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Identification of adenosquamous carcinoma as a rare aggressive HER2-negative subgroup of esophageal/gastroesophageal junction adenocarcinoma

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Abstract

Background: Our purpose was to evaluate the prognostic impact of pathologically-confirmed esophageal adenosquamous carcinoma (ASC) and its association with HER2 status and clinicopathologic characteristics.

Methods: Among 796 patients with esophageal or gastroesophageal junction (GEJ) adenocarcinoma who underwent curative resection, surgical pathology reports were reviewed, and suspected ASC was confirmed utilizing p63 and CK5/6 immunostaining. HER2 status was determined using immunohistochemistry and fluorescence in situ hybridization. Cox models were used to assess the impact of ASC on disease-specific survival (DSS) and overall survival (OS).

Results: Overall, 2.0% (16/796) of patients had esophageal ASC, mostly demonstrating a close intermingling of squamous and adenocarcinoma cells within the same tumor. The percentage of squamous *vs* adenocarcinoma cells in the primary was generally recapitulated in nodal metastases, and intrapatient internodal heterogeneity was uncommon. Patients with esophageal ASC were statistically significantly more likely to be female (*vs* male), have normal (*vs* excess) body-mass index, and harbor HER2-negative (*vs* –positive) tumors, as compared to patients with adenocarcinoma-only. No ASC tumor was HER2-positive as compared to 16% of adenocarcinoma-only tumors (P =.018). Compared to patients with adenocarcinoma-only, those with ASC demonstrated profoundly worse DSS (5-year event-free rate: 34% *vs* 6%; multivariate

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AUTHOR CONTRIBUTIONS

Zhaohui Jin, MD: Investigation, methodology, project administration, visualization, writing - review and editing. Marie Holubek: Conceptualization, data curation, formal analysis, investigation, methodology, writing - original draft, and writing - review and editing. William R. Sukov, MD: Investigation, writing - review and editing. Christopher A. Sattler, CG(ASP): Investigation, writing - review and editing. Anne E. Wiktor: Investigation, writing - review and editing. Robert B. Jenkins, M.D., Ph.D.: Investigation, writing - review and editing. Tsung-Teh Wu, M.D., Ph.D.: Conceptualization, investigation, methodology, visualization, writing - review and editing. Harry H. Yoon, MD MHS: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing - original draft, and writing - review and editing.

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hazard ratio 2.87 [95% confidence interval 1.59 to 4.76]; *P*=.0010) and OS (*P*=.0027) that was independent of known prognostic factors and HER2 status.

Conclusion: Adenosquamous carcinoma identifies a rare aggressive HER2-negative subgroup of esophageal/GEJ adenocarcinoma.

PRECIS

This study identifies esophageal adenosquamous carcinoma as a rare aggressive HER2-negative subtype with divergent clinicopathologic characteristics from pure esophageal adenocarcinoma. These data support further elucidation of its molecular landscape.

Keywords

Esophageal adenosquamous carcinoma; prognosis; frequency; HER2/ERBB2; esophageal cancer; esophageal adenocarcinoma; gastric cancer; gastric adenocarcinoma; body-mass index; tumor heterogeneity

INTRODUCTION

Esophageal carcinoma mostly comprises two distinct histopathologic entities: adenocarcinoma (EAC) and squamous cell carcinoma (ESCC). Pure EAC typically originates from the distal esophagus or GEJ preceded by a premalignant inflammatory condition called Barrett's metaplasia. EAC incidence has increased in Western countries, and major risk factors are gastroesophageal reflux and obesity.[1] By contrast, ESCC typically originates from the mid- or proximal esophagus, and its incidence has stabilized in the last 30 years with ESCC now far less common in the U.S. than EAC. Smoking, alcohol, low intake of fresh fruits and vegetables, low socioeconomic status have been implicated as risk factors for ESCC.[2] ESCCs have been reported in large surgical series to have a worse prognosis than EACs.[3]

