Statistical Analysis

All randomly assigned patients were assessed for efficacy; following the intent-to-treat principle, patients were analyzed per the treatment and stratum to which they were assigned on randomization. Safety was assessed in all patients who received at least one dose of study drug and had at least one postbaseline assessment.

Primary end point was OS, defined as the time from randomization to the time of death (any cause). Secondary end points included PFS, defined as the time from randomization to first documented disease progression or death (any cause); overall response rate (ORR); time to definitive deterioration of ECOG PS; time to definitive 5% deterioration in the global health status/quality of life (QoL) and physical, social, and emotional functioning scales of the European Organisation for Research and Treatment of Cancer QLQ-C30 questionnaire; pharmacokinetics; and safety. (See Appendix [online-only] for information on how missing values were handled.)

Between-arm comparisons of OS and PFS were performed using log-rank tests stratified by the two randomization stratification factors at a one-sided cumulative 2.5% significance level. OS analyses were repeated in several patient subgroups (Appendix); no interaction test was performed. Comparisons of time to definitive deterioration in ECOG PS and time to definitive 5% deterioration in QoL were performed using log-rank tests stratified by the two randomization stratification factors at a two-sided 5% significance level. No other adjustments were performed. A hierarchical testing strategy was implemented such that formal significance for PFS could be declared only if the between-group difference in OS was significant. Subsequent levels of the hierarchy were deterioration in ECOG PS; deterioration in the QLQ-C30 global health status/QoL scale; and deterioration in the QLQ-C30 physical, social, and emotional functioning scales (successively compared). No statistical comparisons were performed for ORR or for pharmacokinetic or safety parameters. For all time-to-event end points, median values were estimated using the Kaplan-Meier method. Hazard ratios (HRs) and 95% CIs were derived from Cox proportional hazards models stratified by the two randomization stratification factors. Exact 95% CIs for ORR were calculated using the Clopper-Pearson method.

The study was designed to detect an improvement in median OS from 4.0 months with placebo to 5.4 months with everolimus (HR, 0.74). Considering the two-look Lan-DeMets group sequential design with an O'Brien-Fleming–type boundary,[32] 526 deaths were required at final analysis (90% power, stratified log-rank test, one-sided cumulative 2.5% significance). Assuming a 24-month recruitment period, 5% loss to follow-up, and 2:1 randomization in favor of everolimus, it was estimated that 633 patients would need to be enrolled. (See Appendix, online only, for results of interim analysis.)

RESULTS

Patient Disposition and Characteristics

From July 2009 to November 2010, 656 patients from 137 centers in 23 countries were enrolled and received everolimus plus BSC (n = 439) or placebo plus BSC (n = 217; Fig 1). As of the analysis cutoff date (September 5, 2011), 11 patients (2.5%) in the everolimus arm and no patients in the placebo arm were still receiving study treatment. The most common reason for treatment discontinuation was disease progression (66.5% in the everolimus arm and 77.9% in the placebo arm). A higher percentage of patients discontinued everolimus because of AEs (21.4% *v* 15.7% with placebo) or consent withdrawal (4.6% *v* 3.2%). Median follow-up duration (ie, time from randomization date of median patient enrolled to date of data cutoff) was 14.3 months.

Baseline demographics and disease characteristics were generally well balanced between treatment groups, although minor differences were observed (Table 1). Compared with the everolimus arm, more patients in the placebo arm had the proximal stomach tumor location, an ECOG PS of 2, and liver metastases. Overall, 47.7% of patients received one previous line of chemotherapy and 52.3% received two previous lines of chemotherapy (Table 1). The most commonly administered chemotherapy regimens contained fluoropyrimidines (96.0%), platinum derivatives (85.8%), and taxanes (38.4%). Other previous therapy included total (21.8%) and partial (28.4%) gastrectomy and radiotherapy (12.0%) (Table 1).

Table 1. Baseline Patient Demographics and Disease Characteristics of All Randomly Assigned Patients

Characteristic	Everolimus Plus BSC (n = 439) No. of Patients	%	Placebo Plus BSC (n = 217) No. of Patients	%
Age, years				
Median	62		62	
Range	20-86		26-88	
< 65	260	59	129	59
≥ 65	179	41	88	41
Male sex	322	73	161	74
Race				
White	166	38	75	35
Black	3	< 1	1	< 1
Asian	251	57	126	58
Other	19	4	15	7
Region and No. of previous chemotherapy lines				
Asia, 1 line	98	22	48	22
Asia, 2 lines	145	33	72	33
Rest of the world, 1 line	112	26	55	25
Rest of the world, 2 lines	84	19	42	19
Time since initial diagnosis, months				
≤ 12	176	40	93	43
> 12 to ≤ 24	156	36	71	33
> 24	107	24	53	24
Anatomic site of cancer				
Proximal stomach	162	37	94	43
Distal stomach	276	63	123	57
Missing	1	< 1	0	0
Gastroesophageal junction involvement	118	27	69	32
Histologic grade				
Well differentiated	33	8	21	10
Moderately differentiated	137	31	69	32
Poorly differentiated	198	45	89	41
Poorly differentiated/undifferentiated	6	1	4	2
Unknown	65	15	34	16
Measurable disease according to RECIST	379	86	192	88
Metastatic site				
Lung	92	21	37	17
Liver	190	43	109	50
ECOG performance status				
0	144	33	70	32
1	269	61	120	55
2	25	6	27	12
Missing	1	< 1	0	0
Prior gastrectomy				
No	216	49	111	51
Partial	126	29	60	28
Total	97	22	46	21
Prior radiotherapy	54	12	25	12

Abbreviations: BSC, best supportive care; ECOG, Eastern Cooperative Oncology Group.

Table 2. Exposure to Study Treatment in the Everolimus Plus Best Supportive Care Treatment Arm in the Safety Population

Characteristic	No. of Patients	Duration of Exposure (weeks)		Mean Dose Intensity (mg/d)
		Median	Range	
Overall population	437	7.1	0.1-79.6	8.9
Gastrectomy				
Yes	224	8.0	0.9-70.7	8.8
No	213	6.7	0.1-79.6	9.1
Sex				
Male	322	7.1	0.4-79.6	8.9
Female	115	7.0	0.1-74.7	8.9
Age, years				
< 65	258	6.9	0.1-79.6	9.1
≥ 65	179	8.0	0.9-58.3	8.6
Race				
Asian	251	8.0	0.1-79.6	8.8
White	164	6.6	0.9-74.7	9.1
Other	22	6.1	0.9-42.4	9.5
Ethnicity				
Chinese	110	6.4	0.1-53.0	9.1
Japanese	74	11.4	1.0-70.7	8.3
Hispanic/Latino	35	7.0	0.9-46.3	9.1
Indian	2	7.4	6.3-8.4	7.8
Mixed	1	6.4	—	10.0
Other	215	7.1	0.6-79.6	9.0
Region				
Asia	243	7.9	0.1-79.6	8.9
ROW	194	6.8	0.9-74.7	9.0

Abbreviation: ROW, rest of world.

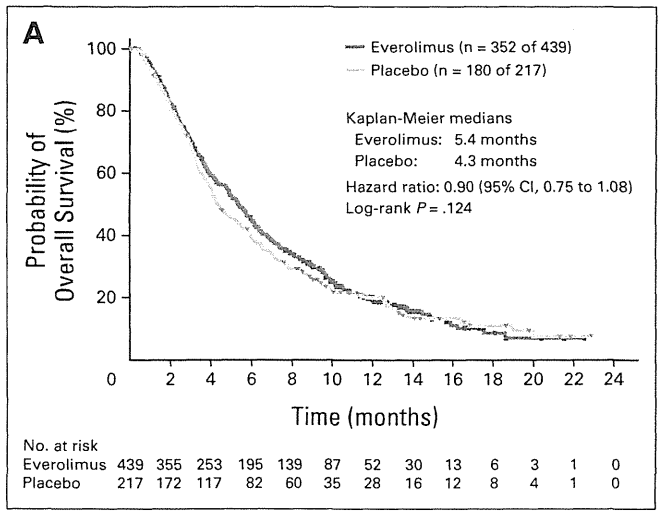

Fig 2. Overall and progression-free survival for all randomly assigned patients. (A) Kaplan-Meier plot of overall survival. (B) Forest plot of overall survival in subgroups. (C) Kaplan-Meier plot of progression-free survival. (D) Longitudinal mean scores of the global health status/quality-of-life scale of the European Organisation for Research and Treatment of Cancer QLQ-C30 questionnaire. ECOG PS, European Cooperative Oncology Group performance status; GE, gastroesophageal; n, number of patients with event (of the number of patients at risk); ROW, rest of world. Appendix Table A3 (online only) lists details on the events experienced.

Study Drug Exposure

Median duration of study drug exposure was 7.1 weeks for everolimus (range, 0.1 to 79.6 weeks) and 6.4 weeks for placebo (range, 0.4 to 90.9 weeks). Mean duration of exposure was 11.5 weeks (standard deviation [SD], 12.1 weeks) and 8.5 weeks (SD, 8.8 weeks), respectively. Median exposure was slightly longer in patients with versus without gastrectomy, patients at least 65 years old versus those younger than 65 years, Asians versus white patients or patients of other races, Japanese versus other ethnicities, and patients enrolled in Asia versus ROW (Table 2). Dose interruptions or reductions were more common with everolimus (48.5% *v* 16.7% with placebo). The most common reasons for dose interruption or reduction were AEs (34.6% and 11.6% with everolimus and placebo, respectively) and laboratory test abnormalities (14.0% and 0.5%, respectively). The median relative dose intensity was 1.0 for both treatment arms. The mean dose intensity was 8.9 mg/d with everolimus (SD, 1.7 mg/d) and 9.7 mg/d with placebo (SD, 1.0 mg/d).

