

Randomized Phase II Trial of Seribantumab in Combination With Paclitaxel in Patients With Advanced Platinum-Resistant or -Refractory Ovarian Cancer

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A B S T R A C T

Purpose

Seribantumab is a fully human immunoglobulin G2 monoclonal antibody that binds to human epidermal growth factor receptor (HER) 3 (ErbB3), blocking heregulin (HRG) –mediated ErbB3 signaling and inducing ErbB3 receptor downregulation. This open-label randomized phase II study evaluated progression-free survival (PFS) with seribantumab in combination with once-per-week paclitaxel compared with paclitaxel alone in patients with platinum-resistant or -refractory ovarian cancer. A key secondary objective was to determine if any of five prespecified biomarkers predicted benefit from seribantumab.

Patients and Methods

Patients with platinum-resistant or -refractory epithelial ovarian, fallopian tube, or primary peritoneal cancer were randomly assigned at a ratio of two to one to receive seribantumab plus paclitaxel or paclitaxel alone. Patients underwent pretreatment core needle biopsy; archival tumor samples were also obtained to support biomarker analyses.

Results

A total of 223 patients were randomly assigned (seribantumab plus paclitaxel, $n = 140$; paclitaxel alone, $n = 83$). Median PFS in the unselected intent-to-treat population was 3.75 months with seribantumab plus paclitaxel compared with 3.68 months with paclitaxel alone (hazard ratio [HR], 1.027; 95% CI, 0.741 to 1.425; $P = .864$). Among patients whose tumors had detectable HRG mRNA and low HER2 ($n = 57$ [38%] of 151 with available biomarker data), increased treatment benefit was observed in those receiving seribantumab plus paclitaxel compared with paclitaxel alone (PFS HR, 0.37; 95% CI, 0.18 to 0.76; $P = .007$). The HR in patients not meeting these criteria was 1.80 (95% CI, 1.08 to 2.98; $P = .023$).

Conclusion

The addition of seribantumab to paclitaxel did not result in improved PFS in unselected patients. Exploratory analyses suggest that detectable HRG and low HER2, biomarkers that link directly to the mechanism of action of seribantumab, identified patients who might benefit from this combination. Future clinical trials are needed to validate this finding and should preselect for HRG expression and focus on cancers with low HER2 levels.

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INTRODUCTION

Ovarian cancer is a leading cause of cancer-related death among women, with approximately 240,000 cases diagnosed annually.¹ Although most patients respond to initial treatment, most eventually develop resistance to platinum-based therapy. At

this stage, single-agent therapies such as once-per-week paclitaxel confer a median progression-free survival (PFS) benefit of approximately 3 to 6 months.²⁻⁶

Patient outcomes could potentially be improved by identifying mechanisms of drug resistance and developing drugs that effectively combat such mechanisms. Many cancers become

desensitized to therapy by upregulating autocrine or paracrine growth factors that activate antiapoptotic signaling pathways.⁷⁻⁹ Notably, heregulin (HRG)-driven ErbB3 signaling mediates insensitivity to a broad range of therapies by activating the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway.¹⁰⁻¹⁵ In ovarian cancer, ErbB3 promotes cell proliferation in preclinical models, and approximately 30% of patients show evidence of an HRG/ErbB3 autocrine loop in tumor cells derived from malignant ascites.¹⁶ Together, these data suggest that blocking HRG/ErbB3 may increase sensitivity to therapy when this pathway is active.

Seribantumab (MM-121; Merrimack Pharmaceuticals, Cambridge, MA) is a fully human immunoglobulin G2 antibody that targets ErbB3, blocks HRG from binding, and downregulates the receptor.¹⁷ Here, we describe the results of a phase II study in which women with advanced platinum-resistant or -refractory ovarian cancer were randomly assigned to receive once-per-week paclitaxel or paclitaxel in combination with seribantumab. Additionally, this study was designed to examine five potential biomarkers of ErbB3 activation that had been previously highlighted by computational modeling: HRG, ErbB3, human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), and betacellulin (BTC).^{15,17} In preclinical studies, the levels of these proteins predicted response to seribantumab in mouse xenograft models.¹⁵ The hypotheses underlying these biomarkers are summarized in Appendix Table A1 (online only). Because it was not known how these biomarkers are altered by disease progression, they were measured in both archived tissue and mandatory pretreatment biopsies.

PATIENTS AND METHODS

Study Design

This was a multicenter, open-label, randomized phase II study of seribantumab combined with paclitaxel versus paclitaxel alone in patients with advanced platinum-resistant or -refractory ovarian cancer. Patients were randomly assigned at a ratio of two to one to receive seribantumab with paclitaxel or paclitaxel alone, with two stratification factors: Eastern Cooperative Oncology Group (ECOG) performance status¹⁸ (0 to 1 ν 2) and number of prior therapies (one to two ν three or more). The primary end point was PFS as assessed by RECIST (version 1.1).¹⁹ Secondary objectives included correlation of PFS with each of five biomarkers, efficacy of combination treatment with regard to overall survival (OS) and objective response rate (ORR), assessment of treatment according to health-related quality-of-life (HRQOL) scores, further characterization of the safety profile of seribantumab, and examination of predictive biomarkers.

Patients

Eligibility criteria included confirmed advanced or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer that was platinum resistant or refractory per Gynecologic Oncology Group criteria.^{20,21} Patients underwent mandatory pretreatment core needle biopsy and also submitted archived tumor samples, as available. All patients supplied written informed consent, and local institutional review boards and/or ethics committees approved the study protocol.

Study Procedures

Patients were randomly assigned at a ratio of two to one to either the experimental (seribantumab plus paclitaxel) or control arm (paclitaxel alone). Seribantumab was administered intravenously (40 mg/kg loading dose, then 20 mg/kg once per week). Paclitaxel was administered

intravenously (80 mg/m² during cycle one, with optional modification in subsequent cycles to 80 mg/m² once per week for 3 weeks followed by 1 week of rest). Disease status was assessed every 8 weeks. After discontinuation from study, patients were observed to determine OS. Data were collected every 4 months from the date of the 30-day follow-up visit.

Assessment of Response and Toxicity

The primary PFS end point was based on the intent-to-treat (ITT) and evaluable populations. The evaluable population was defined as all randomly assigned and treated patients who met all inclusion criteria and were evaluable for response (underwent one or more tumor evaluations while receiving treatment or exhibited early disease progression, including symptomatic deterioration or cancer-related death). Tumor assessment was per RECIST (version 1.1). HRQOL was assessed using the Functional Assessment of Cancer Therapy–Ovarian and –Taxane questionnaires. Safety analyses were performed on the safety population (all patients who received \geq one dose of study drug), and adverse events (AEs) were graded according to National Cancer Institute Common Terminology Criteria for Advanced Events (version 4.0) and MedDRA system organ class.