A recent advance in the treatment of EAC was the validation of human epidermal growth factor receptor 2 (HER2/ERBB2) as a therapeutic target in esophagogastric adenocarcinoma.[4] HER2 is a transmembrane tyrosine kinase receptor involved in controlling cell growth, survival, differentiation, and migration, and its expression/amplification has been detected in ~15% of EACs as we[5, 6] and others[7] have shown. Its frequency appears to be less frequent in ESCCs.[8, 9]

Adenosquamous carcinomas (ASCs) of the esophagus are uncommon tumors (0.37%-1% of esophageal carcinomas) in which the pathologic features of both adenocarcinoma and squamous cell carcinoma reside within the same tumor[10–13]. The prognostic impact, clinicopathologic and molecular characteristics of esophageal ASC are poorly understood. Barriers to further study include the lack of large cohorts of EAC with robust survival and clinicopathologic data. Here we examined the frequency, histologic characteristics, and survival of esophageal ASC in a large cohort of previously untreated patients who underwent surgical resection of their EAC. To better understand the pathogenesis of ASC as compared to EAC, we also compared ASCs and EACs in relation to smoking, BMI, and HER2 expression/amplification.

METHODS

Study population

In the current study we analyzed data from the Mayo Esophageal Cancer Outcome Database, which was previously described.[14] Briefly, adult patients (N = 796) were diagnosed with tissue-confirmed adenocarcinoma of the esophagus, gastroesophageal junction or gastric cardia (Siewert type I or II) and consecutively underwent surgery with curative intent at Mayo Clinic in Rochester, Minnesota (January 1, 1980 to December 31, 1997). Subcardial tumors and tumors lacking an adenocarcinoma component were excluded. Data on clinicopathologic characteristics, exposures, and patient survival were systematically collected from individual medical records. Smoking data were collected pre-surgery via self-reported written institutional questionnaires and verified during a visit with their primary care physician at Mayo. Height and weight were measured at the same time of surgery.

Identification of ASC

Twenty cases of ASC were selected as diagnosed in the original pathology reports. The glass slides were then retrieved and reviewed by a GI pathologist (T-T.W) and the paraffin blocks pulled in order to cut fresh sections. The new sections were stained with antibodies against p63 and cytokeratin CK5/6 to identify the squamous cell carcinoma component. Stains for chromogranin and synaptophysin to exclude a neuroendocrine component were also performed. In this fashion ASC was confirmed in 16 patients. Thirteen ASC had node-positive disease, and the percentage of squamous vs adenocarcinoma cells in both primary and nodes was evaluable in 9 patients. The correlation in the percentage of tumor cells staining positive for p63 and CK5/6 was very high (data not shown); data for p63 are shown.

HER2

HER2 expression by immunohistochemistry (IHC; HercepTest [Dako]) and *HER2* gene amplification by fluorescence *in situ* hybridization (PathVysion) were assessed using gastroesophageal-specific criteria, as previously described.[5, 6, 15] For IHC, each case was scored by two pathologists (T-T.W., W.R.S.), as follows: high ($IHC3^+$), strong intensity in 10% or more of cancer cells; medium ($IHC2^+$), weak-moderate intensity in 10% or more; low ($IHC1^+$), faint intensity in 10% or more; absent (IHC0). A specimen with an HER2/CEP17 ratio of 2.0 or more in invasive cells was classified as *HER2*-amplified. HER2-positive was defined as IHC3+ or IHC2+ with gene amplification, consistent with guidelines.[15]

Statistical Analysis

Statistical significance of the results was determined by chi-square. Agreement in the percentage of SCC in the primary *vs* nodes was determined using the intraclass correlation coefficient (ICC) with values <.40, 0.40-0.75, and >.75 considered poor, fair-to-good, and excellent, respectively.[16] Univariate analysis of survival was performed using the Kaplan Meier method. Cox proportional hazards models were used to estimate the univariate and multivariate association between predictor variables and outcomes. Overall survival was

calculated as the time from surgery to death from any cause. Disease-specific survival was calculated as the time from surgery to death due to index cancer. Events beyond 5 years were censored. P <.05 was considered significant. Data were collected using REDCap electronic data capture tools and statistical analysis was performed using JMP (JMP Pro 10.0.0 SAS Institute Inc. 2012) and MedCalc (version 14.12.0) software.