Median everolimus C_{min} and C_{max} were 13.8 ng/mL and 67.4 ng/mL, respectively, for patients who received everolimus 10 mg/d (Appendix Table A1). There was no apparent difference in steady-state everolimus concentrations between patients enrolled in Asia and ROW or those with and without gastrectomy (Appendix Table A1).

Efficacy

The estimated median OS was 5.4 months with everolimus plus BSC (95% CI, 4.8 to 6.0 months) and 4.3 months with placebo plus BSC (95% CI, 3.8 to 5.5 months; HR for OS, 0.90; 95% CI, 0.75 to 1.08; $P = .124$; Fig 2A). A trend for reduction in the risk of death was observed with everolimus in patients enrolled in ROW (15% reduction in risk) and patients enrolled in ROW with two previous chemotherapy lines (26% reduction in risk; Fig 2B); these trends in ROW seemed to be driven by patients enrolled outside of Europe (Fig 2B). Across the remaining subgroups analyzed, results were consistent with those of the overall population (Fig 2B). The percentage of patients who started other antineoplastic therapy after study treatment discontinuation was slightly higher with placebo (45.2% *v* 39.2% with everolimus; Appendix Table A2).

Estimated median PFS was 1.7 months with everolimus (95% CI, 1.5 to 1.9 months) and 1.4 months with placebo (95% CI, 1.4 to 1.5 months). Although everolimus reduced the risk of disease progression or death compared with placebo (HR, 0.66; 95% CI, 0.56 to 0.78; $P < .001$; Fig 2C), formal statistical significance could not be declared per the hierarchical testing strategy. The estimated percentage of patients progression free at 6 months was approximately three times greater with everolimus (12.0%; 95% CI, 9.0% to 15.4%; *v* 4.3%; 95% CI, 2.1% to 7.7%).

Among patients with measurable disease at baseline, one patient in the everolimus arm experienced a CR, versus no patients in the placebo arm (Table 3). The ORR (percentage of patients with CR or PR) was 4.5% with everolimus (95% CI, 2.6% to 7.1%) and 2.1% with placebo (95% CI, 0.6% to 5.3%). The disease control rate (percentage of patients with CR, PR, or stable disease) was approximately two-fold higher with everolimus (everolimus: 43.3%; 95% CI, 38.2% to 48.4%; *v* placebo: 22.0%; 95% CI, 16.3% to 28.5%). Tumor shrinkage was observed in approximately three times as many patients treated with everolimus (37.8% *v* 12.3% with placebo).

Time to deterioration of ECOG PS did not differ significantly between treatment arms (median time to deterioration, 2.3 months for everolimus *v* 2.2 months for placebo; HR, 0.96; 95% CI, 0.76 to

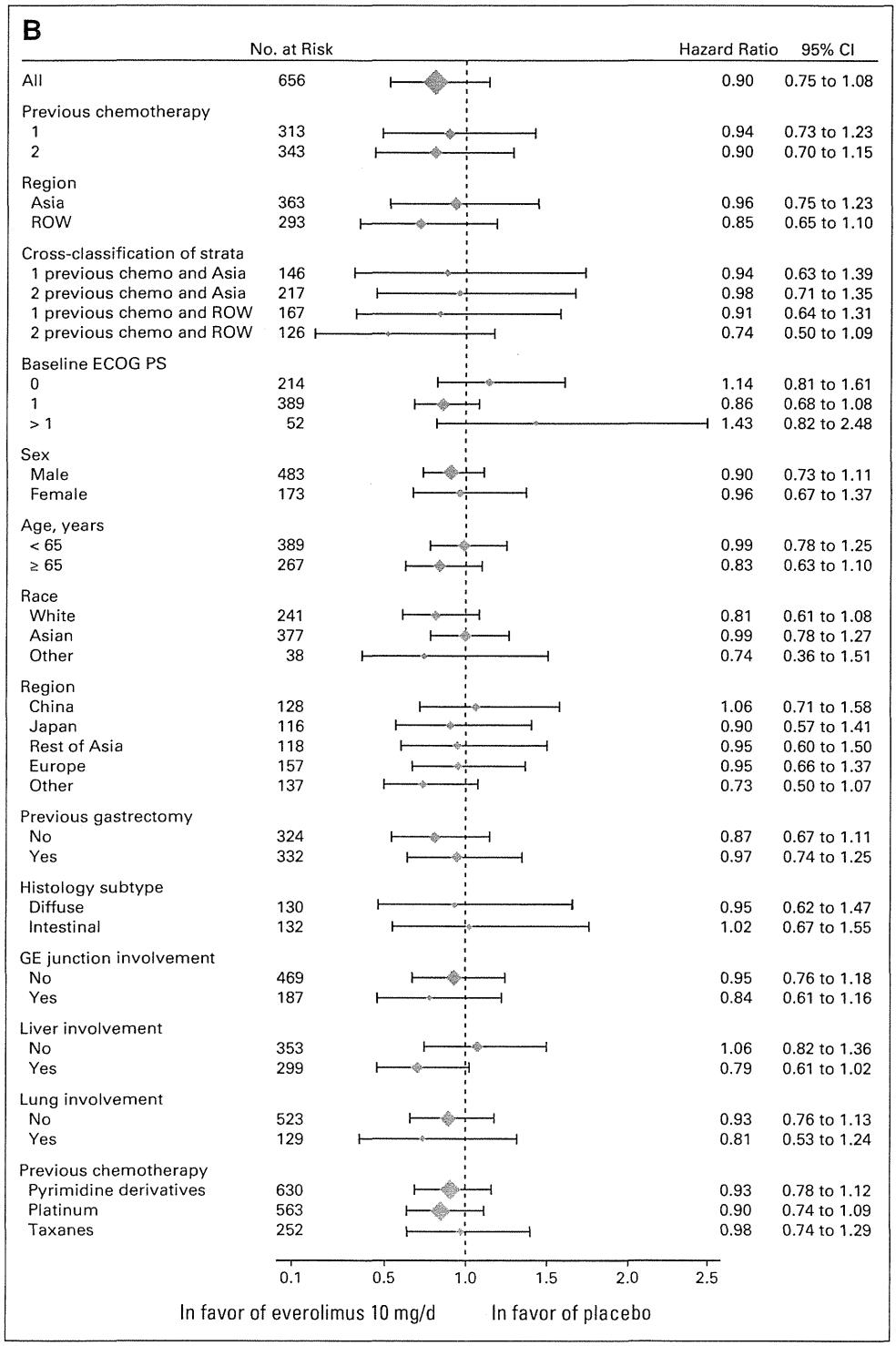

Fig 2. (Continued).

1.20; $P = .693$). A trend for a slightly longer time to ≥ 5% deterioration in global QoL was observed for everolimus (median time to ≥ 5% deterioration, 1.51 months *v* 1.45 months; HR, 0.84; 95% CI, 0.69 to 1.03; $P = .094$). Over time and versus placebo, everolimus recipients had higher mean scores for the global health status/QoL scale of the QLQ-C30 questionnaire (Fig 2D).

Safety

Almost all patients experienced at least one AE (99.1% in the everolimus arm and 96.7% in the placebo arm). The most common AEs (any grade) reported with everolimus were decreased appetite, stomatitis, fatigue, and nausea (Table 4). AEs that occurred in at least 10% of everolimus recipients were decreased appetite,

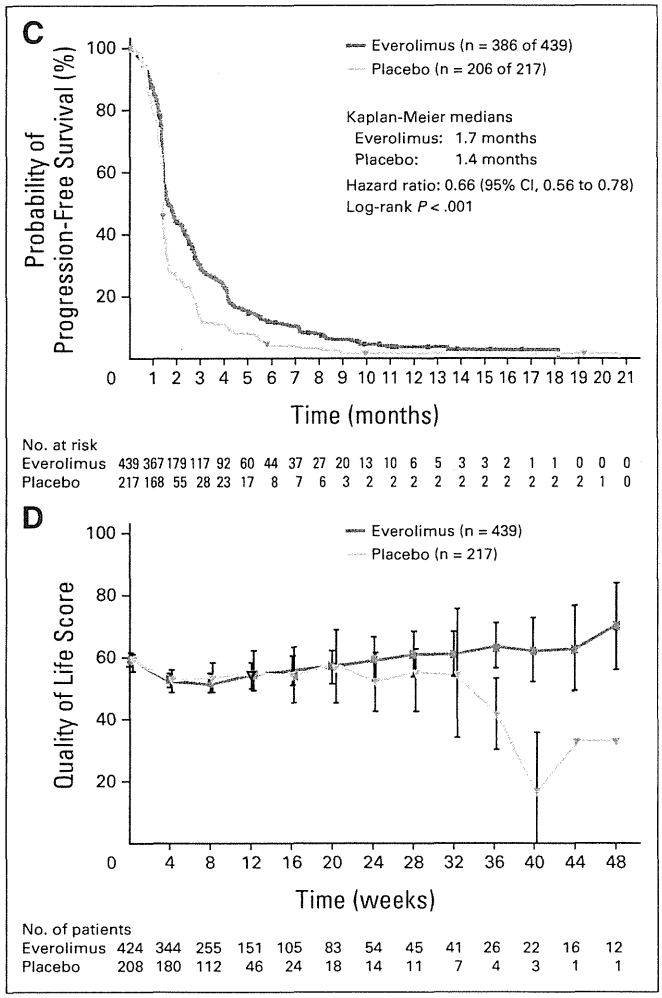

Fig 2. (Continued).

stomatitis, thrombocytopenia, rash, diarrhea, and decreased weight. The most common grade 3/4 AEs with everolimus were anemia, decreased appetite, and fatigue (Table 4). The proportion of patients who experienced grade 3/4 AEs was similar in all patient subgroups assessed (Table 5). All-grade and grade 3/4 pneumonitis were relatively uncommon, with incidences in the everolimus arm of 3.0% (n = 13) and 0.7% (n = 3), respectively. Pneumonitis was not observed in the placebo arm.