Biomarker Assays

Five biomarkers (HRG, ErbB3, HER2, EGFR, and BTC) were analyzed using one or more of the following assays: fluorescence-based quantitative immunohistochemistry (IHC), chromogenic IHC, chromogenic RNA in situ hybridization (ISH), and reverse-transcriptase polymerase chain reaction. Detailed descriptions of assays, methods, and scoring criteria are provided in the Data Supplement.

Statistical Analyses

A total of 210 patients were needed to provide 164 PFS events for the primary efficacy analysis. With a two-sided α of 0.20, this provided 88% power to detect a hazard ratio (HR) of 0.67. The primary efficacy analysis was performed using a stratified log-rank test, adjusting for number of prior therapies but not for ECOG status, because there were too few patients with ECOG status of 2. Survival analyses were performed using Kaplan-Meier estimates, and HR estimates were calculated using a stratified Cox proportional hazards model.

Biomarker analyses were performed according to a prespecified plan, using data from pretreatment biopsies in the safety population ($n = 220$). Biomarkers were initially evaluated by fitting to a Cox proportional hazards model of biomarker-by-treatment interaction. Biomarkers showing a treatment interaction ($P < .4$) were subsequently evaluated using two-variable models. Additional details of the analysis are included in the Data Supplement.

RESULTS

Study Population

A total of 223 patients in North America and Europe were randomly assigned between February 28, 2012, and March 12, 2013, to receive seribantumab plus paclitaxel ($n = 140$) or paclitaxel alone ($n = 83$). Trial flow, ITT population, and subgroups for analysis are shown in Figure 1. Patient demographics and baseline characteristics were well balanced between treatment arms (Table 1).

Efficacy

The data cutoff for the primary analysis was August 1, 2013. Median PFS in the ITT population was 3.75 months in the experimental arm compared with 3.68 months in the control arm

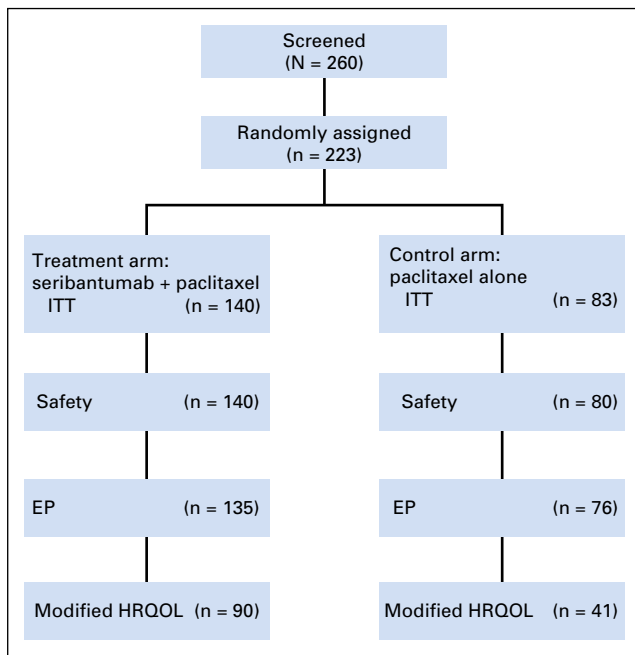


Fig 1. CONSORT diagram. EP, evaluable population; HRQOL, health-related quality of life; ITT, intent to treat.

(HR, 1.027; 95% CI, 0.741 to 1.425; stratified log-rank $P = .864$; events, 76.7%). The study therefore did not meet its primary end point of prolonging PFS in unselected patients. Median OS was 13.70 months in the experimental arm compared with 10.12 months in the control arm (HR, 0.991; 95% CI, 0.62 to 1.584; stratified log-rank $P = .972$; events, 35.4%). ORR was 13.6% (95% CI, 7.9% to 19.6%) in the experimental arm compared with 18.1% (95% CI, 9.8% to 26.4%) in the control arm, with no complete responses in either arm. HRQOL assessments detected no significant changes from baseline between treatment arms.

Safety

The overall safety profile was proportionate across study arms, with 140 patients (100%) in the experimental arm and 79 (98.8%) in the control arm reporting at least one treatment-emergent AEs (TEAEs). An overall increase in all-grade GI toxicities was observed; a majority of events, however, were reported as mild to moderate in severity (Table 2). Grade 3 or greater TEAEs occurred in 50 patients (35.7%) in the experimental arm and 24 (30.0%) in the control arm. Serious TEAEs occurred in 59 patients (42.1%) in the experimental arm and 25 (31.3%) in the control arm, whereas AEs leading to death were noted in 11 (7.9%) and two patients (2.5%) in the experimental and control arms, respectively. None of these deaths was related to study treatment, except in one patient who received seribantumab plus paclitaxel and died as a result of an infection or infestation.

A review of venous thromboembolic events (VTEs) revealed a difference in the rate of occurrence of pulmonary embolism, but not overall VTEs, between the experimental and control arms. Eight patients (5.7%) in the experimental arm and six (7.5%) in the control arm had at least one treatment-emergent VTE. Among patients in the experimental arm, six pulmonary embolisms

(4.3%) occurred, with five (3.6%) reported as grade 3. All these patients received corrective therapy and recovered. One nonserious AE (1.3%) of pulmonary embolism (grade 3) occurred on the control arm, which did not resolve. Overall, the safety profile was consistent with expected toxicities associated with ErbB inhibitors and combination with paclitaxel.

Biomarkers

All biomarker analyses, unless otherwise noted, were performed using data from pretreatment biopsies. In total, five biomarkers were measured using 13 assays (Appendix Table A2, online only). Although pretreatment biopsies were mandated for all patients, some samples did not contain sufficient tumor material. The number of patients with available data for each assay is summarized in Figure 2A.

Univariable biomarker analysis. The strength of biomarker-by-treatment interaction was used to rank the biomarkers, assays, and scoring methods (Fig 2A). On the basis of a predefined criterion of $P < .4$, four biomarkers and their associated assays were prioritized for further analysis: HRG chromogenic RNA ISH, ErbB3 chromogenic IHC, BTC chromogenic RNA ISH, and HER2 fluorescence-based quantitative IHC. Relationships between biomarker values and HRs are summarized in Figures 2B and 2C. Three of the biomarkers (HRG, BTC, and ErbB3) are semi-quantitative, and cut points were chosen from a limited set of options based on prevalence (Data Supplement). Patients with detectable levels of HRG (score ≥ 1 ; 60% of 159 patients with evaluable samples) had a PFS HR that favored the experimental arm, whereas those with undetectable levels of HRG (score < 1 ; 40% of 159 patients) favored the control arm (Fig 2B). In contrast, patients with detectable levels of BTC (score of ≥ 1 ; 78% of 137 patients) showed no benefit from seribantumab (PFS HR, approximately 1), and those with undetectable levels (score < 1 ; 22% of 137 patients) favored the control arm (Fig 2B). For ErbB3, medium to high levels (score ≥ 2 ; 80% of 141 patients) favored the experimental arm, whereas low or undetectable levels (score < 2 ; 20% of 141 patients) favored the control arm (Fig 2B). For HER2, which was measured quantitatively, the relationship between HER2 levels and HR was visualized by plotting HR as a function of HER2 (Fig 2C, blue dots; Data Supplement). Low HER2 favored the experimental arm, with benefit from seribantumab observed at HER2 levels below approximately 5.0 on a log₁₀ scale (100,000 receptors per cell).

