Quantitative measurement of HER2 levels by targeted proteomics predicts survival in gastric cancer patients treated with trastuzumab

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BACKGROUND and PURPOSE

- Trastuzumab-based chemotherapy is the standard treatment for HER2-positive gastric cancer (GC).[1]
- Previous reports[2,3] showed HER2 gene amplification by fluorescent in situ hybridization (FISH) was correlated with sensitivity to trastuzumab.
- A predictive value of HER2 protein expression levels for trastuzumab sensitivity in a HER2 positive population has not been reported.
- We quantitated HER2 protein expression levels using a targeted proteomic[4] assay and identified a cutoff predictive of improved response to trastuzumab.

PATIENTS and METHODS

Patients

- 249 patients from Seoul National University Hospital, 2005-2014
- Histologically confirmed recurrent or metastatic GC
- Formalin-fixed, paraffin-embedded (FFPE) biopsies were collected prior to treatment
- HER2 analyzed by immunohistochemistry (IHC) and/or FISH
- 95 patients treated with trastuzumab-based chemotherapy were used for survival analysis

Methods

Figure 1. Liquid Tissue®-SRM workflow for protein analysis from FFPE tissue. Tumor tissue was microdissected and solubilized for downstream mass spectrometry analysis. Protein levels were quantitated using selected reaction monitoring mass spectrometry (SRM-MS)

RESULTS

Table 1. Patient characteristics

|		Total N=249	HER2+ Tmab+ N=95
Age	median years (range)	63 (22-85)	64 (22-85)
Sex	Male	189 (75.9)	78 (82.1)
	Female	60 (24.1)	17 (17.9)
ECOG	0	33 (13.9)	19 (20.0)
	1	181 (76.4)	66 (69.5)
	2	23 (9.7)	10 (10.5)
Palliative setting	Metastatic	159 (63.9)	64 (67.4)
	Recurrent	90 (36.1)	31 (32.6)
Tumor location	Stomach	219 (88.0)	89 (93.7)
	GEJ	30 (12.0)	6 (6.3)
HER2 status	Positive	158 (63.5)	95 (100)
	Negative	91 (36.5)	0
Pathology	Adenocarcinoma	229 (92.0)	90 (94.7)
	PCC	16 (6.4)	2 (2.1)
	Others	4 (1.6)	3 (3.2)
Signet ring cell component†	No	213 (85.5)	86 (90.5)
	Yes	36 (14.5)	9 (9.5)
Lauren classification	Intestinal	59 (23.7)	22 (23.2)
	Diffuse	39 (15.7)	5 (5.3)
	Mixed	10 (4.0)	4 (4.2)
	Unknown	141 (56.6)	64 (67.4)
Overall survival	median months (95% CI)	14.1 (12.5-16.4)	22.5 (17.5-31.9)
PFS of Tmab	median months (95% CI)	-	9.0 (7.0-11.2)
Follow-up duration	median months (range)	48.5 (7.1-112.6)	39.1 (7.1-110.2)

