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An Exploratory Analysis of HER-2 Amplification and Overexpression in Advanced Endometrial Carcinoma: A Gynecologic Oncology Group Study

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Abstract

Objectives—To investigate the frequency and potential prognostic or predictive value of HER-2 amplification or overexpression in advanced and recurrent endometrial cancers.

Methods—Immunohistochemical staining (IHC; DAKO Herceptest®) and fluorescence *in situ* hybridization (FISH; Vysis Inc. PathVysion® DNA Probe Kit) were performed on specimens collected on a randomized Gynecologic Oncology Group (GOG) protocol testing the addition of paclitaxel to doxorubicin/cisplatin.

Results—HER-2 overexpression (either 2+ (moderate) or 3+ (strong) immunostaining) and *HER-2* gene amplification (a ratio of *HER-2* copies to chromosome 17 *(CEP17*) copies ≥ 2) were detected in 44% (104 of 234; 58 were 2+ and 46 were 3+) and 12% (21 of 182) of specimens, respectively. There was a significant increased frequency of overexpression in serous tumors versus all others (23 of 38, 61% versus 81 of 196, 41%, respectively, *P*=0.03). *HER-2* amplification also appeared to be more common in serous tumors, but results were not significant (6 of 28, 21% versus 15 of 141, 11%, *P*=0.12). There was a significant association between grade and *HER-2* amplification among non-serous tumors, with grades 1, 2, and 3 cancers demonstrating 3%, 2% and 21% amplification, respectively (*P*=0.003). Neither overexpression nor amplification predicted overall survival (OS) after adjusting for treatment and performance status.

Conclusions—*HER-2* amplification was more common in high grade tumors with a trend to being more common in serous tumors. There was no clear evidence for a survival difference or a difference in benefit from the addition of paclitaxel for women with HER-2 amplified or overexpressed tumors, however power to detect clinically meaningful differences was low.

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INTRODUCTION

Endometrial cancer is the fourth most common cancer among women in the United States.[1] While early stage endometrial cancer is usually curable with surgery, advanced disease has a poor prognosis. Median survival on recent GOG protocols for advanced or recurrent disease is about a year.[2] A recent randomized phase III study, GOG #177, enrolled patients with measurable stage III, stage IV, or recurrent endometrial carcinoma, and randomly assigned treatment as either doxorubicin and cisplatin (AP) or doxorubicin, cisplatin, and paclitaxel with G-CSF (TAP). The primary results of this trial have been published and showed a superior overall response (57% vs 34%), progression-free survival (median 8.3 vs 5.3 months), and OS (median 15.3 vs 12.3 months) with the three-drug combination.[3]

HER-2 is one of the most-studied molecular markers in anticancer therapy. *HER-2* (*HER-2/neu, ERBB2*) encodes an 185kD transmembrane cell surface receptor glycoprotein with tyrosine kinase activity that belongs to the epidermal growth factor receptor family. HER-2 amplification or overexpression has been reported in 4% to 69% of endometrial carcinomas, and some series note it to be overexpressed more often in tumors of serous histology, which are aggressive cancers with a propensity to metastasize early.[4]

The clinical relevance of HER-2 is best established in breast cancer. HER-2 amplification/overexpression is found in 15–30% of human breast cancers and is an independent prognostic factor for increased likelihood of relapse in early stage disease.[5] It has also been reported to be a predictive marker of resistance to tamoxifen therapy, benefit from doxorubicin-based adjuvant chemotherapy and benefit from trastuzumab (anti-HER-2 monoclonal antibody) in both the adjuvant and metastatic disease setting.[6–8]

A number of clinical trials have also suggested that HER-2 status is predictive for response to taxanes.[9–12] However, not all the evidence is supportive. Van Poznak et al evaluated tumor samples from patients treated on a series of trials using single agent taxanes for metastatic breast cancer, and found no correlation between HER-2 status and response to therapy, although they did observe resistance to therapy in tumors expressing phosphorylated HER-2.[13,14] The objectives of this study were to examine the effects of HER-2 expression and amplification on overall outcomes in women with advanced endometrial carcinoma and explore the possibility of a differential treatment effect for paclitaxel in patients with advanced HER-2 positive endometrial carcinoma.

MATERIALS AND METHODS

Patient Materials

Tumor blocks or cut slides from either primary or recurrent tumor were available for 234 of 263 eligible patients participating in GOG #177. Sufficient sample remained after IHC for FISH analysis in 182 of these 234 cases. Central pathology review documenting cell type and grade was performed.