AEs leading to study drug discontinuation occurred in 21.5% of everolimus and 15.8% of placebo recipients; those leading to dose adjustments/interruptions occurred in 55.4% of everolimus and 21.4% of placebo recipeints. The most common AEs leading to study drug discontinuation (everolimus *v* placebo) were fatigue (2.1% *v* 1.4%), gastrointestinal hemorrhage (1.4% *v* 0.9%), and abdominal pain (1.1% *v* 0.5%). The AEs most commonly leading to dose adjustment or interruption were thrombocytopenia (everolimus: 10.3% *v* placebo: 0.5%), stomatitis (everolimus: 7.8% *v* placebo: 0.5%), and neutropenia (everolimus: 6.6% *v* placebo: 0%). Three patients in the everolimus arm died and their deaths were suspected to be a result of study treatment (n = 1 each for sudden death, grade 3 pneumonitis, and grade 4 gastrointestinal hemorrhage). In the placebo arm, two patients died and their deaths were suspected to be a result of study treatment (n = 1 each for multiorgan failure and cerebrovascular accident).

Table 3. Best Overall Tumor Response According to RECIST for Patients With Measurable Disease

Response	Everolimus Plus BSC (n = 379) No. of Patients	%	Placebo Plus BSC (n = 191) No. of Patients	%
Best overall response				
CR	1	< 1	0	0
PR	16	4	4	2
SD	147	39	38	20
PD	157	41	119	62
Unknown*	58	15	30	16
ORR (CR and PR)	17	4	4	2
DCR (CR, PR, and SD)	164	43	42	22

Abbreviations: BSC, best supportive care; CR, complete response; DCR, disease control rate; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.
*Tumor response data not available.

DISCUSSION

GRANITE-1 did not demonstrate a significant survival benefit for everolimus versus BSC in patients with advanced gastric cancer whose

Table 4. Adverse Events Irrespective of Relationship to Study Treatment With ≥ 10% Incidence in the Everolimus Plus BSC Treatment Arm in the Safety Population

Adverse Event	Everolimus Plus BSC (n = 437) Any Grade No. of Patients	%	Everolimus Plus BSC (n = 437) Grade 3/4 No. of Patients	%	Placebo Plus BSC (n = 215) Any Grade No. of Patients	%	Placebo Plus BSC (n = 215) Grade 3/4 No. of Patients	%
Decreased appetite	208	48	48	11	78	36	12	6
Stomatitis	174	40	20	5	23	11	0	0
Fatigue	150	34	34	8	65	30	11	5
Nausea	132	30	16	4	69	32	8	4
Diarrhea	115	26	15	3	33	15	2	1
Anemia	114	26	70	16	42	20	27	13
Abdominal pain	107	24	21	5	57	27	13	6
Vomiting	107	24	13	3	62	29	9	4
Constipation	91	21	3	< 1	42	20	3	1
Rash	87	20	1	< 1	19	9	0	0
Weight decreased	86	20	11	3	19	9	0	0
Pyrexia	81	19	3	< 1	24	11	2	1
Thrombocytopenia	80	18	22	5	5	2	3	1
Asthenia	70	16	20	5	22	10	9	4
Dyspnea	61	14	18	4	23	11	9	4
Upper abdominal pain	53	12	6	1	27	13	2	1
Peripheral edema	53	12	1	< 1	23	11	2	1
Hypokalemia	52	12	26	6	9	4	2	1
Insomnia	51	12	2	< 1	22	10	0	0
Cough	50	11	1	< 1	17	8	0	0
Back pain	48	11	10	2	16	7	2	1
Neutropenia	47	11	17	4	6	3	1	< 1
Pruritus	47	11	0	0	9	4	0	0

NOTE. All data are sorted by descending frequency in the everolimus plus BSC treatment group.
Abbreviation: BSC, best supportive care.

Table 5. Incidence of Grade 3/4 Adverse Events by Patient Subgroup in the Safety Population

	Everolimus Plus BSC		Placebo Plus BSC	
Patient Subgroup	No. of Patients	%	No. of Patients	%
Overall population	437	71	215	53
Gastrectomy				
Yes	224	70	107	48
No	213	72	108	59
Sex				
Male	322	69	161	55
Female	115	76	54	48
Age, years				
< 65	258	71	128	54
≥ 65	179	71	87	53
Race				
Asian	251	67	125	44
White	164	77	74	64
Other	22	77	16	81
Ethnicity				
Chinese	110	62	56	48
Japanese	74	70	41	39
Hispanic/Latino	35	74	15	60
Indian	2	50	0	0
Mixed	1	0	3	67
Other	215	76	100	61
Region				
Asia	243	65	119	45
ROW	194	78	96	65

Abbreviations: BSC, best supportive care; ROW, rest of world.

disease progressed on one or two lines of previous systemic chemotherapy. The lack of significant benefit for everolimus may be partially attributable to the slightly higher percentage of placebo recipients who initiated antineoplastic therapy after study drug discontinuation (45.2% *v* 39.2% for everolimus). OS results were consistent across subgroups, although a trend toward a reduced risk of death with everolimus was noted for patients enrolled in ROW (15% reduction in risk) and patients enrolled in ROW who received two previous systemic chemotherapy lines (26% reduction in risk). These trends, which may be a result of chance alone, were mostly driven by patients enrolled outside Europe. A 34% reduction in the risk of disease progression or death with everolimus was observed. Notably, the estimated percentage of patients remaining progression free at 6 months was higher with everolimus (12.0% *v* 4.3%), as were the disease control rate (43.3% *v* 22.0%) and the tumor shrinkage rate (37.8% *v* 12.3%). These results suggest everolimus has activity in this heavily pretreated population.

Identification of specific biomarkers for various patient subpopulations with advanced gastric cancer may help define those patients who would receive the most benefit from everolimus treatment. Despite extensive efforts, including those of a phase II study of everolimus in gastric cancer,[33] identification of gastric cancer biomarkers predictive of benefit from everolimus has been elusive. Results of ongoing biomarker analyses of GRANITE-1 are eagerly awaited.

Advanced gastric cancer, particularly that which progresses after systemic chemotherapy, is associated with a poor prognosis. The fact that 96.7% of placebo recipients in our study experienced at least one AE highlights the large number of comorbidities and overall high level of underlying risk in patients with heavily pretreated advanced gastric cancer. Although cross-study comparisons should be performed with caution, it is interesting that the median OS reported for everolimus in our trial (5.4 months) is similar to, or even longer than, that reported for second-line chemotherapy in two recent phase III studies, whereas the median OS reported for placebo in our study (4.3 months) is similar to, or even longer than, that reported for the control arms.[11,12] In a study of irinotecan versus BSC in 40 patients with advanced gastric cancer previously treated with only one line of systemic chemotherapy, irinotecan significantly reduced the risk of death (HR, 0.48; 95% CI, 0.25 to 0.92; $P = .012$).[11] Median OS was 4.0 months with irinotecan and 2.4 months with BSC; the disease control rate was 53% with irinotecan but was not reported for BSC. In the second study, 202 patients with advanced gastric cancer previously treated with one chemotherapy regimen that included both a fluoropyrimidine and platinum derivative or two chemotherapy regimens, of which one contained a fluoropyrimidine derivative and the other a platinum derivative, were randomly assigned to receive chemotherapy (docetaxel or irinotecan) or BSC.[12] Results of this study showed that second-line chemotherapy significantly reduced the risk of death (HR, 0.66; 95% CI, 0.49 to 0.89; $P = .007$). Median OS was 5.3 months with second-line chemotherapy versus 3.8 months with BSC. These results highlight the need to standardize chemotherapy regimens when designing clinical trials following first-line therapy. Notably, the use of post–first-line chemotherapy and types of regimens used differ owing to between-country differences in approved/preferred agents and reimbursement systems.

The everolimus AE profile observed in our study was generally consistent with that previously observed for everolimus in cancer, with no new safety signals identified.[18-20,28] Although stomatitis and pneumonitis, AEs commonly associated with everolimus, were observed in 39.8% and 3.0% of patients, respectively, they led to treatment discontinuation in only three patients (n = 2 for stomatitis, n = 1 for pneumonitis). The median duration of everolimus exposure was longer in patients with versus without gastrectomy, patients age at least 65 years versus those younger than 65 years, Asian versus white patients or patients of other races, Japanese versus other ethnicities, and patients enrolled in Asia versus ROW. AE incidence was mostly similar across patient subgroups.