Bivariable biomarker analysis. Six two-biomarker models were constructed using the four biomarkers that passed significance testing. For each pair of biomarkers, cumulative HR (calculated using all patients with biomarker levels above or below a given cutoff) was plotted as a function of one biomarker and then repeated at different values of the other biomarker (Fig 2D; Data Supplement). On the basis of the tradeoff between HR and prevalence, HRG and HER2 were identified as the most favorable pair of biomarkers (Fig 2D). To explore this interaction further, local HR was plotted as a function of HER2 level for all patients with available HRG and HER2 data, as well as for the subpopulation of HRG-positive patients (Fig 2C). Notably, HR was consistently lower (ie, more favorable) in the HRG-positive subpopulation, particularly at low levels of HER2.

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Seribantumab + Paclitaxel (n = 140)		Paclitaxel Alone (n = 83)	
	No. of Patients	%	No. of Patients	%
Age, years				
Mean	58.5		60.6	
Median	60.0		62.0	
Range	30-82		28-85	
Race				
White	111	79.3	66	79.5
Black	2	1.4	1	1.2
Asian	1	0.7	2	2.4
Other	18	12.9	8	9.6
ECOG PS*				
0-1	133	95.0	80	96.4
2	7	5.0	3	3.6
Histologic subtype				
Serous	103	73.6	55	66.2
Endometrioid	7	5.0	5	6.0
Clear cell	6	4.3	2	2.4
Transitional cell	2	1.4	0	—
Mixed epithelial	0	—	1	1.2
Undifferentiated	7	5.0	0	—
Other	12	8.6	18	21.7
Prior treatment				
Platinum	139	99.3	82	98.8
Anthracycline	72	51.4	38	45.8
Topotecan	54	38.6	24	28.9
Taxane	137	97.9	81	97.6
Alkylating agent	20	14.3	15	18.1
Antiendocrine	13	9.3	11	13.3
Antiangiogenesis	43	30.7	20	24.1
Other	94	67.1	52	62.7
No. of platinum-based therapies				
1	25	17.9	17	20.5
≥ 2	114	81.4	65	78.3
No. of prior therapies*				
1-2	37	26.4	27	32.5
≥ 3	103	73.6	56	67.5

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.
*Stratification factor.

On the basis of the HR scan in HRG-positive patients, a cut point was chosen for HER2 as the level below which the experimental arm was favored relative to the control arm (HR < 1). This corresponded to an HER2 level of 5.1 on a log₁₀ scale, or approximately 126,000 receptors per cell. Selection of this cut point was confirmed by performing five-fold cross validation (Data Supplement). Coincidentally, this closely matches the threshold separating a score of 1+ from 2+ on a standard HercepTest assay (Dako, Carpinteria, CA),²² suggesting a potentially standardized way to identify patients in future trials. For all additional analyses, a biomarker-positive subpopulation was defined as having HRG scores of 1 or greater and HER2 levels of 126,000 or fewer receptors per cell. This definition resulted in a biomarker-positive prevalence of 38%.

Characteristics of biomarker-positive subpopulation. In total, HRG and HER2 data were available for 151 patients (biomarker-evaluable population). The PFS HR of this population was 1.09 (95% CI, 0.74 to 1.62; *P* = .719), which compared well with the safety population (HR, 1.06; 95% CI, 0.76 to 1.48; *P* = .801). Of these 151 patients, 57 (38%) were biomarker positive, and 94 (62%) were biomarker negative.

Kaplan-Meier PFS plots are shown in [Figure 3](#). Corresponding analyses for OS and ORR are summarized in Appendix [Table A3](#) (online only) and Appendix [Figure A1](#) (online only). In the paclitaxel arm, the biomarkers seemed prognostic of rapid progression, with a PFS HR of 2.21 (biomarker positive *v* negative: 95% CI, 1.08 to 4.51; *P* = .029; [Fig 3B](#)). Comparing experimental and control arms, the biomarkers also seemed predictive of benefit from seribantumab; in the biomarker-positive subpopulation, the PFS HR was 0.37 (95% CI, 0.18 to 0.76; *P* = .007; [Fig 3C](#)). Notably, the control arm was favored in the biomarker-negative subpopulation, with a PFS HR of 1.80 (95% CI, 1.08 to 2.98; *P* = .023; [Fig 3D](#)).

The robustness of these results was evaluated by determining how clinical variables affected PFS and how these variables were distributed between study arms in the biomarker-positive subpopulation compared with the safety population. Overall, similar distributions were observed for each covariate (Appendix [Fig A2](#), online only).

Biomarker status in archived versus pretreatment samples. To determine if the biomarkers changed from time of initial diagnosis to time of study entry, HRG and HER2 were measured in archived tissue blocks and pretreatment biopsies ([Fig 4A](#)). Although 86% of patients were HER2 low (< 126,000 receptors per cell) at initial