IHC assay

Immunostaining was performed on 5-μm-thick formalin-fixed, paraffin-embedded specimens using DAKO Herceptest® Kit (supplied by DAKO Corp., Carpenteria, CA). Scoring was performed according to manufacturer recommendations. Zero: undetectable staining or membrane staining in <10% of the tumor cells. 1+: faint and incomplete membrane staining in >10% of the tumor cells; 2+: weak to moderate complete membrane staining in >10% of the tumor cells; 3+: strong complete membrane staining observed in >10% of the tumor cells. HER-2 protein expression was categorized as negative (scores 0 and 1+), or positive (scores 2+ and 3+), consistent with most of the literature in endometrial carcinoma.[15,16]

FISH assay

Fluorescence *in situ* hybridization for detection of *HER-2* gene amplification was performed on tissue sections adjacent to those analyzed by IHC using the Vysis, Inc. PathVysion *HER-2* DNA Probe Kit (Vysis/Abbot Inc., Des Plaines, IL), which is a hybridization mixture of a *HER-2* probe labeled with Spectrum Orange, and a chromosome 17 enumeration probe *CEP17*, labeled with Spectrum Green. Slides were pretreated using the Vysis/Abbot Inc. Paraffin Pretreatment Kit. In each tumor sample an average of 82 (30–200) well-defined malignant nuclei were scored. Both the absolute number of *HER-2* signals and the ratio of *HER-2* signals to *CEP17* signals were recorded. Tumors with a *HER-2:CEP17* signal ratio < 2 were considered non-amplified; those with a ratio of 2 or greater were considered amplified. The chromosome 17 copy number alteration was estimated using two approaches: 1: calculation of the percentage of cells with given copy number as described previously; 2: calculation of the mean copy number per cell according to Santin et al.[17,18] In the latter method monosomy was defined as mean *CEP17* copies per cell less than 1.5, disomy was defined as mean *CEP17*/cell of 1.5 to 2.5, and polysomy was defined as mean *CEP17*/cell of 2.5 or more.

Clinical endpoint

OS was defined as the length of life measured from the date of entry on to the clinical trial. Clinical response was defined as either complete disappearance of all gross disease or at least a 50% reduction in the product of perpendicular diameters of each lesion for a minimum of four weeks.[3]

Statistical Analysis

Fisher's Exact test was used to test for independence between the baseline categorical and *HER-2* covariates and CEP17 aneusomy (1.5 or fewer copies per cell, 1.5–2.5 copies per cell and greater than 2.5 copies per cell). The Kruskal-Wallis test was used to compare the distributions of the number of *HER-2* and *CEP17* copies per cell, mean copy number ratio of *HER-2* to *CEP17*, and the proportions of polysomic, monosomic and aneusomic cells between cell type groups. Additionally, the distributions of patient age were compared between HER-2 status groups using the Kruskal-Wallis test. The Kaplan-Meier method was used to estimate and plot the failure time distributions by HER-2 status. Cox proportional hazard models were used to explore the relationship between OS and both HER-2 expression and *HER-2* amplification. All models included treatment and the main effect terms for either HER-2 expression or *HER-2* amplification. Treatment by HER-2 interaction terms were tested in each proportional hazards model. Hazard ratio estimates are reported with 95% Wald confidence intervals. Logistic regression modeling was used to explore the relationship between clinical response to treatment and both HER-2 expression and amplification. The effect of HER-2 overexpression and amplification on the odds of response was estimated as an odds ratio (OR) adjusted for treatment. Treatment-by-HER-2 interaction terms were also tested. OR estimates are reported with 95% Wald confidence intervals. Except for subgroup analyses, performance status was included in all models as a covariate (0 or 1 vs. 2).

HER-2 expression was analyzed in two different ways. The first grouped 0 and 1+ together versus 2+ and 3+. The second separated out the 2+ from 3+ patients, creating three categories: 0 or 1+, 2+, and 3+. The relationships between survival and clinical response endpoints and *HER-2* amplification were also analyzed in two ways: using *HER-2* as a binary variable with a cut off ratio ≥2, and using *HER-2* as a continuous variable.

All analyses were considered exploratory in nature. Unless otherwise indicated, a level of 0.05 was used to designate statistical significance. All *P*-values reflect the significance of two-tail tests. No adjustments were made to account for multiple comparisons. Patients who were not evaluated for either assay were excluded from all analyses. Additionally, patients with non-

informative FISH results were excluded from statistical analyses involving *HER-2* amplification.

In analyses of grade, twelve tumors were included in the "grade 3" category which actually had a grade noted as "not specified". Nine of these were serous or clear cell tumors; some pathologists consider grading of these subtypes inappropriate.

RESULTS

Clinical and tumor characteristics of the overall group of patients, the group on whose tumors IHC staining was performed, and the group available for FISH analysis were similar. FISH was interpretable in 169 of 182 (93%) cases. Thirteen tumors were non-informative due to technical problems related either to poor tissue morphology and tissue conservation or FISH-related problems.

Tumor cells from 104 of 234 patients (44%) showed positive (2+/3+) cellular membrane HER-2 expression on IHC staining (Table 1A). Of these, 46 tumors (20%) were strongly HER-2-positive (3+) and 58 tumors (25%) were moderately positive (2+). There was a statistically significant association between HER-2 overexpression with histologic type of tumor; 23 of 38 (61%) serous tumors were IHC-positive versus 81 of 196 (41%; $P = 0.03$) non-serous tumors (Table 1A). Ten of 38 (26%) serous tumors were strongly IHC-positive (3+) versus 36 of 196 (18%) other histologic subtypes. There were seven patients whose tumors were of clear cell histology; five overexpressed HER-2 (71%). *HER-2* amplification was detected in 21 (12%) of 169 endometrial carcinomas (Table 1B). Of these, 10 tumors showed high levels of *HER-2* amplification (ratio ≥ 5.0). The proportion of *HER-2*-amplified tumors among serous carcinomas was six of 28 (21%), versus 15 of 141 (11%) among all other histologic subtypes ($P = 0.12$). *HER-2* amplification was observed in three of seven patients with clear cell histology.