- Data presented as N (%) unless otherwise specified.
- Abbreviations: CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; GEJ, gastroesophageal junction; HR, hazard ratio; PCC, poorly cohesive carcinoma; PFS, progression-free survival; Tmab, trastuzumab.
- † Contains signet ring feature: either pure signet ring cell carcinoma (i.e. poorly cohesive carcinoma) or adenocarcinoma containing signet ring features.
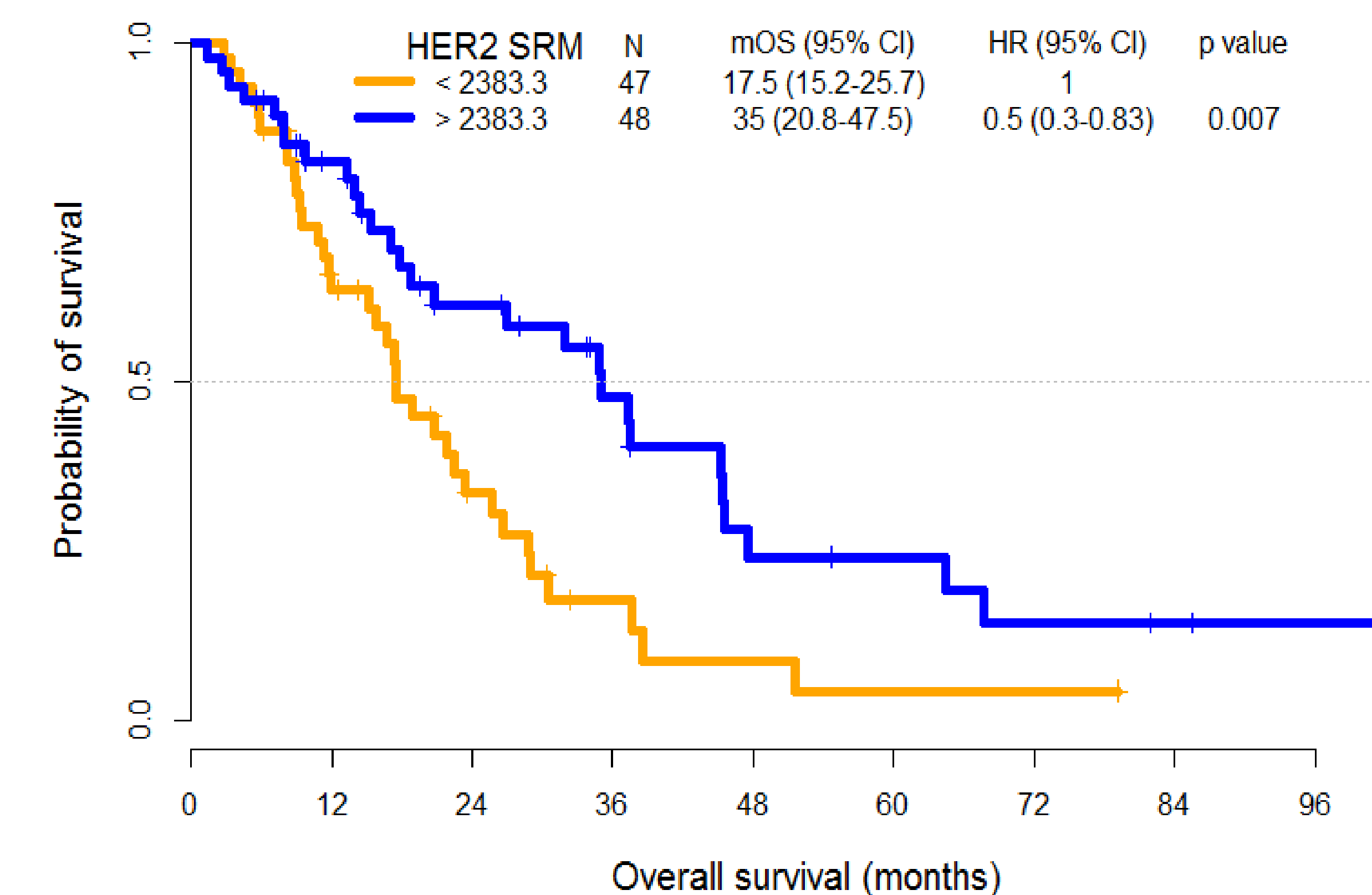
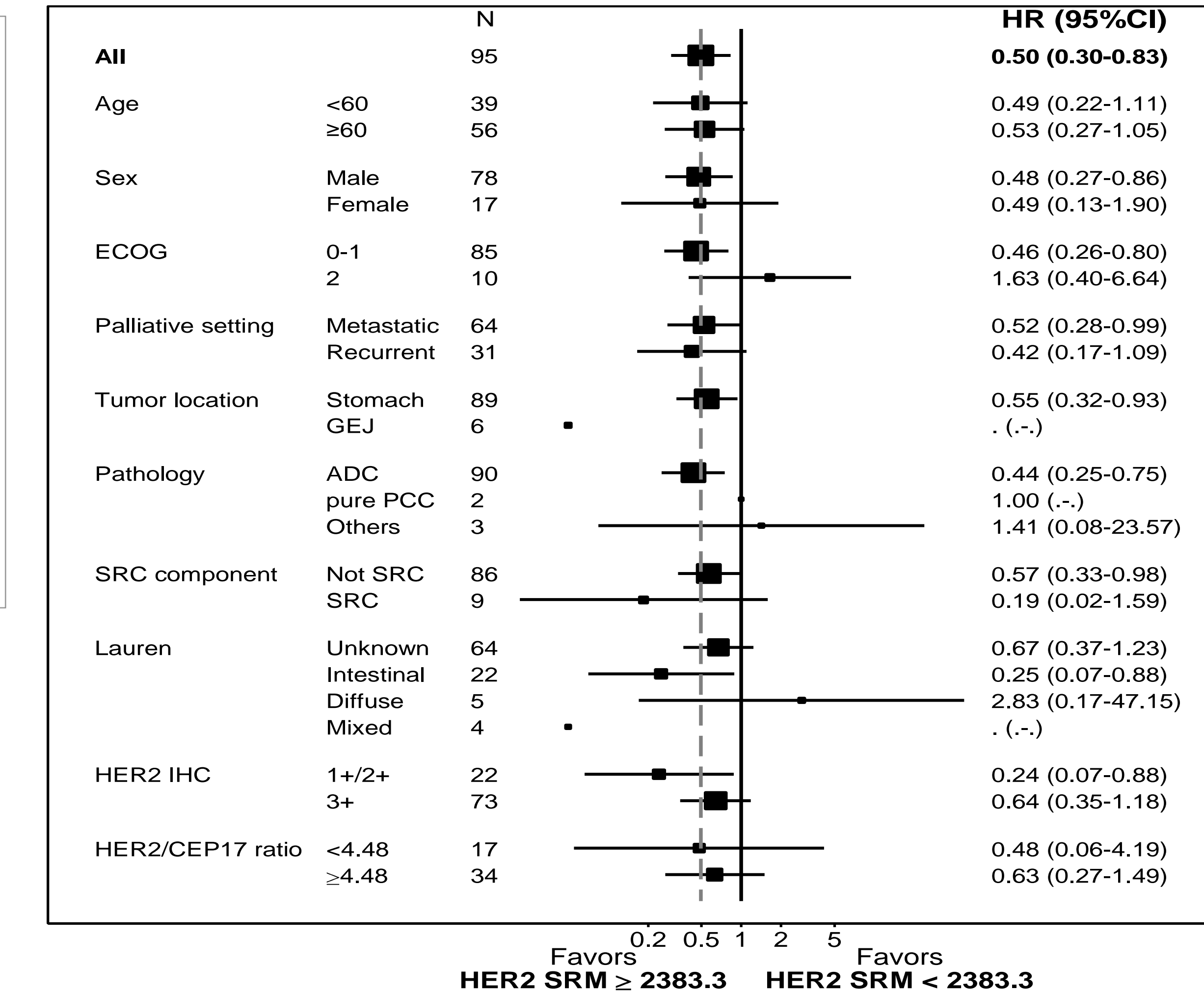### Figure 2. Quantitative distribution of HER2 SRM
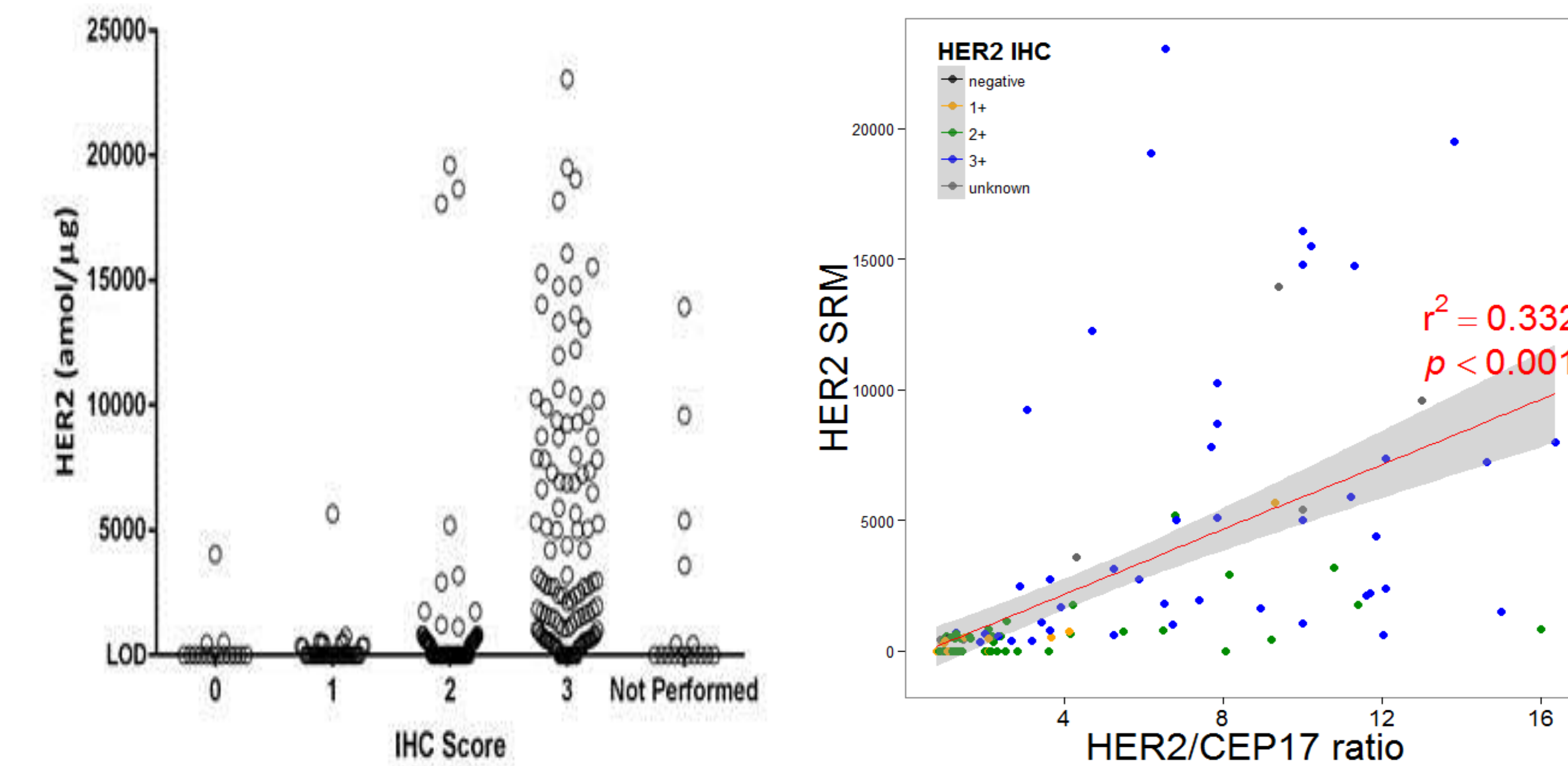
- HER2 protein level by SRM positively correlated with HER2 IHC (left) and HER2 FISH (right).

