12 ; Resection: 27	Biopsy:12 ; Resection: 19	Biopsy:146 ; Resection: 158	hofmann & Ruschoff	For biopsies, HER2 positive was defined by IHC3+ or IHC2+ and BDISH positive (EMEA definition for approval of trastuzumab). For resections, two definitions of HER2 positive were analysed; (i) IHC3+ or IHC2+ and BDISH positive (EMEA) or (ii), IHC3+ or BDISH positive with any IHC result, (US FDA definition for trastuzumab approval), as all samples had been subjected to both IHC and BDISH	NR
433	Phillips	2013	IHC/ISH/CISH	No genfitinib: 56; Genfitinib: 48	NR	NR	No genfitinib: 12; Genfitinib: 19	NR	NR	hofmann	Positive HER2 was defined as IHC 3+, or IHC 2+ reflexed to with amplified ISH, in either biopsy or resection specimen. IHC 0–1+ specimens,irrespective of the ISH results, were considered to be HER2-negative.	NR
434	Lee	2013	IHC/ISH/FISH	128	121	48	25	56	17	dako	IHC 0/1+ were considered negative for HER2 overexpression, and IHC 2+/3+ were considered positive. The HER2 gene was considered to be amplified if HER2/CEP 17 ratio was P2.0. A ratio of 4 or more was defined as high-level amplification and a ratio P2.0 and <4.0 as low-level amplification.	Intratumoral heterogeneity of HER2 overexpression was defined as the presence of >5% and <50% of the tumour cells with HER2 IHC 2+ or 3+, whereas cases showing IHC 2+ or 3+ in >50% of the tumour cells were considered to have homogeneous HER2 overexpression in the whole section. Of the 322 cases, 54 (16.8%) and 19 (5.9%) showed heterogeneous and homogeneous HER2 overexpression, respectively, as per the definition. Forty-six (95.8%) of the 48 cases with IHC 2+ and 8 (32.0%) of the 25 cases with IHC 3+ showed heterogeneous overexpression, and the intratumoral heterogeneity of HER2 overexpression was significantly associated with IHC 2+(P < 0.001).
471	Zhou	2012	IHC/ISH/FISH	189	4	11	23	27	200	hofmann	Scores of 0 and 1+ were considered negative for HER-2/ neu overexpression, and scores of 3+ were considered positive. Scores of 2+ were considered	NR

											overexpression if FISH confirmed amplification [4,17]. Gene amplification was defined as cancer cell nuclei exhibiting a ratio of HER-2/neu to CEP17 (centromeric probe 17) ≥2, or when an HER-2/neu signal cluster was observed	
494	Yoon	2012	IHC/ISH/FISH	NR	NR	NR	NR	117	558	NR	NR	FollowingCAP breast cancer guidelines, a sample was considered to have heterogeneous HER2 amplification if there were more than 5% but less than 50% infiltrating tumor cells with an HER2/ CEP17 ratio greater than 2.2 (results were the same for a 2.0 cut point).
496	Terashima	2012	IHC/ISH/DISH	443	210	101	75	IHC 2+/dual-ISH negative, 63 (7.6%); IHC 2þ/dual-ISH positive, 38 (4.6%); IHC 3þ/dual-ISH negative, 2 (0.2%); and IHC 3þ/dual-ISH positive, 72 (8.7%)	NR	hofmann	For EGFR, an IHC score of 3+ was defined as positive, and IHC scores of 0, 1+, and 2+ were defined as negative. For HER2, an IHC score of 3+ or an IHC score of 2+ with a dualISH HER2/CEP17 ratio of 2.0 or more was defined as positive, and IHC scores of 0 and 1+ or a score of IHC 2+ with a dual-ISH HER2/CEP17 ratio of less than 2.0 were defined as negative	NR
510	Janjigian	2012	IHC/ISH/DISH	NR	NR	NR	78	78	NR	hofmann &ruschoff	Tumors showing 3+ protein expression or gene amplification were considered HER2 positive.	NR
526	Gomez-Martin	2012	IHC/ISH/FISH/ dc-SISH	122	5	6	15	F: 27; ds-S: 32	F: 121; ds-S: 116	ruschoff	HER2+ disease was defined as IHC3+ or FISH+ (FISH ratio >2) or dc-SISH+ (dc-SISH ratio >2).	NR
528	Tamura	2012	IHC/ISH/FISH	SV2-61gamma: 61; Dako HercepTest: 35	SV2-61gamma: 1; Dako HercepTest: 10	SV2-61gamma: 2; Dako HercepTest: 11	SV2-61gamma: 9; Dako HercepTest: 17	NR	NR	hofmann & bang	NR	NR