No significant relationships were detected between either HER-2 expression or *HER-2* amplification and patient race/ethnicity or age, performance status, or disease status at study entry. However, tumor grade was significantly related to *HER-2* amplification with 3%, 4%, and 21% of grade 1, 2, and 3 tumors respectively, demonstrating amplified *HER-2* (P=0.002) (Table 1B). This held true even when serous tumors were excluded; grades 1, 2, and 3 nonserous cancers had 3%, 2% and 21% of cases amplified, respectively (P=0.003).

Concordance between FISH and IHC was observed in 101 of 169 (60%) cases where both assays were successfully performed: 13 were positive and 88 were negative by both methods (Table 2). Of the ten tumors with high FISH amplification ratios (≥ 5), six showed strong immunostaining (3+), and four stained 0–1+. Photomicrographs of representative tumors showing strong (3+) immunostaining (Panels A and B), moderate (2+) immunostaining (Panel C) and no (0) immunostaining shown in Figure 1.

Of the twenty FISH negative cases staining 3+ by IHC only 4 samples showed a gain of HER-2 copy number due to polysomy for chromosome 17. Table 3 shows that serous tumors were more likely both to amplify HER-2/*neu* and to be polysomic for chromosome 17.

There was no significant effect of HER-2 expression or amplification on survival in the group as a whole (Figure 2) after adjusting for treatment and performance status. For the binary categorization, the estimated ratio of the hazards of death of HER-2 2+, 3+ relative to 0, 1+ on survival in the group as a whole was 1.17 (95% confidence interval (CI) 0.88 to 1.55; P=0.28). Similarly, for the three-way categorization, the estimated effect of HER-2 2+ relative to 0, 1+ was 1.16 (95% CI 0.83 to 1.61; $P = 0.40$) and the estimated effect of HER-2 3+ relative to 0, 1+ was 1.18 (95% CI 0.82 to 1.70; P=0.36).

For the model using a binary categorization for FISH, the estimate of the death hazard ratio of a *HER-2/CEP17* of ratio ≥ 2 relative to < 2 was 0.95 (95% CI 0.59 to 1.55; *P*=0.84) and for the continuous covariate, it was 1.001 (95% CI 0.94 to 1.06; *P*=0.97).

Evidence of a qualitative treatment by *HER-2* amplification interaction with respect to clinical response did not reach statistical significance (interaction OR: 0.15 *P*=0.06). The estimate of the effect of TAP was positive within the subgroup with nonamplified tumors (OR for treatment TAP relative to AP: 2.75 (95% CI 1.40 to 5.40) and negative within the subgroup with amplified tumors (OR for treatment TAP relative to AP 0.43 (95% CI 0.17 to 1.09). Evidence of a quantitative treatment by HER-2 expression interaction with respect to clinical response in the opposite direction also did not reach statistical significance (interaction OR: 2.42 *P*=0.11). The estimated ratio of the odds of response to TAP relative to AP is 1.54 (95% CI 0.76 to 3.14) for tumors staining 0 or 1+ and 3.74 (95% CI 1.63 to 8.58) for tumors staining 2+ or 3+ (Table 4B). Table 4A provides the crude estimates of response and number of patients within HER-2 and treatment subgroups.

There was also no clear evidence of any treatment – HER-2 interaction with respect to survival (Table 4B). The *P*-values for the tests of interaction terms were: 1) *P*=0.71 for IHC: 0, 1+ vs 2+, 3+; 2) *P*=0.69 for IHC: 0, 1+ vs. 2+ vs. 3+ (both interaction terms were tested jointly); 3) *P*=0.16 for FISH: <2 vs. ≥ 2; and 4) *P*=0.21 for FISH, continuous. However, with only 21 tumors classified as having amplified HER-2 and 19 deaths among them (Table 4A), the power under clinically relevant alternative hypotheses was very low.

DISCUSSION

Our study is unique in that we examined only patients with recurrent or advanced measurable disease, and examined outcomes in the setting of uniform first-line chemotherapy treatment. *HER-2* amplification was more common in USC relative to other histologic cell types, (21% versus 11%) and, among nonserous tumors, was more common in grade 3 tumors (21%) than in grade 1 or 2 tumors (3% and 2%). The overall results for levels of *HER-2* amplification (12%) and overexpression (44%) and the concordance of the two assays are within the ranges reported by others.[4,13,14,19–22] The series in the literature consistently note that the percentage of cases with moderate to high immunostaining is higher than the percentage with gene amplification. Saffari et al, working in the laboratory of Dr Michael Press, who pioneered the immunohistochemical detection of HER-2 in breast cancer, reported that 21% of endometrial cancer cases demonstrated gene amplification and 52% demonstrated moderate or high immunostaining;[15] more recently Morrison et al, in the largest series to date, reported that 6.6% of 483 cases demonstrated gene amplification and 14% demonstrated moderate or high immunostaining.[16] In GOG 181B, a trial which used a central commercial laboratory to screen patients with advanced stage or recurrent endometrial cancer for a clinical trial of trastuzumab, 13% of cases demonstrated gene amplification and 37% demonstrated moderate or high immunostaining.[20] Some of the variability in the percentage of endometrial cancers demonstrating gene amplification is likely due to the mix of tumors in the various series; those such as the current one, which are composed of patients with advanced or recurrent disease, will contain more patients with high grade and serous tumors, which are more likely to demonstrate *HER-2* gene amplification. The variability in HER-2 immunostaining results in the literature is probably related both to a different mix of patients in different series, and the wide variety of antibodies and staining conditions used. It is well-known that anti-HER-2 antibodies have very variable sensitivity in formalin-fixed, paraffin embedded tissue, and that other technical issues are also critical.[21] It is possible that it is technical issues with immunohistochemistry that result in some part of the discordance between immunohistochemistry and FISH results in both our and other series. Our results do not support the possibility that gain of *HER-2* copies due to polysomy for chromosome 17 is frequently