In conclusion, the phase III GRANITE-1 study did not meet its primary objective of demonstrating a significant survival benefit for everolimus compared with BSC in patients with advanced gastric cancer whose disease progressed after one or two lines of previous systemic chemotherapy. The everolimus AE profile was consistent with that observed for everolimus in other cancers.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy,

please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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Expert Testimony: None **Patents:** None **Other Remuneration:** Heind Smith, Novartis

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Final approval of manuscript: All authors

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Acknowledgment

We thank the patients who participated in the GRANITE-1 trial; the investigators, the study nurses, and the clinical research associates from the individual trial centers who provided ongoing support; Melanie Leiby, PhD (ApotheCom, Yardley, PA), for assistance with manuscript preparation; and Novartis Pharmaceuticals for supporting this trial and for funding medical editorial assistance on the manuscript.

Appendix

Supportive methodology: Handling of missing values. For the primary end point of overall survival, if a patient was not known to have died, survival was censored at the date of last contact. For the secondary end point of progression-free survival (PFS), if a patient was not known to have died or experienced disease progression at the date of the analysis cutoff or when he/she received further antineoplastic therapy, PFS was censored at the time of the last adequate tumor assessment before the analysis cutoff date or the date of the start of new antineoplastic therapy, whichever occurred first. If a PFS event was observed after at least two missing tumor assessments, then the date of progression was censored at the date of the last adequate tumor assessment. If a PFS event occurred after a single missing tumor assessment, the actual date of disease progression was used. For the secondary end points of time to definitive deterioration of Eastern Cooperative Oncology Group (ECOG) performance status and time to definitive 5% deterioration in the global health status/quality of life scale of the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30, if a patient died before definitive deterioration but within 8 weeks (ie, twice the planned period between two assessments), the date of death was considered as the event date; patients who died after more than 8 weeks were censored at the date of their last available assessment. If definitive deterioration was observed after at least two missing assessments, the event was backdated to the first missing assessment before deterioration. For each EORTC QLQ-C30 subscale, the raw scores were standardized as described in the third edition of the EORTC QLQ-C30 manual (Fayers P et al: The EORTC QLQ-C30 Scoring Manuscript [ed 3]. Brussels, Belgium, EORTC, 2001). No specific methodology was applied to handle individual missing answers to specific questions of the EORTC QLQ-C30.

Subgroup analyses. For the primary end point of overall survival, analyses were performed for the following subgroups: number of prior chemotherapy lines (1 or 2), region (Asia or rest of world [ROW]), cross-classification of the number of prior chemotherapy lines and region (one prior regimen plus Asia; two prior regimens plus Asia; one prior regimen plus ROW, or two prior regimens plus ROW), baseline ECOG performance status (0, 1, or $\geq$ 2), sex (male or female), age ($<$ 65 years or $\geq$ 65 years), race (white, Asian, or other), specific region (China, Japan, rest of Asia, Europe, or other), prior gastrectomy (yes or no), histology subtype (diffuse or intestinal), gastroesophageal junction involvement (yes or no), liver involvement (yes or no), lung involvement (yes or no), and prior chemotherapy (pyrimidine derivatives, platinum, or taxanes).

Results of the interim analysis. A single interim analysis was performed after approximately 60% of the number of deaths required for final analysis was observed. At the time of the interim analysis, which occurred after 382 deaths were observed, the observed hazard ratio was 0.93 (95% CI, 0.75 to 1.16), and the *P* value from the stratified log-rank test was .266. This *P* value was greater than the .008 threshold required to stop the study for outstanding efficacy.

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Table A1. Everolimus Steady-State Blood Concentrations by Actual Dose in the Safety Population

	C_{min} (ng/mL)			C_{max} (ng/mL)		
Population	No. of Patients	Median	Range	No. of Patients	Median	Range
Everolimus, 10 mg/d						
Overall	201	13.8	0-81.8	218	67.5	15.3-282.0
Asia	127	15.1	0-54.9	132	69.4	18.3-167.0
ROW	74	11.4	2.2-81.8	86	63.7	15.3-282.0
With gastrectomy	118	13.8	0-81.8	125	72.9	19.9-282.0
Without gastrectomy	83	13.2	2.6-60.3	93	53.8	15.3-157.0
Everolimus, 5 mg/d						
Overall	18	9.3	2.1-24.3	16	34.7	6.3-98.9
Asia	11	9.8	2.1-21.2	10	29.1	6.3-81.0
ROW	7	6.3	4.0-24.3	6	34.7	12.3-98.9
With gastrectomy	10	9.0	4.9-24.3	9	41.9	14.8-81.0
Without gastrectomy	8	10.0	2.1-17.2	7	12.3	6.3-98.9

Abbreviations: C_{max}, maximum concentration in whole blood; C_{min}, minimum concentration in whole blood; ROW, rest of world.

Table A2. Antineoplastic Therapies Since Discontinuation of Study Treatment in the Full Analysis Set

Type of Therapy	Everolimus Plus BSC (n = 439)		Placebo Plus BSC (n = 217)	
	No. of Patients	%	No. of Patients	%
Any	172	39.2	98	45.2
Type of therapy*				
Chemotherapy	155	35.3	89	41.0
Immunotherapy	1	0.2	0	0
Radiation therapy	13	3.0	6	2.8
Surgery	0	0	1	0.5
Targeted therapy	5	1.1	1	0.5
Other	3	0.7†	4	1.8‡

Abbreviation: BSC, best supportive care.
*Patients could receive > 1 type of therapy.
†Includes Chinese traditional medicine (n = 2) and Java Brucea fruit fat injection (n = 1).
‡Includes Chinese traditional medicine (n = 1), antineoplastic agents (n = 1), fluorouracil (n = 1), and PDK1 inhibitor (n = 1).

Table A3. Analysis of Survival in the Full Analysis Set

Survival	Everolimus Plus BSC (n = 439)		Placebo Plus BSC (n = 217)		Hazard Ratio	95% CI	*P*
	No. of Patients	%	No. of Patients	%			
Overall survival							.1244
Deaths	352	80.2	180	82.9	0.90	0.75 to 1.08	
Censored	87	19.8	37	17.1	—		
PFS							
Total events	386	87.9	206	94.9	0.66	0.56 to 0.78	< .001
Progression	315	71.8	174	80.2	—		
Deaths	71	16.2	32	14.7	—		
Censored	53	12.1	11	5.1	—		

Abbreviations: BSC, best supportive care; PFS, progression-free survival.

Annals of Oncology 24: 2560–2565, 2013
doi:10.1093/annonc/mdt238
Published online 24 July 2013

Impact of excision repair cross-complementing gene 1 (ERCC1) on the outcomes of patients with advanced gastric cancer: correlative study in Japan Clinical Oncology Group Trial JCOG9912

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Received 5 March 2013; revised 17 May 2013; accepted 21 May 2013

Background: Since the best chemotherapy regimen for each patient with advanced gastric cancer is uncertain, we aimed to identify molecular prognostic or predictive biomarkers from biopsy specimens in JCOG9912, a randomized phase III trial for advanced gastric cancer.

Patients and methods: Endoscopic biopsy specimens from primary lesions were collected in 445 of 704 randomized patients in JCOG9912. We measured the mRNA expression of excision repair cross-complementing group 1 (ERCC1), thymidylate synthase, dihydropyrimidine dehydrogenase, and five other genes, then, categorized them into low and high groups relative to the median, and examined whether gene expression was associated with efficacy end point.

Results: Multivariate analyses showed that high ERCC1 expression [HR 1.37; 95% confidence interval (CI) 1.08–1.75; $P = 0.010$], performance status ≥1 (HR 1.45; 95% CI 1.13–1.86; $P = 0.004$), and number of metastatic sites ≥2 (HR 1.66; 95% CI 1.28–1.86; $P < 0.001$) were associated with a poor prognosis, and recurrent disease (versus unresectable; HR 0.75; 95% CI 0.56–1.00; $P = 0.049$) was associated with a favorable prognosis. None of these molecular factors were a predictive marker for choosing irinotecan plus cisplatin or 5-fluorouracil rather than S-1.

Conclusion: These correlative analyses suggest that ERCC1 is an independent prognostic factor for overall survival in the first-line treatment of gastric cancer.

Clinical Trial Number: C000000062, www.umin.ac.jp.

Key words: dihydropyrimidine dehydrogenase, excision repair cross-complementing gene 1, gastric cancer, prognostic factor, thymidylate synthase, vascular endothelial growth factor

introduction

Fluoropyrimidine and platinum-based combination therapies are the most commonly used and acceptable first-line therapies all over the world. Poor performance status (PS), liver metastases, peritoneal metastases, and higher value of plasma alkaline phosphatase have been identified as clinical prognostic factors for local and advanced gastric cancer [1]. However, these prognostic factors are not predictive markers for selecting the optimal regimens for systemic chemotherapy. Therefore, we need to have a better understanding of biological prognostic markers of conventional cytotoxic agents to so that we can give patients the optimal drugs to prolong their survival and improve their quality of life, since cytotoxic drugs are not effective in every patient and often have severe adverse effects.