544	Halon	2012	IHC	35	20	17	6	NR	NR	Remmele scale (IRS) and hofmann	NR	NR
553	Chua	2012	IHC/ISH/FISH/ CISH					NR	NR		NR	NR
555	Kim	2012	IHC/ISH/FISH	64	34	7	6	10	101	hofmann	HER2 positivity was defined as IHC 3+ or HER2 gene amplification by FISH.	
595	Kunz	2012	IHC/ISH/FISH	ASCO/ CAP: 126; TOGA: 124	ASCO /CAP: 29; TOGA : 18	ASCO /CAP: 5; TOGA : 12	ASCO /CAP: 5; TOGA : 10	16	144	ToGA & ASCO/CAP breast cancer guideline	NR	Heterogeneous HER2 immunoreactivity was the most frequent cause of discrepant results between FISH and HER2 IHC. In total, 8 of 169 (4.7%) carcinomas demonstrated heterogeneous HER2 immunoreactivity with some areas of tumor displaying strong HER2 staining adjacent to areas lacking HER2 reactivity.

Table 21: Outcomes 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/ median	Range										
41	Otsu	2015	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
206	Chen	2014	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
226	Yoshida	2014	NA/NR	NA/NR	NA/NR	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	57.0 % (κ=0.224) btw surg and biop spec 72.7 % (κ=0.313) btw surg and biop spec	NA/NR
239	Geppert	2014	43mo	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
303	Prins	2014	27.1mo	2.7-271.1mo	10	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
307	Wang	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
388	Gordon	2013	NA/NR	NA/NR	NA/NR	IHC/FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	90	NA/NR
409	Shan	2013	NA/NR	NA/NR	NA/NR	IHC/FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	98.5	NA/NR
419	He	2013	NA/NR	NA/NR	26	IHC V FISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	88.83; 88.83	NA/NR
421	Prins	2013	27.1mo	2.7-271.1mo	10	IHC/FISH Vs. IHC/SISH	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	90% 92% (SISH & FISH was 95%)	NA/NR

422	Okines	2013	NA/NR	NA/NR	NA/NR	IHC/BDISH Vs. Biopsy/resection	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	96% 92.9%	NA/NR
433	Phillips	2013	49	26-93	NA/NR	Biopsy/resection	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	91%	NA/NR
434	Lee	2013	15mo	1-82mo	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
471	Zhou	2012	64mo	1-108mo	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
494	Yoon	2012	12.6		NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
496	Terashi ma	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
510	Janjigia n	2012	18.2	3.3-44.1	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
526	Gomez -Martin	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
528	Tamura	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
544	Halon	2012	The followup was scheduled every 3 months for the first 2 years and then every 6 months		NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
553	Chua	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
555	Kim	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
595	Kunz	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR

Table 22: Outcome 2

Ref id	First Author	Year	Comparisons	HR (CI)	Median OS (Her2+)	Median OS (Her2-)	p value for OS	HR for PFS (CI)	Median PFS (Her2+)	Median PFS (Her2-)	p value for PFS	Quality	Algorithm	xtra info
41	Otsu	2015	IHC	NA/NR	NA/NR	NA/NR	0.88	NA/NR	NA/NR	NA/NR	0.045	NA/NR	NA/NR	There was a significant association between HER2 expression and clinicopathological characteristics. patients with HER2-positive tumors were older (p=0.004) predominantly males (p=0.0035), and had a higher tumor grade (p=0.0005). HER2 expression was significantly associated with histological differentiation (p=0.0007). 163 patients had intestinal