responsible for protein overexpression.[24] We also saw no evidence of particular biologic significance for the subset of tumors that was both amplified and overexpressed, as was suggested by Morrison et al.[16]

Previous studies have varied widely in their conclusions about whether HER-2 is of prognostic value in endometrial cancer.[4,21,22,25–28] Coronado et al found the prognostic value of HER-2 overexpression to be higher in early stages than in advanced stages of disease.[29] This might relate to the higher levels of overexpression found in serous and grade 3 cancers. Serous histology is a poor prognostic indicator in early-stage disease, but in the setting of GOG trials for advanced and recurrent disease it is only a weak prognostic factor (relative hazard ratio for OS 1.2) and does not predict for chemotherapy response.[30] Saffari et al, whose retrospective series of 90 cancers (82% of which were stage I or II) included only three serous tumors, none of which were *HER-2* amplified, also noted HER-2 overexpression to be a predictor of poor OS on multivariable analysis, and additionally found that adjuvant chemotherapy or radiotherapy were associated with improved survival only among tumors that overexpressed HER-2.[15] Our study does not suggest any effect of *HER-2* amplification or expression on survival in the setting of advanced or recurrent endometrial carcinoma undergoing first-line chemotherapy. It could be hypothesized that any survival difference for women with an aggressive subset of tumors will be less evident in a setting where everyone receives chemotherapy. However, the number of cases with *HER-2* amplification in both our series and in the other series in the literature is small, limiting the precision of results.

There was also no clear evidence from the current study to suggest a differential effect of paclitaxel on survival between patients with *HER-2* amplified tumors and patients with *HER-2* non-amplified tumors. Given the imprecision of the subgroup treatment effect estimates, these results must be viewed with even more caution. Of particular interest is whether overexpression or amplification of HER-2 might predict for response of endometrial cancers to trastuzumab. A complete response to single agent trastuzumab in a patient with USC has been reported.[31] Using entry criteria of IHC 2+ or 3+ staining, GOG #181B found no activity of trastuzumab as a single agent in heavily pretreated women with recurrent or metastatic endometrial cancer.[20] Entry criteria were therefore revised to include only those patients whose tumors were FISH +, but the study was recently closed due to slow enrollment, illustrating the challenges posed in developing tailored treatments to specific biologic subsets in less common tumors.