Table 2. TEAEs Occurring in $\geq 10\%$ of Study Population

Adverse Event	No. (%)			
	Seribantumab + Paclitaxel (n = 140)		Paclitaxel Alone (n = 80)	
	Grade 1 or 2	Grade ≥ 3	Grade 1 or 2	Grade ≥ 3
At least one TEAE	55 (46.4)	85 (60.7)	36 (45.0)	43 (53.8)
Diarrhea	93 (66.4)	10 (7.1)	30 (37.5)	4 (5.0)
Fatigue	52 (37.1)	11 (7.9)	26 (32.5)	4 (5.0)
Nausea	55 (39.3)	5 (3.6)	36 (45.0)	3 (3.8)
Abdominal pain	39 (27.9)	2 (1.4)	17 (21.3)	3 (3.8)
Alopecia	51 (36.4)	2 (1.4)	26 (32.5)	0
Vomiting	42 (30.0)	2 (1.4)	12 (15.0)	3 (3.8)
Anemia	26 (18.6)	13 (9.3)	17 (21.3)	7 (8.8)
Decreased appetite	34 (24.3)	1 (0.7)	18 (22.5)	2 (2.5)
Rash	34 (24.3)	0	9 (11.3)	1 (1.3)
Asthenia	31 (22.1)	3 (2.1)	15 (18.8)	2 (2.5)
Hypokalemia	23 (16.4)	11 (7.9)	5 (6.3)	2 (2.5)
Edema	31 (22.1)	2 (1.4)	16 (20.0)	0
Epistaxis	33 (23.6)	0	14 (17.5)	0
Stomatitis	29 (20.7)	2 (1.4)	8 (10.0)	0
Mucosal inflammation	29 (20.7)	2 (1.4)	1 (1.3)	0
Hypomagnesemia	28 (20.0)	3 (2.1)	12 (15.0)	0
Constipation	25 (17.9)	1 (0.7)	20 (25.0)	1 (1.3)
Peripheral neuropathy	24 (17.1)	2 (1.4)	19 (23.8)	1 (1.3)
Pyrexia	23 (16.4)	2 (1.4)	19 (23.8)	2 (2.5)
Cough	25 (17.9)	0	18 (22.5)	0
Dyspnea	18 (12.9)	6 (4.3)	10 (12.5)	2 (2.5)
Myalgia	23 (16.4)	0	7 (8.8)	0
Neutropenia	13 (9.3)	9 (6.4)	9 (11.3)	8 (10.0)
Dizziness	22 (15.7)	0	4 (5.0)	0
Dyspepsia	18 (12.9)	1 (0.7)	6 (7.5)	0
Urinary tract infection	16 (11.4)	3 (2.1)	11 (13.8)	1 (1.3)
Sensory neuropathy	18 (12.9)	1 (0.7)	4 (5.0)	1 (1.3)
Muscle spasm	18 (12.9)	0	6 (7.5)	0
Headache	17 (12.1)	1 (0.7)	13 (16.3)	0
Dysgeusia	18 (12.9)	0	16 (20.0)	0
Erythema	18 (12.9)	0	2 (2.5)	0
Paresthesia	17 (12.1)	0	8 (10.0)	0
Anxiety	15 (10.7)	1 (0.7)	6 (7.5)	0
Dysphonia	15 (10.7)	0	3 (3.8)	0
Hyperglycemia	12 (8.6)	3 (2.1)	3 (3.8)	3 (3.8)
Dehydration	11 (7.9)	3 (2.1)	3 (3.8)	0
Back pain	11 (7.9)	3 (2.1)	12 (15.0)	0

Abbreviation: TEAE, treatment-emergent adverse event.

diagnosis, 35% of these patients had HER2 levels above this threshold in their pretreatment biopsies. Patients who were HRG positive in their archived tissue (35% of samples) tended to remain HRG positive (78%), whereas approximately 50% of patients who were HRG negative in their archived samples were HRG positive in their pretreatment biopsies.

Because patients with detectable HRG in their archived tissue tended to remain HRG positive, the effect of HRG level on PFS HR was assessed using results from the HRG reverse-transcriptase polymerase chain reaction assay performed on archived tissue (Fig 4B). Consistent with the RNA ISH results in pretreatment biopsies, favorable HRs were observed at high levels of HRG mRNA.

DISCUSSION

In the general population, the addition of seribantumab to paclitaxel did not result in an improvement in PFS, the primary study end

point. A potential PFS and OS benefit was observed, however, in patients whose tumors were positive for HRG and low for HER2. Preclinical studies have shown that HRG induces a more aggressive and treatment-insensitive phenotype in tumor cells.^{10-12,23,24} Seribantumab inhibits HRG from binding to ErbB3, thereby reversing this aggressive phenotype. In our study, five potential biomarkers of ErbB3 activation were investigated.¹⁵ Univariable analyses revealed that three of these biomarkers (HRG, HER2, and ErbB3) differentially affected the experimental and control arms, and all three effects were directionally consistent with preclinical predictions (Appendix Table A1). Notably, the cut point for HER2 of approximately 126,000 receptors per cell agreed well with the HER2 level above which seribantumab exhibits decreased potency²⁵ (Data Supplement). A relatively lenient criterion ($P < .4$) was used to identify biomarkers for bivariate analyses, because prior preclinical studies had indicated that more than one biomarker may be needed to accurately predict the activity of seribantumab.¹⁵ Although the importance of HRG was expected, we did not anticipate such