														type of cancer. Patients with HER2 positive tumor were mainly males (p=0.0036), and with a more advanced tumor stage (p=0.045). RFS of patients with HER2-positive tumors was worse than that of those with HER2-negative ones (p=0.011).
206	Chen	2014	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR		Her2 IHC over expression rate was 5.1%. Univariate analyses showed none of the clinicopathological characteristics was associated with the IHC Her2 over expression compared to negative expression (0/1+)(p>0.05), with the exception of a higher rate (12.2%) of IHC Her2 (3+) in moderate differentiation subset (p=0.02).
226	Yoshida	2014	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Same as the one used in the Toga trial	HER2 gene amplification was detected in 31 % (61/200) of surgically resected tumors and 32 % (47/147) of biopsy specimens. Except for immunohistochemistry (IHC) equivocal (2+) cases, the concordance rates between IHC and FISH was 90.9 % in surgically resected tumors and 90.2 % in biopsy specimens. In IHC 2+ cases, the rate of HER2 gene amplification was 56 and 38 % in surgically resected tumors and biopsy specimens, respectively. IHC-FISH discordance was mainly due to intratumoral heterogeneity and low-level gene amplification. Polysomy 17 was detected in 5.5 and 7.5 % of surgically resected tumors and biopsy specimens and significantly correlated with IHC score, but polysomy 17 could explain one IHC score 3+ and FISH-negative tumor only.
239	Gepper t	2014	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	The best stratification into favourable and unfavourable prognoses was shown by P1, percentage of cells with less than two ZNF217 signals; P2, percentage of cells with fewer ERBB2- than ZNF217 signals; and P3, overall ratio of ERBB2-/ZNF217 signals. Median survival times for P1 were 32 vs 73 months, 28 vs 73. esophageal adenocarcinoma. Applied multicolor FISH for 4 gene loci to TMA. months for P2; and 27 vs 65 months for P3. Regarding each tumour grade P2 subdivided patients into distinct prognostic groups independently within each grade, with different median survival times of at least 35 months
303	Prins	2014	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	A high automated HER2/ CEP17 ratio (≥1.8) was significantly associated with worse survival (HR 1.731; 95% CI 1.075 to 2.786; p=0.024). However, agreement between automated and conventional FISH was only 72.2% and 71.4% between automated FISH and SISH, compared with 94.6% for conventional FISH/SISH. Therefore, thresholds for HER2/CEP17 amplification were sequentially raised from HER2/CEP17 ratio 1.8 till 5.0. A HER2/CEP17 ratio threshold of ≥3.6 had similar prognostic significance as conventional FISH (HR 1.880; 95% CI 1.060 to 3.332; p=0.031 vs HR 1.828; 95% CI 1.102 to 3.033; p=0.020), yielded comparable amplification rates as

														conventional FISH (14.3% vs 18.1%) and comparable agreement to SISH/Immunohistochemistry (IHC). primarily a comparison of FISH methods and study of HEr2 as a prognostic marker in esophageal adenocarcinoma
307	Wang	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	the expression of her2 is closely related to differentiation degree, infiltration depth, lymph nodes metastasis and pTNM stage. The level of her2 expression is also an independent prognostic factor in esophageal cancer pts.study of esophageal carcinomas. 66 were squamous cell carcinoma; only 6 adenocarcinomas were studied. Only 26 cases were from distal esophagus.
388	Gordon	2013	Surg alone Vs. post-op chemo		22 16	24 44	0.76 0.025		11mo 13mo	17mo 34mo	0.60 0.026	NA/NR	NA/NR	There was a significant interaction between HER2 amplification and treatment with respect to both disease-free survival (DFS) (P = 0.020) and overall survival (OS) (P = 0.034). HER2 status was not a prognostic marker among patients who received no postoperative chemoradiation.study concludes that patients with tumors not HER2 amplified benefit from treatment with post-operative chemoradiation compared to surgery alone. No survival benefit was demonstrated for patients with HER2-amplified tumors treated with chemoradiation (only 28 cases available for analysis)
409	Shan	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2 overexpression (3+) was detected in 9.8% of carcinomas and more frequently observed in GEJ cancer cases, in the intestinal type, and in the well or moderately differentiated type (P=0.003, 0.000, and 0.000, respectively). HER2 equivocal (2+) was detected in 14.4% of cases
419	He	2013	NA/NR	NA/NR	NA/NR	NA/NR	p=0.006	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	The positivity rates in intestinal type and well-differentiated gastric cancer were higher than those in diffuse/mixed type and poorly-differentiated gastric cancer respectively (28.57% vs 13.43%, P = 0.0103; 37.25% vs 11.64%, P < 0.0001), but were not correlated with gender, age, tumor location or TNM stage, depth of invasion, lymph node metastases and distant metastasis. In poorly-differentiated gastric cancer patients, those without lymph node metastasis showed a higher HER2 positivity rate than those with lymph node metastasis (26.47% vs 7.14%, P = 0.0021). This association was not present in those patients with well-differentiated gastric cancer (28.57% vs 43.33%, P = 0.2832). in patients presenting well-differentiated tumors, the overall survival of the HER2-positive group was significantly worse than that of the HER2-negative group (P = 0.0123)