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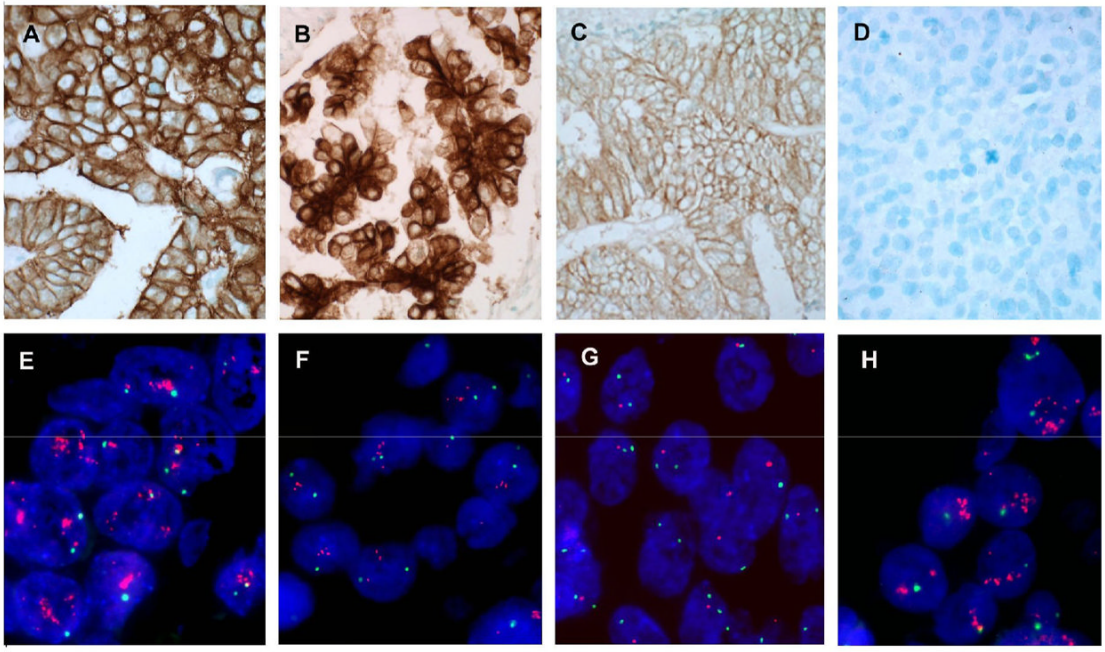
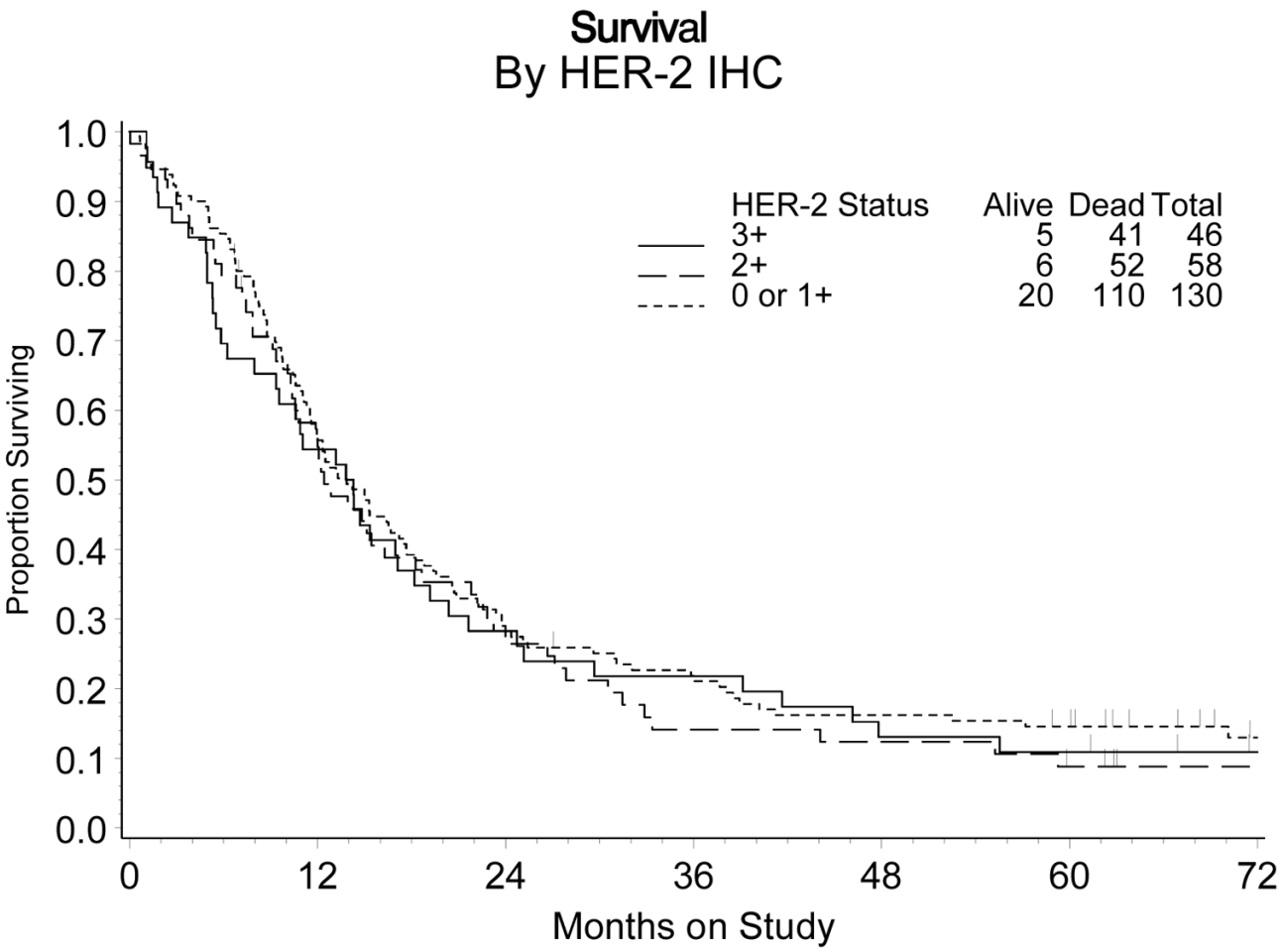
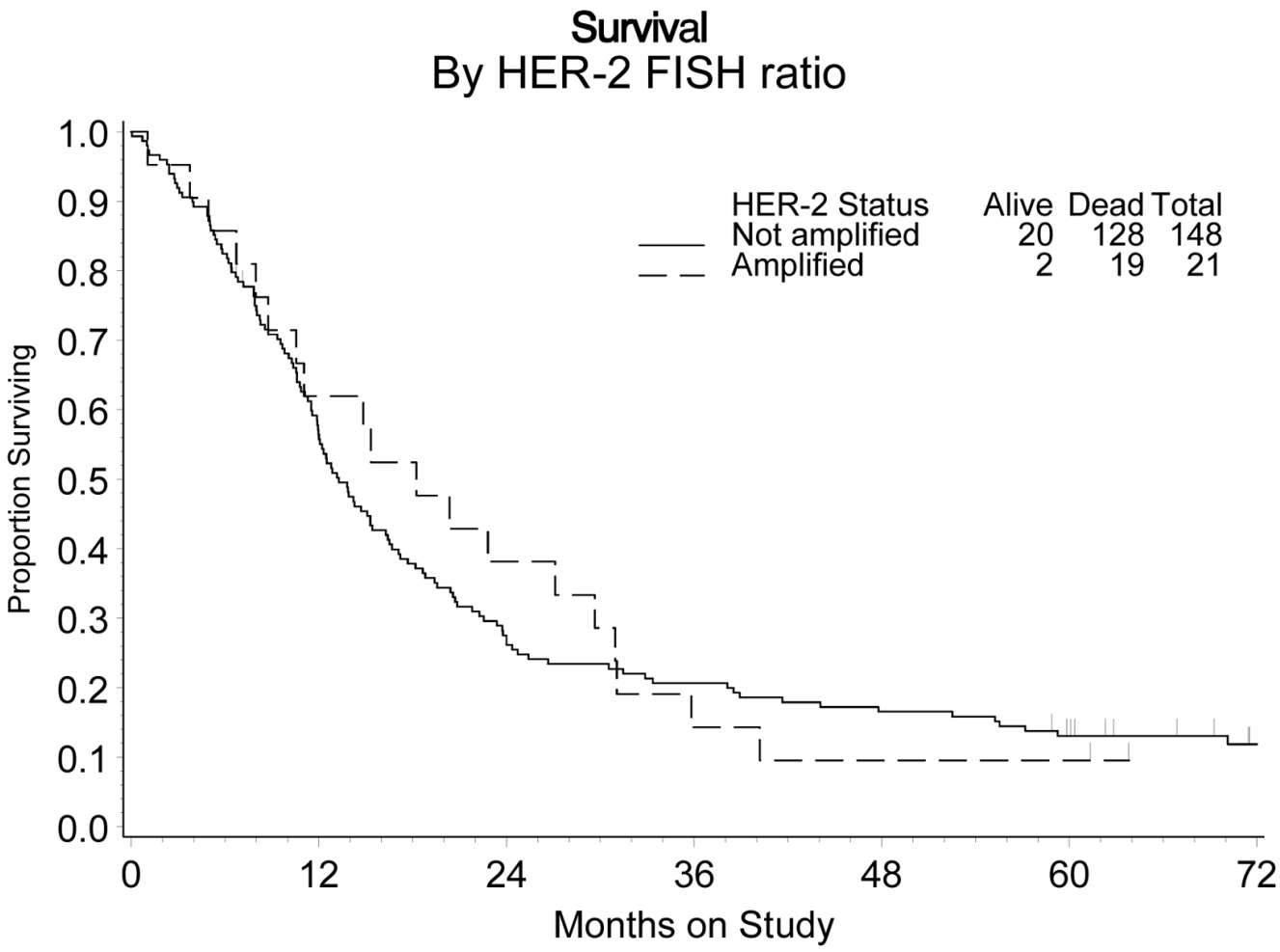