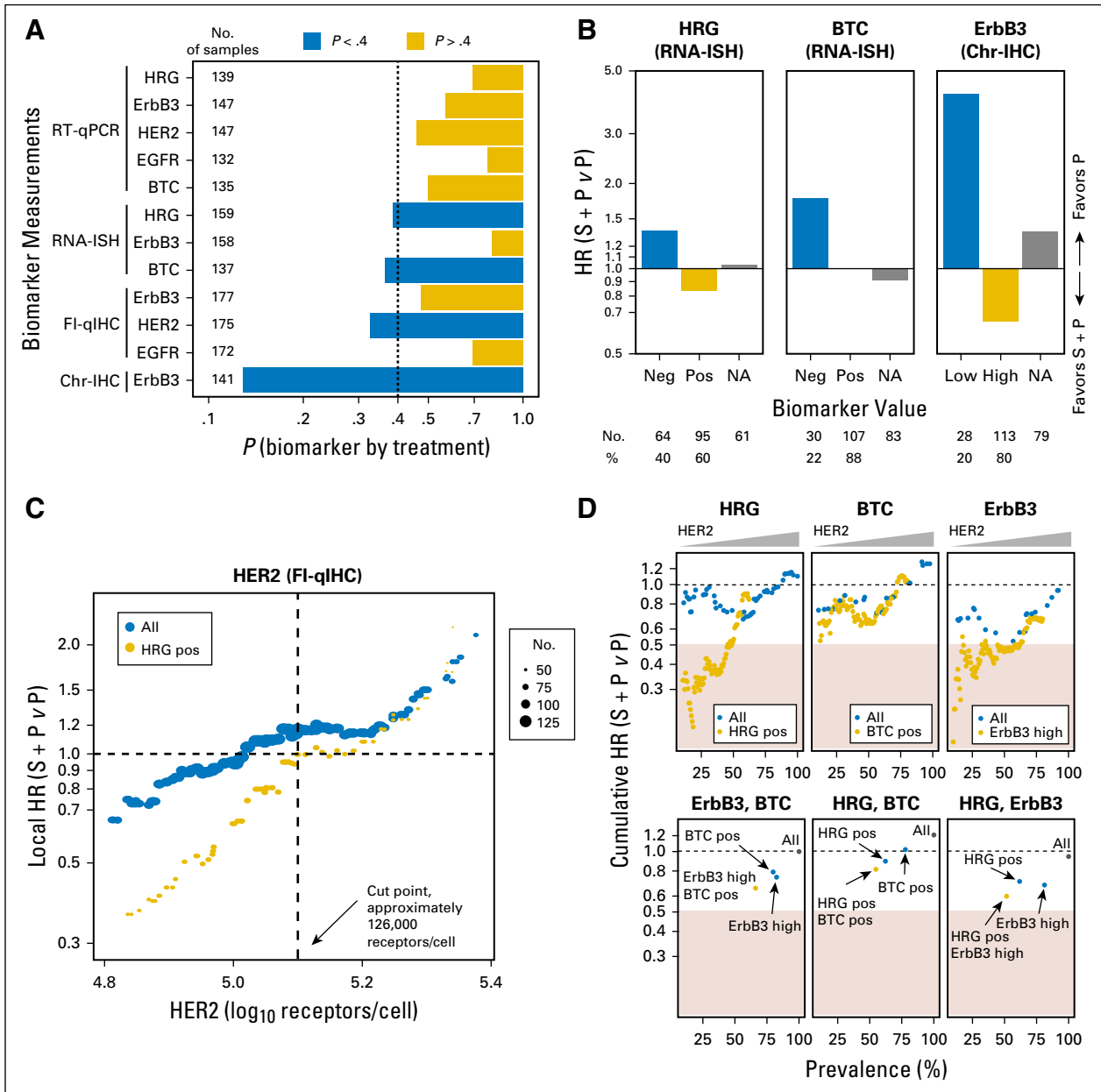


Fig 2. Biomarker analyses of progression-free survival. (A) Significance testing of biomarkers and assays. Assays showing a treatment interaction ($P < .4$) are shown in blue. The number of patients for whom biomarker data were available for each assay is shown to the left. (B) Hazard ratio estimations by biomarker status for heregulin (HRG), betacellulin (BTC), and human epidermal growth factor receptor 3 (ErbB3) in all patients for whom biomarker data were available. HRG negative (neg), HRG score < 1 ; HRG positive (pos), HRG score ≥ 1 ; BTC neg, BTC score < 1 ; BTC pos, BTC score ≥ 1 ; ErbB3 low, ErbB3 score < 2 ; ErbB3 high, ErbB3 score ≥ 2 . (C) Local hazard ratio (HR) scan of human epidermal growth factor receptor 2 (HER2) in all patients with available data (blue) or in the subset of these patients who were HRG pos (gold). Local HR was estimated by including all patients with HER2 levels within one standard deviation of the measurement error. The number of patients used for each HR calculation is indicated by the size of the dot. (D) Cumulative HR scans of two-way combinations of biomarkers that passed significance testing by univariable analysis. In the top three plots, cumulative HR was estimated as a function of HER2 threshold, either in all patients for whom biomarker data were available (blue) or in patients positive for the second biomarker (gold; HRG pos, BTC pos, or ErbB3 high [score ≥ 2]). In the bottom three plots, points represent the estimated HR for all patients with available biomarker data (gray), patients positive for one of the biomarkers (blue), or patients positive for both biomarkers (gold). In all six plots, the x-axis indicates the percentage of patients who meet the criteria. Yellow rectangles indicate HR < 0.5 . Chr-IHC, chromogenic immunohistochemistry; FI-qIHC, fluorescence-based quantitative immunohistochemistry; ISH, in situ hybridization; RT-qPCR, reverse-transcriptase quantitative polymerase chain reaction.

a prominent role for HER2 expression in platinum-resistant ovarian cancer, based on its observed levels at the time of diagnosis.

The combination of HRG and HER2 as biomarkers provided the optimal balance between benefit and prevalence, with a PFS HR of 0.37 and prevalence of 38%. This biomarker pair also identified

a subgroup of patients in the control arm who were particularly insensitive to paclitaxel. Interestingly, the PFS curve of biomarker-positive patients receiving seribantumab plus paclitaxel closely resembles that of biomarker-negative patients receiving paclitaxel alone (Figs 3B and 3C). This suggests that in this setting,