421	Prins	2013	IHC Vs. SISH FISH		20.7 21 21	35.6 39.4 41.3	0.020 0.042 NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	All IHC 3+ cases were amplified by SISH and in 93% by FISH. Of the IHC 2+cases, this was 33% (SISH) and 50% (FISH). Of the IHC 1+ cases, still 6% (SISH) and 8% (FISH) showed amplification.
422	Okines	2013						NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2 positive rate (IHC3+, or IHC2+/BDISH positive) was 10.9% in the whole cohort and 10.4% in resection specimens. A further 4.0% of resections were IHC negative/BDISH positive. HER2 status was neither prognostic, nor (in pre-treatment biopsies) predicted enhanced benefit from chemotherapy [HER2 positive HR 0.74 (0.14–3.77); HER2 negative HR 0.58 (0.41–0.82), interaction P = 0.7]. However, the power of the predictive analysis was limited by the small number of HER2 positive pre-treatment biopsies
433	Phillips	2013	No genfitinib Vs. Gefitinib	NA/NR	25% 11%	18% 33%	0.28 0.28	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	HER2 positivity was found in 23%; 28% with GEJ primaries and 15% with esophageal primaries (P = 0.10). There was no statistical difference in clinicopathologic features between HER2-positive and negative patients except HER2-negative tumors were more likely to be poorly differentiated (P < 0.001). After adjusting for treatment in multivariable Cox analysis, HER2 status was not associated with outcomes (hazard ratio [HR] 0.74 [95% confidence interval (CI) 0.28–1.99; P = 0.55] locoregional recurrence; HR 1.18 [95% CI 0.73–1.91; P = 0.49] distant recurrence; HR 1.09 [95% CI 0.67–1.75; P = 0.74] any recurrence; HR 0.84 [95% CI 0.53–1.33; P = 0.47] mortality). Findings were similar after adjusting for both treatment and tumor differentiation.
434	Lee	2013	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	IHC scoring was performed microscopically by two independent pathologists who were blinded to the clinical details of individual patients. The case-by-case final consensus result was discussed and determined in a common	NA/NR	Kaplan–Meier survival analysis revealed that the heterogeneous overexpression was significantly associated with longer disease-free survival times than the homogeneous, and the high average GCN was most associated with poor outcome. Also, there was a strong correlation between the IHC and FISH results for each spot. Quantitative polymerase chain reaction (PCR) analysis of the cancer tissues and the cell-free plasma showed that HER2 gene copy by quantitative PCR on tissue correlated well with those by FISH, but plasma HER2 level was not.

												session.		
471	Zhou	2012	IHC	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	All samples on the TMA sections from gastric cancers were reviewed by two pathologists independently to determine scores of IHC. For discordant opinions, the samples were re-examined by the two pathologists to achieve a consensus score	NA/NR	In univariate analysis, Lauren classification, differentiated histology, tumor size (≥5 cm), invasion depth, lymph node metastasis, TNM stage, vessel invasion and HER-2/ neu overexpression/amplification were all associated with poor survival (P <0.05). A multivariate Cox proportional hazards model identified Lauren classification, vessel invasion, TNM stage and tumor size (≥ 5cm) as bearing prognostic importance (P <0.05); however, HER-2/neu overexpression/amplification was not an independent prognostic factor in this model
494	Yoon	2012	NA/NR	2.04			p=.025	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Overall, 117 EACs (17%) demonstrated HER2 amplification, of which 20 (17%) showed HER2 heterogeneity. All HER2-heterogeneous tumors were amplified. Among HER2-amplified tumors, heterogeneous tumors had significantly higher frequency of poor histologic grade and polysomy 17. In multivariable models that included number of metastatic lymph nodes, grade, tumor stage, and polysomy 17, only HER2 heterogeneity and node number were prognostic among HER2- amplified tumors, with heterogeneity showing worse DSS (hazard ratio, 2.04; 95% CI, 1.09 to 3.79; P ? .025) and OS (P ? .026). Among HER2-nonamplified EACs, polysomy 17 was independently associated with worse DSS (P ? .012) and OS (P ? .023). All-cause and disease-specific deaths within 5 years of surgery were experienced by 492 patients (72.9%) and 458 patients (67.9%), respectively.
496	Terashima	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Five-year OS and relapse-free survival (RFS) were 73.6% [95% confidence interval (CI)=69.3%–77.9%] and 66.7% (95% CI = 62.1%–71.3%), respectively, in the S-1 group, compared with 61.9% (95% CI =57.1%–66.7%) and 53.7% (95% CI = 48.8%–58.7%) in the surgery-only group, respectively. HER2-positive status was not associated with outcomes in either the S-1 group or the surgery-only group.Multivariate analysis showed that EGFR positivity correlated with poor outcomes [HR = 1.504; 95% confidence interval (CI) =