Excision repair cross-complementation group 1 (ERCC1) is an important component of the nuclear excision repair pathway

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which repairs DNA intrastrand, interstrand, and DNA-protein crosslinks caused by cisplatin. High mRNA levels of ERCC1 in primary gastric cancer may be associated with a lower response to cisplatin and poor survival [2]. The overall survival (OS) in patients with low ERCC1 levels was significantly longer than that in patients with high levels [3]. Several potential predictive factors of the response to 5-fluorouracil (5-FU) or prognostic factors have been reported in the metabolic pathway of 5-FU and folic acid. These include thymidylate synthase (TS), which is a target enzyme of 5-FU for the synthesis of DNA, and the cytosolic enzyme dihydropyrimidine dehydrogenase (DPD), which degrades 5-FU in mainly the liver but also in tumor [4, 5]. High mRNA expression of TS and DPD has been shown to predict a poor clinical outcome of treatment with 5-FU [5, 6].

Some studies have suggested that expression of the ERCC1, TS, and DPD genes is clinically useful for predicting the effects of chemotherapy. Other studies [7, 8], however, have failed to confirm that they are associated with the outcome of chemotherapy. Thus, further larger studies are required to identify predictive and prognostic factors to individualize anticancer drugs in patients.

The Japan Clinical Oncology Group (JCOG) trial JCOG9912 was a randomized phase III trial of advanced gastric cancer which revealed the noninferiority of S-1 to 5-FU [hazard ratio (HR) 0.83; 95% confidence interval (CI) 0.68–1.01; $P < 0.001$] with regard to OS, but failed to show the superiority of irinotecan plus cisplatin (IP) (HR 0.85; 95% CI 0.70–1.04; $P = 0.055$) [9].

This study was designed to identify differences in survival and tumor shrinkage after 5-FU, S-1, and IP therapy through the use of molecular markers, and to identify potential prognostic and predictive factors for the clinical outcome from subset analyses in JCOG9912.

patients and methods

Between 2000 and 2006, 704 patients were enrolled in the JCOG9912 trial [9]. After the primary analysis of JCOG9912, endoscopic biopsy specimens taken before treatment were obtained from patients enrolled in JCOG9912. The tumor response was scheduled to be assessed every 8 weeks according to the RECIST ver1.0. OS was defined as the period from the date of randomization until death from any cause. Progression-free survival (PFS) was calculated as the time from randomization until the first objective evidence of disease progression or death from any cause. Written informed consent to be enrolled in JCOG9912 was obtained before registration and the opportunity to refuse to provide tumor samples for this translational research was provided through web sites of the National Cancer Center (NCC) and JCOG according to the Japanese Ethical Guidelines for Clinical Studies. The protocol of this translational study was approved by the institutional review board of NCC and each participating hospital, and complied with the REMARK, reporting recommendations for tumor marker prognostic studies [10].

laboratory methods

The tumor cells on the sections of interest were selectively isolated by laser-captured microdissection (P.A.L.M. Microsystem, Leica, Wetzlar, Germany). ERCC1, TS, DPD, orotate phosphoribosyl transferase (OPRT), and methylene tetrahydrofolate reductase (MTHFR), epidermal growth factor receptor (EGFR), topoisomerase I (Topo-1), vascular endothelial growth factor-A (VEGF-A), and an internal reference gene (beta-actin) were quantified with a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System, TaqMan®, Perkin-Elmer [PE] Applied Biosystems, Foster City, CA). The same primers and probes as previously described were used [7].

statistical analysis

To assess the associations of gene expression levels with the response rate (RR), PFS, and OS, the expression levels of each gene were categorized into low and high values with respect to the median. Categorical data were evaluated using Fisher's exact test. The probability of survival was calculated with the Kaplan–Meier method, and differences between curves were evaluated with the log-rank test. Estimates of hazard ratios with 95% CIs based on a Cox proportional hazards model were used to provide quantitative summaries of the gene expression data.

Variables for the multivariate analysis included the genes with expression levels (high or low) that showed associations in the univariate analyses in this study, as well as the patient's background, such as sex, age, tumor status (recurrent versus unresectable), PS, number of metastatic sites, presence or absence of target lesions according to RECIST version 1.0, macroscopic type (Borrmann 0,1,2 versus 3,4,5), histological classification (intestinal/diffuse), and presence or absence of peritoneal metastasis. All reported P-values are two sided, and the level of statistical significance was set at $P < 0.05$. All analyses were carried out using the SAS statistical package, version 9.1 or 9.2 (SAS Institute, Inc., Cary, NC).

results

patient characteristics and molecular biomarkers

Tissue samples for this gene expression study were collected in 445 of 704 randomized patients in JCOG9912, and assay data were available in 325 (supplementary Figure S1, available at *Annals of Oncology* online). The MST of the 325 patients analyzed in this correlative study was 12.6 months (95% CI 11.5–14.1). The MST was 11.5 months in the 5-FU arm, 14.2 months in the IP arm, and 11.9 months in the S-1 arm. The baseline characteristics were equally distributed among the subsets for each biomarker (supplementary Table S1, available at *Annals of Oncology* online). The numbers of patients assayed were not equal for each biomarker because some samples were not sufficient for all eight assays.

The mRNA expression of ERCC1 and DPD in the diffuse type were higher than those in the intestinal type (Figure 1), while there were no clear associations between histological types and the expression of the other five genes for OPRT, EGFR, MTHFR, Topo-1, and VEGF-A. ERCC1 expression did not show a strong association with TS expression (Spearman's coefficient 0.38) or DPD (0.30). Higher VEGF-A expression was more commonly observed in patients with unresectable disease ($P = 0.060$), target lesions ($P = 0.052$), and liver metastasis ($P = 0.090$) (supplementary Table S2, available at *Annals of Oncology* online).

value of molecular markers and efficacy in each treatment arm

To better understand the association between mRNA levels of selected biomarkers and treatment outcomes with each chemotherapy regimen, we carried out a subgroup analysis in terms of tumor shrinkage (Table 1). The RR of IP in the low ERCC1 group was significantly higher than that in the high

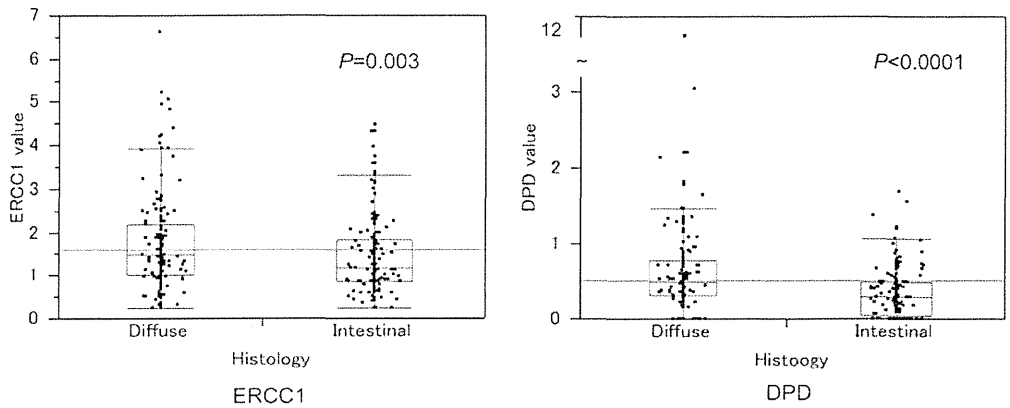

Figure 1. Gene expression levels in diffuse type and intestinal type. Intestinal type, papillary and tubular adenocarcinoma; diffuse type, poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma; ERCC1, excision repair cross-complementation group 1; DPD, dihydropyrimidine dehydrogenase.

ERCC1 group ($P = 0.045$). IP was also more effective in patients with low DPD compared with high DPD ($P = 0.006$). A similar tendency was seen for 5-FU: the RR was 17.5% in the low ERCC1 group and 2.7% in the high ERCC1 group ($P = 0.058$). The RR in patients with low TS treated with 5-FU (16.7%) seemed to be higher than that in patients with high TS (2.9%) ($P = 0.068$). On the other hand, S-1 showed constant activity in terms of the RRs between low and high ERCC1, TS, DPD, and the five other genes. There were no significant findings regarding the associations between the expression levels of the five other genes and the RR.

Although the RR for IP in the low ERCC1 group was better than that in the high ERCC1 group, there was no difference in PFS of IP regardless of the expression level of ERCC1 (HR 1.04; $P = 0.82$). Similarly, there was no difference in PFS of S-1 between the low and high ERCC1 groups. Patients with high ERCC1 showed substantially worse survival than those with low ERCC1 in both S-1 and IP, as did patients with high TS in IP.

value of molecular markers as prognostic factors

A univariate analysis of the whole study population showed that both OS and PFS in the low ERCC1 and low TS groups were better than those in the high ERCC1 and high TS groups (supplementary Tables S3 and S4, available at *Annals of Oncology* online). There were no differences in OS or PFS according to the expression of the six other genes.