Figure 1.

Representative photomicrographs of adjacent tissue sections from different cell types of advanced endometrial carcinomas after HER-2 IHC (*A–D*) and FISH (*E–H*). The *HER-2* gene is localized by *red* fluorescent signals, and the chromosome 17 centromere (*CEP17*) is localized by *green* fluorescent signals. The cells were counterstained with DAPI (4′,6-diamidino-2-phenilindole (blue). Original magnification × 1200. *A, E,* endometrial carcinoma of mixed histology displaying the high level of protein expression (3+) and high level of gene amplification (mean *HER-2*/cell =28.6; mean *CEP17*/cell = 2.5; ratio = 11.3). 52% of cells were polysomic for chromosome 17. *B, F*, USC, exhibiting strong 3+ membranous staining of HER-2 and moderate levels of *HER-2* amplification (mean *HER-2*/cell =5.7; mean *CEP17*/cell = 1.8; ratio = 3.24). 40% of cells were monosomic and 12% of cells were polysomic for chromosome 17. *C, G,* Example of endometrial carcinoma of endometrioid histology, which was moderately positive for HER-2 expression (2+) but negative for *HER-2* amplification (mean *HER-2*/cell =1.5; mean *CEP17*/cell = 2.4; ratio = 0.64). 34% of cells were polysomic for chromosome 17. *D, H,* endometrial carcinoma of endometrioid histology displaying no HER-2 protein expression (0) but high level of gene amplification (mean *HER-2*/cell =16.8; mean *CEP17*/cell = 1.6; ratio = 10.2). 55% of cells were polysomic for chromosome 17.