														1.020–2.149; P=0.040]. Treatment with S-1 improved survival compared with surgery alone, irrespective of EGFR and HER2 status
510	Janjigian	2012	IHC/ISH	NA/NR	13.9mo	11.4mo	0.047		6.5mo	6.4mo	0.889	NA/NR	NA/NR	In the multivariate logistic model, there were significantly higher rates of HER2 positivity in patients with liver metastasis (liver metastasis 31%; no liver metastasis 11%; P = 0.025) and intestinal histology (intestinal 33%; diffuse/mixed 8%; P = 0.001). No significant differences in HER2 positivity were found between resections and biopsies or primaries and metastases. multivariate analysis indicated that HER2 status was not an independent prognostic factor (hazard ratio 0.79; 0.44–1.14; P = 0.194).
526	Gomez-Martin	2012	IHC FISH dc-SISH	0.42 (0.21 - 0.84) 0.49 (0.29-0.83) 0.53 (0.33-0.85)	21.39m 19.61m 19.58m	9.82m 9.72m 9.66m	0.005 0.007 0.009	0.62 (0.34-1.13) 0.58 (0.36-0.94)	7.98mo 7.98 NR	5.45mo 5.35 NR	0.119 NR NR			There were significant differences in HER2+ rates according to histological type when FISH (intestinal, 23%; no intestinal, 4%; p<0.0001) or dc-SISH (intestinal, 26%; no intestinal, 6%; p<0.0001) amplification techniques were used. Factors associated with favourable survival in the multivariate analysis were intestinal type and Her2+ determination by IHC, FISH or dc-SISH
528	Tamura	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	All of the equivocal or discordant cases with a HER2 score of 3+ using Dako HercepTest exhibited amplification of the HER2 gene regardless of the HER2 score determined with SV2 61gamma.
544	Halon	2012	IHC	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	The overexpression of HER-2 was associated with poorly differentiated tumors, but this correlation was not significant (P = 0.064). No relationship was found between HER-2 expression and primary tumor size and degree of spread to regional lymph nodes. Both univariate and multivariate analyses revealed that TNM stage and patient's age were the crucial negative prognostic factors. No correlation was observed between patient survival and expression of HER-2 estimated using both scales
553	Chua	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Forty-four percent of patients had Stage I/II, and 56% had Stage III/IV disease. Immunohistochemistry was most commonly used to assess HER2 expression, identifying a median rate of 18% (range, 4–53%) of gastric cancer demonstrating HER2 overexpression. In patients with and without HER2 overexpression, the median 3-year disease-free survival rate was 58% (range, 50–88%) and 86% (range, 62–97%), respectively. Of the 35 studies reporting the impact of HER2 overexpression on survival, 20 studies (57%) reported no difference in overall survival, two studies

														(6%) reported significantly longer overall survival in patients with HER2 overexpression and 13 studies (37%) reported significantly poorer overall survival in patients with HER2 overexpression. The median overall survival and 5-year survival rate was 21 (range, 10–57) months and 42%, and 33 (range, 13–80) months and 52% in patients with and without HER2 overexpression, respectively.
555	Kim	2012	NA/NR	NA/NR	7.5mo (95% CI=6.1-8.8)	10.8mo (95% CI=9.2-12.3)	0.068	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	In the subgroup of patients without diffuse-type histology by Lauren classification, patients with HER2-positive gastric cancer had a significantly worse TTP than those with HER2-negative gastric cancer (p=0.024). Multivariate analysis including performance status, HER2 status and disease status were performed in this subgroup. HER2 status and performance status were significantly associated with TTP (p=0.014 and p=0.035, respectively). The hazard ratio (HR) of HER2 positivity and ECOG 2 was 2.926 and 2.489, respectively. However, in OS, no factor was statistically significant.
595	Kunz	2012	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	figure 5 in article	The ToGA IHC scoring scheme demonstrated greater concordance with the HER2 FISH analysis compared with the ASCO/CAP IHC scoring scheme. Twelve of 99 (12%) gastric carcinomas were positive for HER2. Of these, HER2 was more often identified in intestinal-type adenocarcinomas (10 of 52, 19%) compared with diffuse (2 of 34, 6%) adenocarcinoma. Seven of 70 (10%) gastroesophageal junction carcinomas were positive for HER2 of which all were intestinal type (7 of 58, 12%). HER2 status or primary tumor site did not correlate with patient survival.