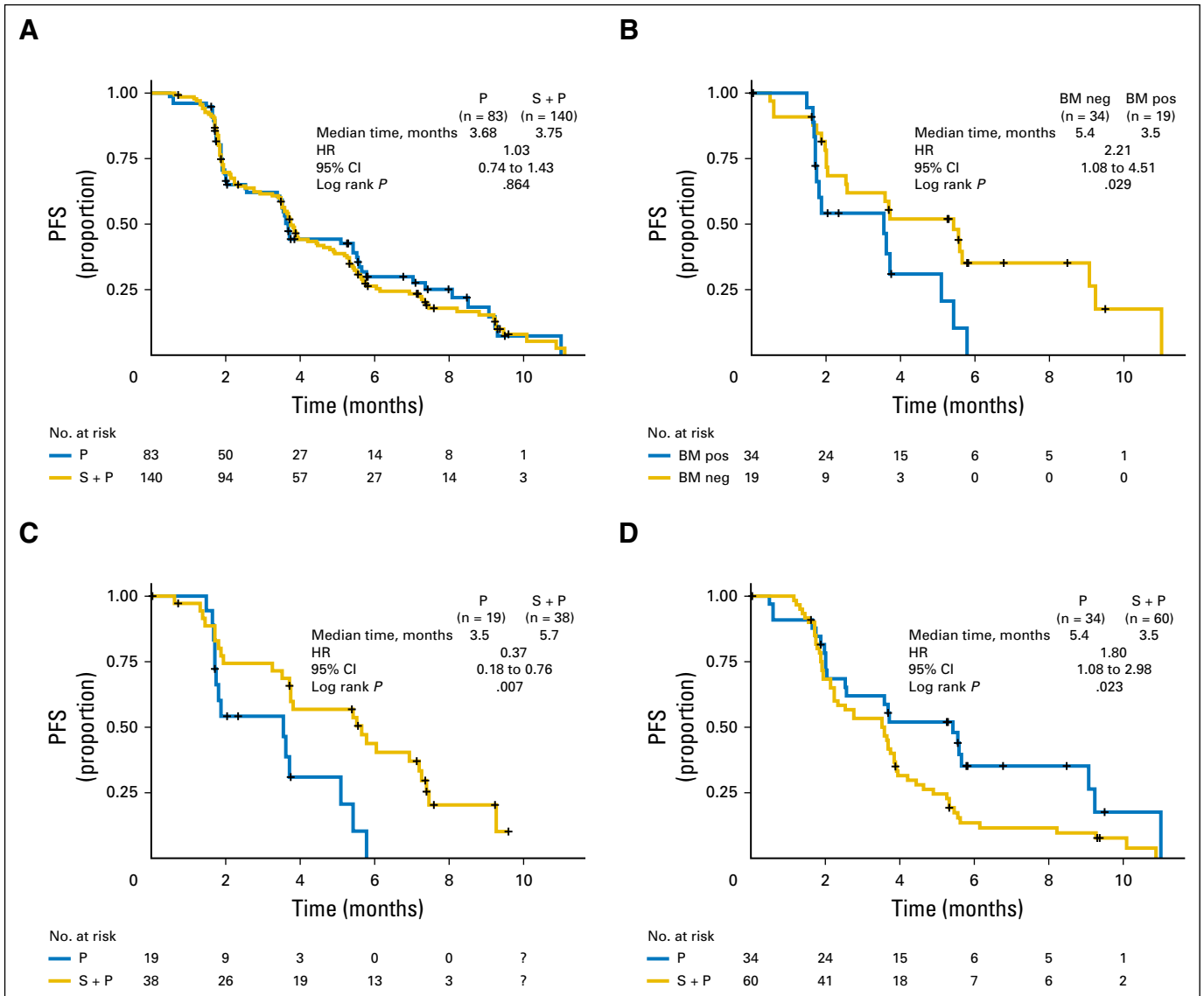


Fig 3. Kaplan-Meier estimated progression-free survival (PFS) curves by biomarker status. (A) PFS for unselected patients in the intent-to-treat population (seribantumab [S] + paclitaxel [P] v P). (B) PFS for control arm patients in the biomarker (BM) –evaluable population (P alone; BM positive [pos] v BM negative [neg]). (C) PFS for BM-pos patients in the BM-evaluable population (S + P v P). (D) PFS for BM-negative patients in the BM-evaluable population (S + P v P). BM pos, heregulin (HRG) score ≥ 1 and human epidermal growth factor receptor 2 (HER2) $< 126,000$ receptors per cell; BM neg, HRG score < 1 and/or HER2 $\geq 126,000$ receptors per cell. HR, hazard ratio.

seribantumab may act primarily by blocking resistance to therapy, rather than by inhibiting a driver of tumor growth.

Patient-matched pairs of biomarker measurements showed that pretreatment samples provided better predictive power than archived tissue. Additionally, patients with HRG-positive primary tumors tended to remain HRG positive, whereas approximately 50% of patients with HRG-negative primary tumors presented as HRG positive in their biopsies. This suggests that HRG expression increases in response to cytotoxic therapy and serves as a mechanism of acquired resistance.¹⁶

One unanticipated finding was that biomarker-negative patients experienced progression faster in the experimental compared with control arm. This effect is not unique to seribantumab; a similar effect was observed with other RTK inhibitors, including the anti-insulin-like growth factor-1 receptor antibodies figitumumab²⁶ and

dalotuzumab,²⁷ the anti-EGFR antibodies cetuximab²⁸ and panitumumab,²⁹ the anti-Met antibody onartuzumab,³⁰ and the anti-HER2/ErbB3 antibody pertuzumab³¹ (Appendix Table A4, online only). It is not currently known why these agents may accelerate progression when their target pathway is inactive. It is possible that some of these agents act as weak ligands, partially activating their target pathways. They may also trigger compensatory signaling through alternative pathways that are otherwise dormant. A third intriguing hypothesis is that these antibodies inhibit their target pathways in nontumor cells, including cells of the immune system. Whether any of these mechanisms occur with ErbB3 inhibitors remains to be determined.

One of the caveats of this study is that multiple biomarkers were analyzed retrospectively, raising the possibility of false discovery. This concern is mitigated by the mechanistic relevance of these findings, as

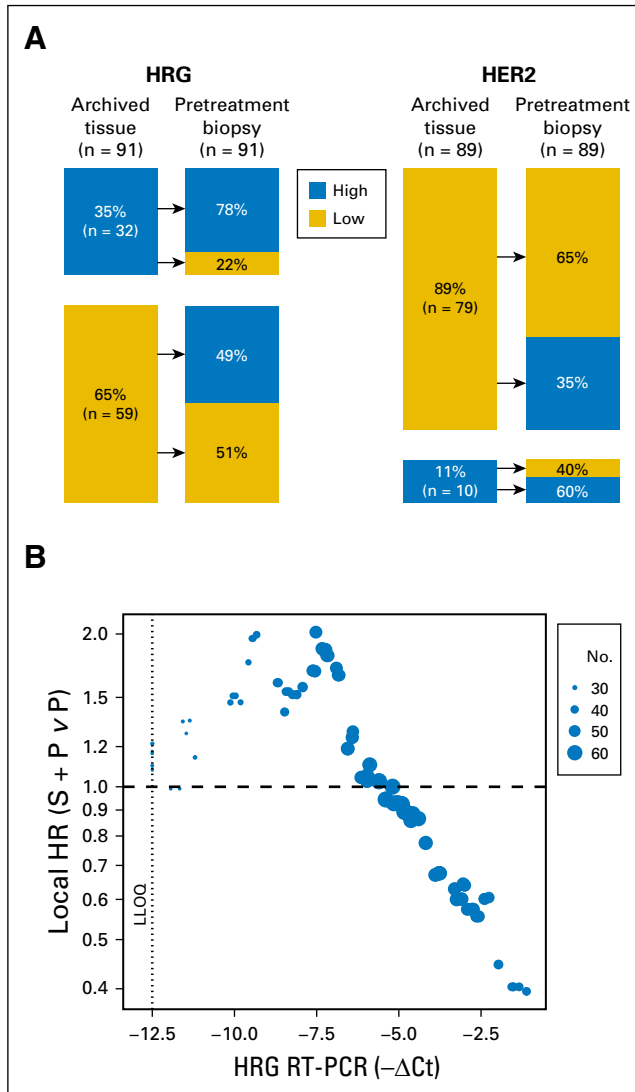


Fig 4. Biomarker analyses in archived tissue. (A) Concordance of heregulin (HRG) and human epidermal growth factor receptor 2 (HER2) status between archived tissue and pretreatment biopsies in patient-matched samples. HRG high, HRG score ≥ 1 ; HRG low, HRG score < 1 ; HER2 high, HER2 $\geq 126,000$ receptors per cell; HER2 low, HER2 $< 126,000$ receptors per cell. (B) Relationship between local progression-free survival hazard ratio (HR; seribantumab [S] + paclitaxel [P] v P) and HRG reverse-transcriptase quantitative polymerase chain reaction (RT-PCR) values in archived tissue. HRs were estimated at different values of HRG by including all patients with HRG levels within one standard deviation of the measurement error. The number of patients used for each HR estimation is indicated by the size of the dot. LLOQ, lower limit of quantification. CT, cycle threshold.

well as by their consistency with preclinical predictions in terms of the direction of the treatment effect in this marker-specific subgroup. In

addition, the findings in this study agree well with those from two other randomized studies of seribantumab: one in combination with exemestane in women with advanced hormone-refractory, HER2-negative breast cancer^{32,33} and one in combination with erlotinib in patients with *EGFR* wild-type advanced non-small-cell lung cancer.³⁴ In both studies, HRG seemed prognostic of decreased PFS in the control arm and predictive of PFS benefit from the addition of seribantumab. Most patients in these studies had low or undetectable levels of HER2. Recently, Mendell et al³⁵ found that HRG seemed predictive of benefit from patritumab, another ErbB3-directed antibody, when combined with erlotinib in non-small-cell lung cancer. In addition, Qian et al³⁶ reported that HRG may be prognostic of decreased OS in oropharyngeal squamous cell carcinoma, further signifying its role in cancer biology. That several independent studies across different cancer types found HRG to be prognostic of poor outcome and predictive of benefit from ErbB3 inhibitors suggests that HRG-mediated resistance may be a broad phenomenon in cancer and that ErbB3 inhibition may be part of a general strategy to combat insensitivity to anticancer agents.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Randomized Phase II Trial of Seribantumab in Combination With Paclitaxel in Patients With Advanced Platinum Resistant- or -Refractory Ovarian Cancer