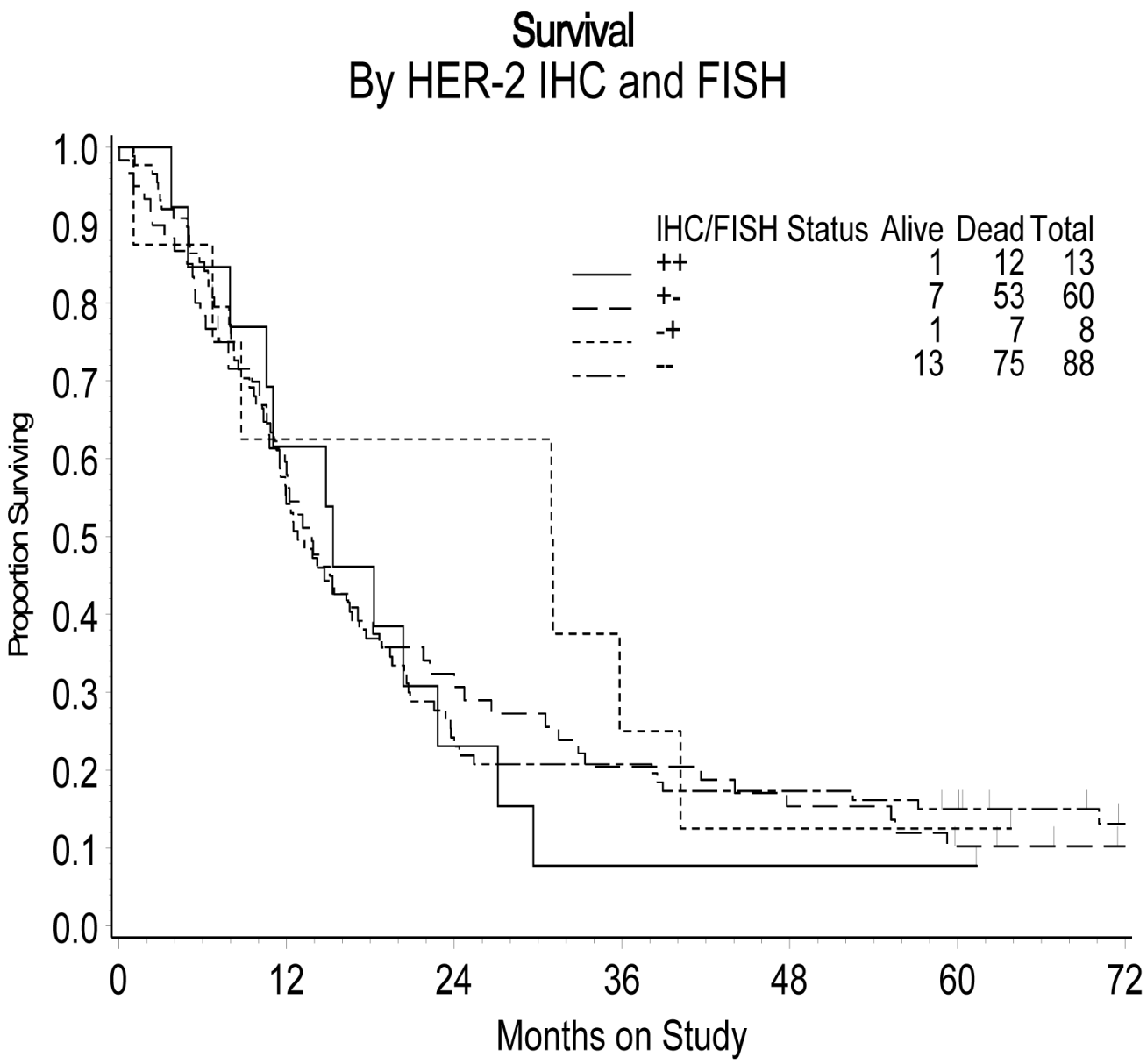


Figure 2.
Survival curves
A. survival by HER-2 IHC status
B. survival by *HER-2* FISH status
C. survival by combined IHC and FISH status

Table 1

Table 1A. Patient Characteristics and HER-2 expression

Characteristics	IHC (n=234) 0, 1+		2+		3+		Total
	N	%	N	%	N	%	N
Age at study entry							
<40	3	100.0	0	0.0	0	0.0	3
40–49	6	50.0	2	16.7	4	33.3	12
50–59	35	54.7	14	21.9	15	23.4	64
60–69	55	56.1	26	26.5	17	17.3	98
70–79	29	55.8	13	25.0	10	19.2	52
>=80	2	40.0	3	60.0	0	0.0	5
Race/ethnicity							
Asian	3	60.0	1	20.0	1	20.0	5
Black/African American	18	54.5	10	30.3	5	15.2	33
Hispanic	1	33.3	2	66.7	0	0.0	3
White	108	56.0	45	23.3	40	20.7	193
Regimen							
AP	58	51.3	34	30.1	21	18.6	113
TAP	72	59.5	24	19.8	25	20.7	121
Performance status							
0	65	59.6	29	26.6	15	13.8	109
1	53	50.0	25	23.6	28	26.4	106
2	12	63.2	4	21.1	3	15.8	19
Cell Type							
Serous Adenocarcinoma	15	39.5	13	34.2	10	26.3	38
Clear Cell	2	28.6	2	28.6	3	42.9	7
All Others	113	59.8	43	22.8	33	17.5	189
Histologic Grade*							
1	28	68.3	6	14.6	7	17.1	41
2	45	57.7	19	24.3	14	17.9	78
3 or not specified	57	50.0	32	28.1	25	21.9	114
Stage or Disease Status							