Multivariate analyses for OS with molecular markers and clinical characteristics showed that ERCC1 (HR 1.37; 95% CI 1.08–1.75, $P = 0.010$), PS, tumor status (recurrent versus unresectable), and the number of metastatic sites were independent prognostic factors for OS (supplementary Table, available at *Annals of Oncology* online). Multivariate analyses for PFS showed that recurrent disease and a histological classification of intestinal type were independent favorable prognostic factors.

value of molecular markers as predictive factors

Supplementary Table S5, available at *Annals of Oncology* online, shows the predictive values of ERCC1, TS, and DPD for choosing 5-FU or IP rather than S-1. Although marginal interaction was seen between ERCC1 and PFS after 5-FU or S-1, S-1 was superior to 5-FU regardless of the expression level of ERCC1. Thus, ERCC-1 cannot be a predictive marker for choosing S-1 or 5-FU from the perspective of PFS. The hazard ratios of IP compared with S-1 for PFS and OS in the low DPD group were 0.87 and 0.84, and those in the high DPD group were 1.13 and 1.21, which suggested that there might be some interaction between DPD and the treatment arm of IP or S-1. Furthermore, ERCC1, TS, and the five other genes had no predictive value for choosing IP rather than S-1 from the perspective of either PFS or OS.

discussion

This study shows that low ERCC1 expression was a significant independent favorable prognostic factor in patients with advanced gastric cancer who were receiving first-line chemotherapy regardless of the treatment regimen in JCOG9912. High ERCC1 expression confers cisplatin resistance and reconstitutes the cell's ability to remove cisplatin from cellular DNA in an animal model [11]. Furthermore, the aberrant methylation of DNA repair genes has been shown to be indicative of sensitivity to chemotherapeutic agents other than cisplatin [12]. Other studies in ovarian [13], pancreatic [14], lung cancer [15] have also suggested that greater activity of ERCC1 was associated with resistance to platinum compounds. In this study, patients with low ERCC1 showed higher RRs than those with high ERCC1 in both IP and 5-FU, while the RRs were similar regardless of the ERCC1 level among patients treated with S-1.

The expression of several DNA repair genes has been shown to be inactivated or decreased in tumors associated with promoter hypermethylation [16], and it has been reported that ERCC1 promoter methylation was inversely associated with mRNA expression [17]. Concurrent hypermethylation of gene promoters is associated with a high microsatellite instability phenotype in gastric cancer [18], and the concordant methylation of CIMP-high is associated with better survival [19]. Overall, in this study, in patients with a high expression of

Table 1. Univariate analyses for clinical outcomes in first-line chemotherapy: correlation with mRNA expression levels

mRNA	Group	All			5-Fluorouracil			S-1			Irinotecan plus cisplatin		
		n	RR (%)	*P*	*n*	RR (%)	*P*	*n*	RR (%)	*P*	*n*	RR (%)	*P*
ERCC1	Low	120	34.2	0.064	40	17.5	0.058	40	32.5	1.00	40	52.5	0.045
	High	123	22.8		37	2.7		42	33.3		44	29.6	
TS	Low	120	33.3	0.15	42	16.7	0.068	36	41.7	0.17	42	42.9	0.82
	High	120	24.2		34	2.9		45	26.7		41	39.0	
DPD	Low	119	31.9	0.39	46	15.2	0.24	37	27.0	0.24	36	58.3	0.006
	High	113	26.6		26	3.9		41	41.5		46	26.1	

mRNA	Group	*n*	mPFS (months)	HR (95%CI)	*P*	*n*	mPFS (months)	HR (95% CI)	*P*	*n*	mPFS (months)	HR (95% CI)	*P*	*n*	mPFS (months)	HR (95% CI)	*P*
ERCC1	Low	162	4.78	1	0.31	53	3.81	1	0.062	55	5.32	1	0.87	54	5.32	1	0.82
	High	160	3.89	1.12 (0.90–1.40)		50	2.07	1.45 (0.98–2.14)		55	4.29	1.03 (0.71–1.51)		56	4.32	1.04 (0.72–1.52)	
TS	Low	159	5.10	1	0.015	54	3.71	1	0.093	48	5.34	1	0.16	57	5.59	1	0.068
	High	158	3.81	1.32 (1.06–1.65)		47	2.10	1.40 (0.94–2.10)		60	4.24	1.32 (0.90–1.94)		51	4.11	1.43 (0.97–2.11)	
DPD	Low	154	4.22	1	0.97	57	2.14	1	0.60	50	4.47	1	0.73	47	5.72	1	0.26
	High	150	4.21	1.00 (0.80–1.26)		37	3.58	0.89 (0.59–1.36)		52	4.35	0.93 (0.63–1.38)		61	4.04	1.25 (0.85–1.84)	

mRNA	Group	*n*	MST (months)	HR	*P*	*n*	MST (months)	HR	*P*	*n*	MST (months)	HR (95% CI)	*P*	*n*	MST (months)	HR	*P*
ERCC1	Low	162	14.9	1	0.016	53	11.8	1	0.41	55	15.0	1	0.10	54	16.1	1	0.066
	High	160	11.5	1.32 (1.05–1.65)		50	10.5	1.18 (0.79–1.75)		54	11.0	1.39 (0.94–2.07)		56	11.7	1.43 (0.97–2.12)	
TS	Low	159	14.2	1	0.034	54	11.0	1	0.53	48	11.9	1	0.36	57	16.8	1	0.0014
	High	158	11.5	1.28 (1.02–1.61)		47	11.8	1.14 (0.76–1.70)		60	12.8	1.21 (0.81–1.81)		51	11.1	1.89 (1.27–2.80)	
DPD	Low	154	11.9	1	0.64	57	11.5	1	0.65	50	11.5	1	0.44	47	15.5	1	0.22
	High	150	13.1	0.95 (0.75–1.20)		37	11.8	0.91 (0.59–1.39)		52	12.1	0.85 (0.56–1.29)		61	14.1	1.28 (0.86–1.90)	

High ERCC1 and High TS were poor prognostic markers in advanced gastric cancer. ERCC1 and DPD were the predictive factors of tumor shrinkage in irinotecan plus cisplatin.

ERCC1, Excision repair cross-complementation group 1; TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; RR, response rate; mPFS, median progression-free survival time; MST, median overall survival time; HR, hazard ratio.

ERCC1 who received first-line chemotherapy, the risk of death was increased by more than 30% compared with that in low ERCC1 patients.

In colorectal cancer, since many studies have examined the molecular predictors of outcomes over the past two decades, TS and DPD were newly listed in 'ASCO 2006 Tumor Marker Guidelines in Gastrointestinal Cancer' [20]. However, due to a lack of sufficient supporting evidence, the guidelines recommend that these biomarkers should not yet be used clinically to predict the prognosis or treatment response. With regard to TS in this study, while patients with high TS showed slightly lower RRs than those with low TS in both 5-FU and S-1, there was no difference in the RR regardless of the expression level of TS in IP. However, PFS and OS in patients with high TS in IP were similar to those in S-1. As a result, TS could not be a predictive marker for choosing IP over S-1. Two previous prospective trials with pharmacogenetic-tailored therapy against colorectal cancer failed to confirm the predictive values of TS and DPD [21, 22]. TS and DPD were not predictive markers for selecting 5-FU/leucovorin or irinotecan/oxaliplatin, since the group of patients who had low TS and low DPD not only had a high RR to 5-FU/leucovorin when compared with irinotecan/oxaliplatin, but they also had a longer OS [21].

As for DPD, S-1 showed a higher RR in patients with high DPD than in those with low DPD, while the reverse association between the DPD level and RRs was observed in 5-FU. However, since S-1 showed better efficacy than 5-FU regardless of the level of DPD, DPD could not be a predictive marker for choosing between S-1 and 5-FU. While IP showed a higher response, PFS and OS in patients with low DPD were slightly longer than those in patients with high DPD, and the efficacy of S-1 was slightly worse in low DPD than in high DPD, the hazard ratios of IP compared S-1 in low DPD for PFS and OS were marginal (0.87 and 0.84) and those in high DPD were 1.13 and 1.21. It is speculated that a low DPD might have some potential as a predictive marker for selecting IP rather than S-1. Similar results were observed in the CAIRO study which compared capecitabine plus irinotecan to capecitabine monotherapy for patients with metastatic colorectal cancer; the irinotecan combined regimen was more efficacious in a low DPD group. Based on our current knowledge, this association between DPD and irinotecan is difficult to explain logically, and further studies are needed to more clearly define the association between DPD and the efficacy of regimens that contain irinotecan.

In this study, patients with low ERCC1 showed a higher RR than those with high ERCC1 in IP, and RRs were similar regardless of the ERCC1 level among patients treated with S-1. On the other hand, there were no differences in PFS or OS among patients with low ERCC1 between IP and S-1. As a result, no predictive marker for selecting 5-FU or IP rather than S-1 could be found in this study. The pattern and extent of DNA damage induced by fluoropyrimidines in human cancer cell lines varies and may be affected not only by the activity of enzymes involved in DNA repair but also by downstream factors such as p53. Wild-type p53 was a strong predictor of sensitivity to 5-FU in cell lines of the National Cancer Institute's Anticancer Drug Screen panel *in vitro* [23]. Many other factors associated with chemosensitivity should be investigated in future studies to identify predictive markers of cytotoxic agents.