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Appendix

Table A1. Predefined Biomarker Hypotheses

Biomarker	Direction	Rationale
HRG	Positive	HRG is the direct ligand of ErbB3 and potently activates ErbB3 signaling; seribantumab works by blocking HRG binding to ErbB3
ErbB3	Positive	ErbB3 is the target of seribantumab, so more ErbB3 should favor more signaling; as a biomarker, however, ErbB3 is complicated by ligand-induced endocytosis, ligand-induced negative transcriptional feedback to ErbB3, and low dynamic range of expression
HER2	Negative	High HER2 levels (> approximately 100,000-200,000 receptors/cell) favor the formation of a high-affinity HER2/ErbB3/HRG ternary complex; when this forms, seribantumab is less able to compete with HRG and block ErbB3 signaling; high HER2 levels also favor formation of HER2/HER2 homodimers, which may also need to be inhibited with HER2-directed therapy
EGFR	Positive	EGFR promotes ErbB3 signaling by favoring the formation of EGFR/ErbB3 dimers; important in EGFR-driven cancers
BTC	Positive	BTC is a ligand of EGFR and promotes activation of ErbB3 in the presence of EGFR; important in EGFR-driven cancers

Abbreviations: BTC, betacellulin; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HRG, heregulin.

Table A2. Biomarkers and Assays

Biomarker	Fl-IHC (protein)	Chr-IHC (protein)	RNA-ISH (mRNA)	RT-qPCR (mRNA)
EGFR	X			X
HER2	X			X
ErbB3	X	Y	Y	X
HRG			Y	X
HRG-1 β 1				X
BTC			Y	X

NOTE. There are five main isoforms of HRG-1 (HRG-1 α , HRG-1 β 1, HRG-1 β 2, HRG-1 β 3, and HRG-1 γ) and a total of 17 splice variants. The isoform that most potently activates ErbB3 is HRG-1 β 1. Two RT-PCR assays were used to detect HRG-1: one that amplifies all five isoforms and all 17 splice variants of HRG-1 (designated here as HRG) and one that amplifies all four splice variants of the HRG-1 β 1 isoform. X indicates continuous variable; Y indicates discrete variable (pathologist scored).

Abbreviations: BTC, betacellulin; Chr-IHC, chromogenic immunohistochemistry; EGFR, epidermal growth factor receptor; Fl-IHC, fluorescence-based quantitative immunohistochemistry; HER2, human epidermal growth factor receptor 2; HRG, heregulin; ISH, in situ hybridization; RT-PCR, reverse-transcriptase polymerase chain reaction; RT-qPCR, reverse-transcriptase quantitative polymerase chain reaction.

Table A3. Best Response Rates by Treatment and Biomarker Status

Best Overall Response	No. (%)					
	Seribantumab + Paclitaxel			Paclitaxel Alone		
	BM Positive	BM Negative	Missing	BM Positive	BM Negative	Missing
PR	6 (15.8%)	2 (3.3%)	11 (26.2%)	2 (10.5%)	7 (20.6%)	6 (22.2%)
SD	21 (55.3%)	34 (56.7%)	16 (38.1%)	8 (42.1%)	16 (47.1%)	12 (44.4%)
PD	8 (21.1%)	18 (30%)	12 (28.6%)	8 (42.1%)	9 (26.5%)	8 (29.6%)
Not evaluable	3 (7.9%)	6 (10%)	3 (7.1%)	1 (5.3%)	2 (5.9%)	1 (3.7%)

NOTE. BM positive, HRG positive and HER2 < 126,000 receptors per cell. BM negative, HRG negative and/or HER2 \geq 126,000 receptors per cell.

Abbreviations: BM, biomarker; HER2, human epidermal growth factor receptor 2; HRG, heregulin; PD, progressive disease; PR, partial response; SD, stable disease.

Seribantumab and Paclitaxel in Platinum-Resistant Ovarian Cancer

Table A4. RTK-Directed Antibodies Seeming to Accelerate Progression in Patients Negative for Reported Biomarker

Antibody	Trial	RTK Target	Biomarker	PFS HR BM	
				Positive	Negative
Figitumumab ²⁶	Phase III NSCLC	IGF-1R	Serum IGF-1	0.93	1.37
Dalotuzumab ²⁷	Phase II/III	IGF-1R	IGF-1 mRNA	0.59	1.56
Cetuximab ²⁸	Phase III CRC (CRYSTAL)	EGFR	<i>KRAS</i>	0.70	1.13
Cetuximab ²⁸	Phase II CRC (OPUS)	EGFR	<i>KRAS</i>	0.57	1.72
Panitumumab ²⁹	Phase III CRC (PRIME)	EGFR	<i>KRAS</i>	0.72	1.28
Onartuzumab ³⁰	Phase II lung	Met	Met	0.53	1.82
Pertuzumab ³¹	Phase II ovarian	HER2/ErbB3	ErbB3 mRNA	0.32	1.68
Seribantumab	Phase II ovarian	ErbB3	HRG mRNA, HER2	0.37	1.54

NOTE. BMs assessed were not associated with a treatment effect on PFS.

Abbreviations: BM, biomarker; CRC, colorectal cancer; CRYSTAL, Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; HRG, heregulin; IGF, insulin-like growth factor; NSCLC, non-small-cell lung cancer; OPUS, Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer; PFS, progression-free survival; PRIME, Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; RTK, receptor tyrosine kinase.