Figure 3. OS of HER2 + patients treated with Tmab

HER2 SRM	N	mOS (95% CI)	HR (95% CI)	p value
< 2383.3	47	17.5 (15.2-25.7)	1	
> 2383.3	48	35 (20.8-47.5)	0.5 (0.3-0.83)	0.007

- The optimal cutoff of HER2 SRM to predict trastuzumab sensitivity was determined by the lowest p value of log rank test.
- Overall survival (OS) of patients with HER2 SRM > 2383.3 amol/ug was significantly longer than that of those with lower HER2 levels.

Figure 4. Subgroup analysis of OS

		N		HR (95%CI)
All		95		0.50 (0.30-0.83)
Age	<60	39		0.49 (0.22-1.11)
	≥60	56		0.53 (0.27-1.05)
Sex	Male	78		0.48 (0.27-0.86)
	Female	17		0.49 (0.13-1.90)
ECOG	0-1	85		0.46 (0.26-0.80)
	2	10		1.63 (0.40-6.64)
Palliative setting	Metastatic	64		0.52 (0.28-0.99)
	Recurrent	31		0.42 (0.17-1.09)
Tumor location	Stomach	89		0.55 (0.32-0.93)
	GEJ	6		. (.-.)
Pathology	ADC	90		0.44 (0.25-0.75)
	pure PCC	2		1.00 (.-.)
	Others	3		1.41 (0.08-23.57)
SRC component	Not SRC	86		0.57 (0.33-0.98)
	SRC	9		0.19 (0.02-1.59)
Lauren	Unknown	64		0.67 (0.37-1.23)
	Intestinal	22		0.25 (0.07-0.88)
	Diffuse	5		2.83 (0.17-47.15)
	Mixed	4		. (.-.)
HER2 IHC	1+/2+	22		0.24 (0.07-0.88)
	3+	73		0.64 (0.35-1.18)
HER2/CEP17 ratio	<4.48	17		0.48 (0.06-4.19)
	≥4.48	34		0.63 (0.27-1.49)

Favors HER2 SRM ≥ 2383.3 — Favors HER2 SRM < 2383.3

- HER2 SRM > 2383.3 amol/ug was significantly correlated with prolonged OS irrespective of clinicopathologic features.

CONCLUSIONS

- Quantitative measurement of HER2 levels measured by targeted proteomics correlated with HER2 IHC and FISH.
- HER2 protein levels were highly variable in HER2 IHC 3+.
- High levels of HER2 (> 2383.3 amol/ug) significantly correlated with prolonged overall survival following trastuzumab.

REFERENCES

1. Bang Y et al. Lancet. 2010; 376:687-97.
2. Gomez-Martin C et al. JCO. 2013; 31:4445-52
3. Ock CY et al. Clin Cancer Res. 2015 Feb 23. [Epub]
4. Hembrough et al. J Mol Diagn. 2013;15:454-65.