In conclusion, our study provides evidence that high mRNA expression of ERCC1 in primary lesions of gastric cancer is associated with significantly worse OS. We did not identify a predictive marker for choosing 5-FU or IP rather than S-1.

acknowledgements

We thank Kathleen D. Danenberg, Response Genetics, Inc., Los Angeles, CA, USA; Peter V. Danenberg, University of Southern California, CA, USA; Teiji Takechi and Takashi Kobunai, Taiho Pharmaceutical Co. Ltd, Tokyo, Japan; Hideko Morita, Hiromi Orita, and Eri Ohnishi, National Cancer Center Hospital, for their technical support and fruitful discussion.

funding

Financial support for this research was provided by the National Cancer Center Research and Development Fund (23-A-16 and 23-A-19); a Grant-in-Aid for Cancer Research (20S-3, 20S-6) from the Ministry of Health, Labour, and Welfare; and Taiho Pharmaceutical Co. The funding sources had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

disclosure

YY has received honoraria from Taiho and Chugai, and has received research funding from Astrazeneca and Novartis. NB has received honoraria from Taiho, Yakult, and Daiichi-Sankyo and research funding from Taiho. All remaining authors declare no conflicts of interest.

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Annals of Oncology 24: 2565–2570, 2013
doi:10.1093/annonc/mdt247
Published online 14 July 2013

A simple prognostic scoring system for patients receiving transarterial embolisation for hepatocellular cancer

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Received 13 January 2013; revised 9 April 2013; accepted 23 May 2013

Background: The prognosis for patients with hepatocellular cancer (HCC) undergoing transarterial therapy (TACE/TAE) is variable.

Methods: We carried out Cox regression analysis of prognostic factors using a training dataset of 114 patients treated with TACE/TAE. A simple prognostic score (PS) was developed, validated using an independent dataset of 167 patients and compared with Child–Pugh, CLIP, Okuda, Barcelona Clinic Liver Cancer (BCLC) and MELD.

Results: Low albumin, high bilirubin or α-fetoprotein (AFP) and large tumour size were associated with a two- to threefold increase in the risk of death. Patients were assigned one point if albumin <36 g/dl, bilirubin >17 μmol/l, AFP >400 ng/ml or size of dominant tumour >7 cm. The Hepatoma arterial-embolisation prognostic (HAP) score was calculated by summing these points. Patients were divided into four risk groups based on their HAP scores; HAP A, B, C

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Clinical Study

Oncology

Oncology 2013;84:57–64
DOI: 10.1159/000337981

Received: October 10, 2011
Accepted after revision: March 5, 2012
Published online: October 30, 2012

Treatment Strategy for Superficial Pharyngeal Squamous Cell Carcinoma Synchronously Combined with Esophageal Cancer

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Key Words
Pharyngeal cancer · Chemotherapy · Esophageal squamous cell carcinoma · Narrow band imaging · Early detection

Abstract
Background: Esophageal squamous cell carcinoma (ESCC) is often synchronously accompanied by pharyngeal squamous cell carcinoma (PSCC). However, treatment strategies for these synchronous cancers have not been established. ***Aim:*** To evaluate retrospectively the effects of both chemoradiotherapy (CRT) targeted for invasive ESCC on synchronous superficial PSCC and additional endoscopic resection (ER) for PSCC. ***Patients and Methods:*** Screening endoscopy in the pharynx was performed in newly diagnosed ESCC patients. CRT combined with 5-fluorouracil (5-FU) and cisplatin (CDDP) was administered to all patients. The effect on superficial PSCC was only evaluated for 5-FU-CDDP chemotherapy that excluded the pharynx from the radiation field. When PSCC was remnant or recurrent in patients evaluated at complete response (CR) of ESCC, ER was performed on the PSCC. ***Results:*** Fourteen cases of superficial PSCC (4.0%) were detected in 348 ESCC patients. Three PSCC reached CR in 8 ESCC-CR patients, while all 3 lesions recurred. No treatment response was found in the remaining 11 PSCC. As a second treatment, ER for 8 PSCC was completed in the 8 ESCC-CR patients, with one complication due to pneumonia. ***Conclusions:*** Standard 5-FU-CDDP CRT targeted for invasive ESCC did not demonstrate a sufficient efficacy for superficial PSCC, while ER even for PSCC after chemotherapy was curative.

Introduction

Esophageal squamous cell carcinoma (ESCC) is often accompanied by pharyngeal squamous cell carcinoma (PSCC) either simultaneously with the primary lesion (synchronously) or after a period of time (metachronously). These findings have been explained by the 'field cancerization' theory that describes how repeated local exposure to carcinogens contributes to the occurrence of multiple cancers in the esophageal and head and neck regions [1]. For more than 5 decades many epidemiological studies have attributed the increased cancer risks associated with alcohol drinking and smoking to this phenomenon

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[2–6]. In 2009, the Working Group of WHO-IARC concluded that acetaldehyde associated with alcoholic beverages was carcinogenic to humans and confirmed the group 1 classification of alcohol consumption [7]. In addition, heterozygous traits found in 40% of Asians, who have an inactive alcohol metabolizing enzyme of aldehyde dehydrogenases 2, accumulate acetaldehyde, with higher relative risks of these cancers [7, 8]. Furthermore, the prevalence of multiple Lugol-unstained lesions (LULs) [9, 10], which are caused by repeated exposure to acetaldehyde, was strongly related to the occurrence of synchronous or metachronous cancers in the esophagus and head and neck regions [11].

In contrast, most patients with PSCC are detected at an advanced stage with a poor prognosis. Even in an operable PSCC case, the extensive surgical resection required may cause a loss of function with respect to swallowing and/or speaking and can lead to cosmetic deformities. Thus it is difficult to determine a final treatment from the viewpoints of both curability and retaining organ function. In cancers combining ESCC and PSCC, the selection of treatment is even more critical. Because of this, the ability to detect pharyngeal lesions at an earlier stage, e.g. as carcinoma in situ, would be of clear benefit to patients. Recently, superficial PSCC has been detected by NBI endoscopy [12].

Systemic 5-fluorouracil-cisplatin (5-FU-CDDP) chemotherapy combined with radiotherapy is the standard treatment for ESCC, and the same treatment is also effective for PSCC patients [13, 14]. The radiation field used in radiotherapy for ESCC does not generally reach the region of the larynx and pharynx, while chemotherapy acts systemically. There have been no reports regarding the efficacy of systemic chemotherapy for patients with superficial PSCC. In this study, we examined the effect on superficial PSCC of chemoradiotherapy (CRT) targeted for invasive ESCC.

Patients and Methods

Patients

Between January 2003 and December 2006, concurrent CRT was performed in 348 patients with invasive ESCC who met the following criteria of this study: (1) newly diagnosed thoracic ESCC; (2) aged between 20 and 75 years; (3) clinical stage I to IVA according to the UICC-TNM classification; (4) absence of previous chemotherapy for malignancy; (5) absence of radiation or surgical treatment for head and neck, and esophageal cancers, and (6) absence of active malignancy except ESCC and PSCC. All patients with invasive ESCC visited our hospital to receive treatment after histological diagnosis of ESCC by endoscopy at another hospital.

Endoscopic Observation of the Oral Cavity and Pharynx

Since January 2003, endoscopic screening of the oral region has been performed in all ESCC patients in order to detect synchronously superficial PSCC. In the initial endoscopic observation in our hospital, narrow band imaging (NBI) or conventional endoscopy was used because both evaluation of ESCC and gastroduodenal screening including oral cavity and pharynx are performed in all patients. When a mucosal abnormality in the oral cavity or pharynx, or multiple LULs in the esophagus, were found in initially conventional endosopcy, the oral cavity and pharynx were observed again by magnifying NBI endoscopy within 2 weeks. Figure 1 shows the NBI findings of an oral cavity and pharynx using a video endoscope system (EVIS LUCERA CV-260, Olympus Optical Co. Ltd., Tokyo, Japan). When a brownish area and an enhancement of the intraepithelial papillary capillary loop were found in the pharynx (fig. 2), an endoscopic biopsy was performed to histologically confirm the carcinoma.

Lugol chromoendoscopy was performed in all patients for both diagnosis of the correct cancer region and evaluation of LULs in the background esophageal epithelium. After ordinary endoscopic observation, 5–10 ml of 2.0% glycerin-free Lugol iodine solution, which is a brown liquid consisting of 2.0 g potassium iodine and 4.0 g iodine in 100 ml distilled water, was sprayed from the upper thoracic esophagus to the gastroesophageal junction using a plastic spray catheter passed through the biopsy channel of the endoscope. Multiple LULs were defined as described in our previous study [15].

Definition of Superficial Pharyngeal Cancer

According to the Japan Society for Head and Neck Cancer [16], a superficial pharyngeal lesion is defined as one in which the invasion depth is comparatively limited and visual changes do not indicate an advanced cancer. The pharynx has no muscularis mucosa, so this somewhat vague definition suggests that the depth of invasion is limited to the epithelium or just beneath the epithelium, but does not extend to the muscle layer.

Treatment Schedule of CRT for ESCC

Chemotherapy consisted of a protracted infusion of 5-FU at a dose of 1,000 mg/m^2 per day on days 1–5 and 22–26, combined with a 2-hour infusion of CDDP at 75 mg/m^2 on days 1 and 22. A 10-MV radiation treatment was administered for 6 weeks (5 days/week) at 1.8 Gy/day with a total radiation dose of 50.4 Gy, concomitantly with chemotherapy.