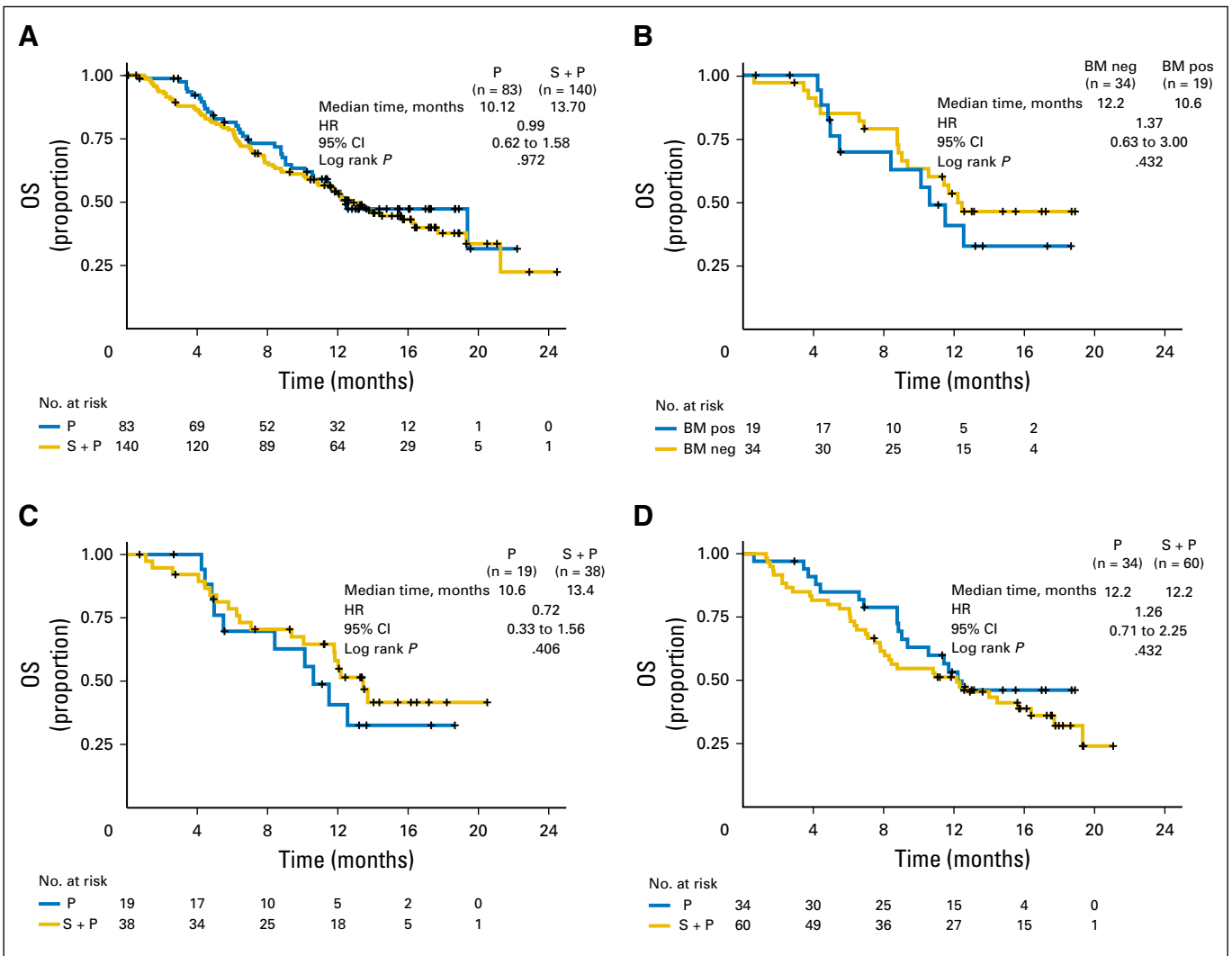


Fig A1. Kaplan-Meier estimated overall survival (OS) curves by biomarker status. (A) OS for unselected patients in the intent-to-treat population (seribantumab [S] + paclitaxel [P] v P). (B) OS for control arm patients in the biomarker (BM) -evaluable population (P alone; BM positive [pos] v BM- negative [neg]). (C) OS for BM-positive patients in the BM-evaluable population (S + P v P). (D) OS for BM-negative patients in the BM-evaluable population (S + P v P). BM pos, heregulin (HRG) pos and human epidermal growth factor receptor 2 (HER2) < 126,000 receptors per cell; BM neg, HRG neg and/or HER2 ≥ 126,000 receptors per cell. HR, hazard ratio.

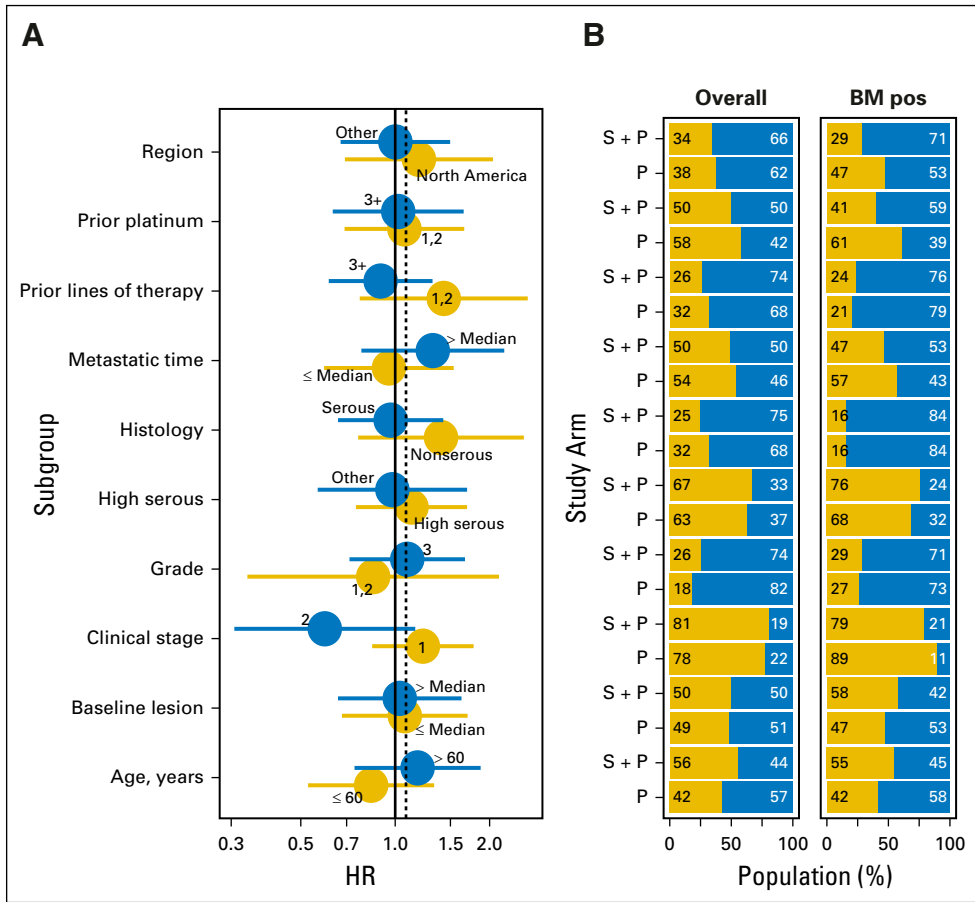


Fig A2. Subgroup analyses by baseline clinical covariates. (A) Forest plot of progression-free survival by baseline clinical covariates in the overall safety population. (B) Number of patients in the treatment (seribantumab [S] + paclitaxel [P]) and control (P) arms by clinical covariates in the overall safety population and in the biomarker (BM) –positive (pos) subpopulation. BM pos, heregulin pos and human epidermal growth factor receptor 2 < 126,000 receptors per cell. HR, hazard ratio.