Table 1A. Patient Characteristics and HER-2 expression

Characteristics	IHC (n=234) 0, 1+		2+		3+		Total
	N	%	N	%	N	%	N
III	13	54.2	7	29.2	4	16.7	24
IV	33	58.9	13	23.2	10	17.9	56
Recurrent	84	54.5	38	24.7	32	20.8	154
Response to treatment							
Complete or Partial	67	60.4	25	22.5	19	17.1	111
All Others	63	51.2	33	26.8	27	22.0	123
Total	130	55.6	58	24.8	46	19.7	234

Table 1B. Patient Characteristics and *HER-2* amplification

Characteristics	FISH (n=169)[1] Not amplified		Amplified		Total
	N	%	N	%	N
Age at study entry					
<40	2	100.0	0	0.0	2
40–49	8	100.0	0	0.0	8
50–59	37	88.1	5	11.9	42
60–69	62	83.8	12	16.2	74
70–79	36	90.0	4	10.0	40
>=80	3	100.0	0	0.0	3
Race/ethnicity					
Asian	2	66.7	1	33.3	3
Black/African American	22	84.6	4	15.4	26
Hispanic	2	100.0	0	0.0	2
White	122	88.4	16	11.6	138
Regimen					
AP	72	88.9	9	11.1	81
TAP	76	86.4	12	13.6	88
Performance status					
0	68	87.2	10	12.8	78

Table 1B. Patient Characteristics and *HER-2* amplification

Characteristics	FISH (n=169)[1] Not amplified		Amplified		Total
	N	%	N	%	N
1	68	87.2	10	12.8	78
2	12	92.3	1	7.7	13
Cell Type					
Serous Adenocarcinoma	22	78.6	6	21.4	28
Clear Cell	3	50.0	3	50.0	6
All Others	123	91.1	12	8.9	135
Histologic Grade*					
1	31	96.9	1	3.1	32
2	49	96.1	2	3.9	51
3 or not specified	67	78.8	18	21.2	85
Stage or Disease Status					
III	17	81.0	4	19.0	21
IV	31	79.5	8	20.5	39
Recurrent	100	91.7	9	8.3	109
Response to treatment					
Complete or Partial	73	85.9	12	14.1	85
All Others	75	89.3	9	10.7	84
Total	148	87.6	21	12.4	169

[1]The 13 individuals with inconclusive FISH results are not included in this table

*Histologic grade is missing for one individual with 2+ staining intensity; grade was not specified for 11 individuals and combined with grade 3

Table 2

The number of patients in each category of HER-2 expression and amplification

	HER-2 Expression IHC			
HER-2 Amplification FISH	**0,1+**	**2+**	**3+**	**Total**
Inconclusive result	9	2	2	13
Amplified	8	5	8	21
No amplification	88	40	20	148
Missing	25	11	16	52
Total	130	58	46	234

Table 3

Mean number of *HER-2* and *CEP17* copies per cell, copy number ratios and proportion of cells aneusomic for chromosome 17 in uterine serous papillary carcinoma (USPC) versus all other histologic subtypes of endometrial cancer.

Parameter	Mean copy number (Standard Deviation)		Pr > Chi- Square*
	UPSC (n=28)	Other EC (n=141)	
HER-2	3.8 (2.45)	3.64 (6.78)	<0.001
CEP17	2.13 (0.56)	1.90 (0.51)	0.04
HER-2/CEP17	1.86 (1.33)	1.89 (3.11)	0.06
	Mean percentage of cells		
Monosomic +	33	39	0.14
Polysomic ++	30	20	0.03
Aneusomic +++	63	59	0.18

* Kruskal-Wallis Test;

+ percentage of cells with reduction of chromosome 17 to one copy;

++ proportion of cells with gain of chromosome 17 to three or more than three copies per cell;

+++ Monosomic+Polysomic.

Table 4

Table 4A. Clinical response and survival information within treatment and HER-2 subgroups

	FISH analysis of *HER-2*				IHC analysis of HER-2			
	Not amplified		Amplified		0 or 1+		2+ or 3+	
	AP	TAP	AP	TAP	AP	TAP	AP	TAP
Number of patients	72	76	9	12	58	72	55	49
Number of deaths	67	61	8	11	52	58	52	41
Median survival (months)	12.0	15.3	22.8	15.1	12.8	15.3	10.8	16.3
Number of responders	26	47	6	6	26	41	15	29
Proportion responding	0.36	0.62	0.67	0.50	0.45	0.57	0.27	0.59

Table 4B. Estimated treatment effects (odds ratio or hazard ratio with 95% confidence interval) of TAP relative to AP within FISH and within IHC subgroups using models with a treatment by HER-2 interaction for each endpoint and adjusted for performance status

	FISH analysis of *HER-2*			IHC analysis of HER-2		
Endpoint	Not amplified	Amplified	$P_{interaction}$	0 or 1+	2+ or 3+	$P_{interaction}$
Survival (HR)	0.62 (0.44, 0.89)	1.27 (0.51, 3.16)	0.16	0.71 (0.49, 1.04)	0.64 (0.42, 0.97)	0.71
Response (OR)	2.75 (1.40, 5.40)	0.43 (0.17, 1.09)	0.06	1.54 (0.76, 3.14)	3.74 (1.63, 8.58)	0.11

HR: death hazard ratio; OR: response odds ratio; $P_{interaction}$: *P*-value for the test of the treatment by HER-2 interaction term

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