Patients who were evaluated for an objective response to this treatment received additional chemotherapy consisting of a continuous infusion of 5-FU at a dose of 1,000 mg/m^2 on days 1–5 and CDDP at a dose of 75 mg/m^2 on day 1. This treatment schedule was administered for 1 week followed by a 3-week break. All patients receiving CRT were monitored by neck, chest and abdominal computed tomography, and by endoscopy to evaluate the efficacy of the treatment on both ESCC and PSCC.

As for response for ESCC, objective responses of measurable metastatic lesions were evaluated according to the response evaluation criteria in solid tumors (RECIST v 1.0) guideline. Response of the primary tumor was evaluated by the criteria of the Japan Esophageal Society [17, 18].

Evaluation of Response for PSCC

All follow-up evaluations after 5-FU-CDDP chemotherapy for PSCC were performed every 2 months for the first year and every 6 months thereafter by magnifying NBI endoscopy, with the same periods of evaluation as for ESCC. For PSCC, complete response (CR) was defined as the disappearance of all visible tumors (brownish areas), including ulceration, for at least 4 weeks, confirmed by normal endoscopic biopsy specimens. The recurrence was defined as the reappearance of a brownish area accompanied by an enhancement of intraepithelial papillary capillary loop by NBI endoscopy, and was confirmed in histological findings by endoscopic biopsy. Non-CR for PSCC was defined as the remnant of brownish areas and was classified into a partial response, stable disease or progressive disease.

In the case of non-CR for PSCC, the second treatment was selected according to the efficacy of CRT for ESCC. When ESCC reached CR with remnant or recurrence of PSCC, endoscopic resection (ER) was performed for PSCC. When the ESCC was evaluated for non-CR, thereafter treatment for ESCC, such as second-line chemotherapy, salvage surgery or palliation was performed.

ER for PSCC after CRT

The ER involved endoscopic mucosal resection using the cup method or an endoscopic subepithelial dissection method with the patient under general anesthesia. An important consideration was that ER for PSCC should be performed with cooperation from the endoscopists and the head and neck surgeons. Some head and neck surgeons participated in the ER to prepare emergency treatment, such as tracheostomy, with evaluation of the degree of laryngeal edema after the procedure.

Statistics

All statistical analyses were performed using IBM SPSS Statistics 18 software (SPSS Inc., Tokyo, Japan). Overall survival data were calculated from the date of commencement of CRT to the date of death or the most recent follow-up visit. Survival curves were plotted according to the Kaplan-Meier method. The significance of differences was assessed using the log-rank test. A p value of <0.05 was considered statistically significant.

Results

Patient Characteristics

Fourteen patients (4.0%) with synchronous superficial PSCC were found among the 348 patients with invasive ESCC (table 1). Of the 14 patients, 13 (93%) were male and the median age was 62 years. The number of patients for ESCC clinical stage I, II, III, and IVA were 5, 2, 6 and 1, respectively. All 14 patients had both daily alcohol consumption and multiple LULs of the esophagus. All PSCC lesions were detected at our institute with no prior detection in other hospitals. Twelve (86%) PSCC lesions were detected using magnifying NBI endoscopy and the other 2 (14%) by conventional endoscopy. The latter 2 lesions were reevaluated with magnifying NBI endoscopy before

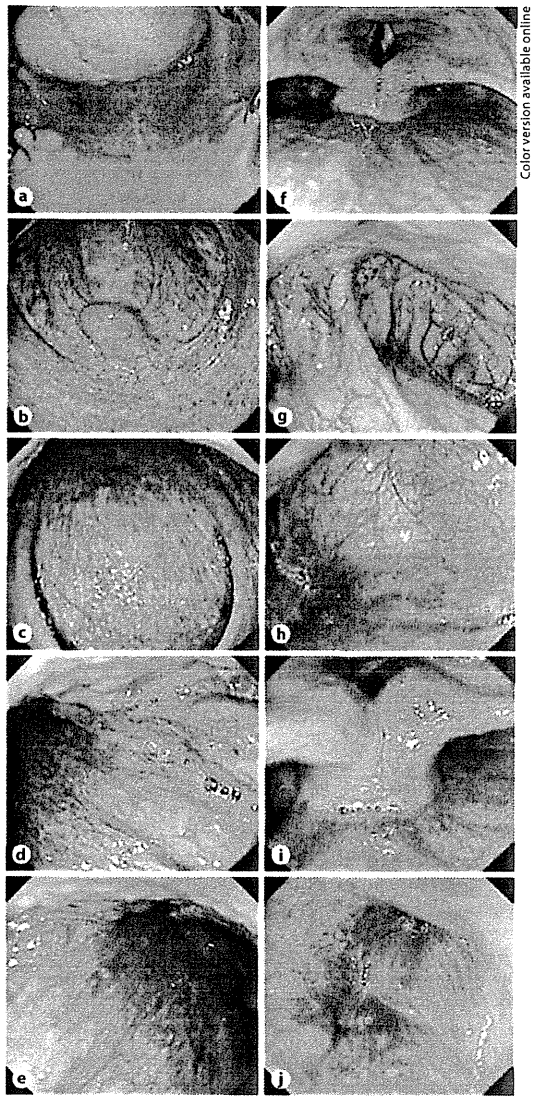

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Fig. 1. Narrow Band Imaging observations in individual regions from the oral cavity to the pharynx. **a** The view seen from the entrance of the oral cavity: dorsal side of tongue, hard palate and soft palate. **b** Uvula, palatoglossal arch and lateral walls of oropharynx. **c** The posterior wall of oropharynx. **d** The right side of base of tongue and lateral wall of oropharynx. **e** The left side of base of tongue and lateral wall of oropharynx. **f** Posterior wall of hypopharynx and larynx. **g** Vallecula of epiglottis, median glossoepiglottic fold. **h** The lateral wall and apex of right piriform sinus. **i** Arytenoids. **j** The lateral wall and apex of left piriform sinus.

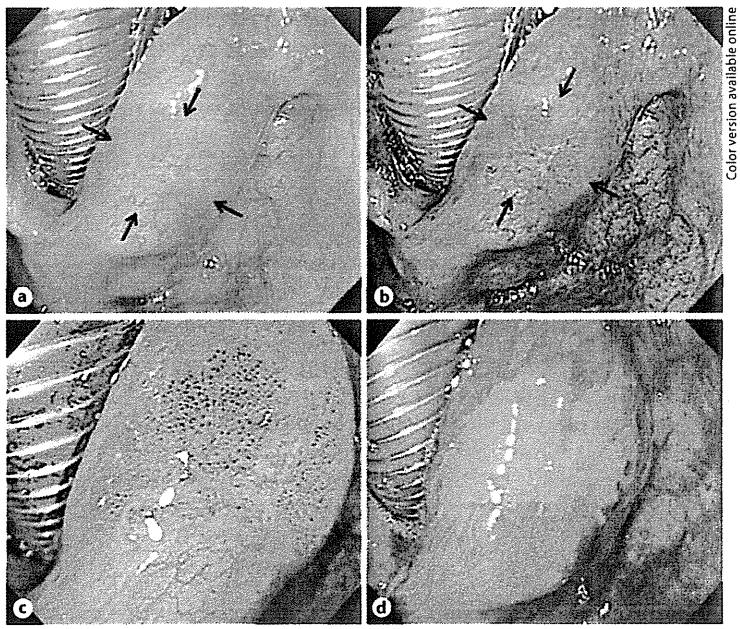

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Fig. 2. Superficial cancer of the right arytenoid. **a** Conventional endoscopic observation. The margin of the cancer is unclear (black arrows). **b** NBI observation. Cancer is shown as a brownish area (black arrows) and the margin is clear. **c** Magnifying NBI observation. The enhanced intraepithelial papillary capillary loop is seen in the cancer area. **d** The view of Lugol staining. Lugol-unstained lesion coincided with the cancer area. Lugol staining method was used to improve lesion visualization during endoscopic treatment. Color refers to the online version only.

Table 1. Patient characteristics

Age, years	Median	62
	Range	47–71
Gender	Male	13
	Female	1
Alcohol consumption	Presence	14
	Absence	0
Cigarette smoking	Presence	12
	Absence	2
Multiple LULs	Presence	14
	Absence	0
PSCC		
Location	Hypopharynx	10
	Oropharynx	4
Size, mm	Median	20
	Range	5–50
Macroscopic findings	Elevated type	5
	Flat type	4
	Depressed type	5
ESCC		
Clinical stage	I	5
	II	2
	III	6
	IVA	1

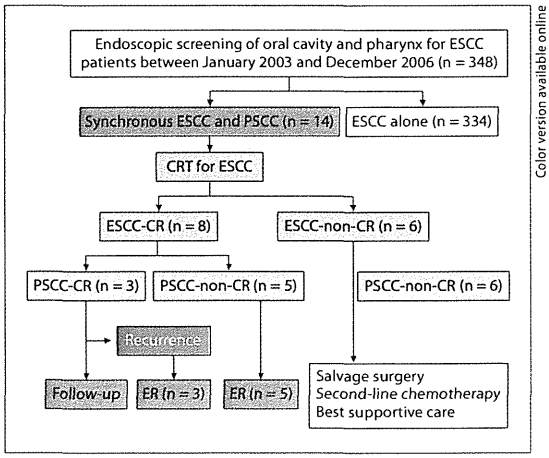

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Fig. 3. Flow chart of this study.