RESULTS

Study Population

Table 1 shows the baseline characteristics of the study population (N = 796). All patient tumors contained adenocarcinoma, and ASC was confirmed in 16 patients. None of the 16 patients with ASC had been diagnosed with a second primary at the time of surgery. Neoadjuvant chemotherapy with concurrent radiotherapy, chemotherapy alone, or radiotherapy alone was administered in 7 patients (0.9%), 1 patient (0.1%), and 1 patient (0.1%), respectively; no patient with ASC received neoadjuvant therapy. Adjuvant chemotherapy with concurrent radiotherapy, chemotherapy alone, or radiotherapy alone was administered in 53 patients (7%), 28 patients (4%), and 26 patients (3%), respectively; among patients with ASC, 2 patients received RT alone and 2 patients received chemoradiation.

ASC in primary tumor and nodes

In most patients with ASC (93% [15/16]) squamous and adenocarcinoma cells were closely intermingled within the same primary tumor, although in one tumor (7% [1/15]) two distinct subpopulations of squamous *vs* adenocarcinoma cells were identified (Figure 1). Across all ASC tumors, SCCs comprised a median 40% of the tumor cells, although considerable variability was observed (range 1-100%; interquartile range 16%-58%). Invasive depth of the SCC component was T3 in most ASC tumors (*ie*, 10 of 14 tumors with evaluable subpopulation depth), T2 in one tumor, and T1b in three tumors. Invasive depth of the adenocarcinoma component was likewise T3 in most ASC tumors (9 of 13 tumors with evaluable subpopulation depth), T4 in one tumor, T2 in one tumor, and T1b in two tumors. In the majority of ASC tumors (62% [8/13]) the invasive depth of the SCC component was the same as that of the adenocarcinoma component.

As shown in Figure 2, paired regional nodes were examined in nine primary ASC cases with node-positive disease that was evaluable for ASC (22 nodes in total) (see Methods). SCC was identified in at least one node in most patients (78% [7/9]), and in those nodes SCC comprised at least 10% of tumor cells. The percentage of squamous *vs* adenocarcinoma cells in the primary showed good agreement with the corresponding percentage in the nodes (ICC =.73 [95% CI 0.19, 0.93]), although discrepant cases were observed (*eg*, Patients 3-5). For most patients with multiple nodes examined, the percentage of SCC cells in a node showed minimal variation between nodes.

Clinicopathologic characteristics of ASC

Patients with esophageal ASC showed a statistically significant higher likelihood of being female (*vs* male), having normal body-mass index (*vs* excess), and having a tumor that was

HER2-negative (*vs* HER2-positive), as compared to patients whose esophageal tumors showed adenocarcinoma-only (Table 1). Other characteristics, including the presence of adjacent intestinal metaplasia, were not noticeably different between ASC and adenocarcinoma-only patients.

ASC and patient survival

Median follow-up duration for surviving patients was 12.8 years. In univariate analysis ASC (*vs* adenocarcinoma-only) was associated with shorter DSS (5-year DSS rate = 6% *vs* 34%, respectively; $P < .0001$ log-rank) and OS (5-year OS rate = 6% *vs* 31%, respectively; $P = .0001$ log-rank) (Table S1, Figure 3). As expected, increasing age, T stage, number of malignant nodes, and tumor grade were also associated with worse survival, supporting the generalizability of our cohort (Table S1). After adjustment for all covariates, ASC (*vs* adenocarcinoma-only) remained statistically significantly associated with shorter DSS (HR 2.87 [95% CI 1.59-4.76]; $P = .0010$) and OS (HR 2.57 [95% CI 1.43-4.26]; $P = .0027$) (Table 2). Sensitivity analyses in multivariable models excluding patients who received neoadjuvant and/or adjuvant therapy revealed stable results (Table S2).

DISCUSSION

To our knowledge, we report the first data on the prognostic impact of pathologically confirmed ASC in a large cohort of EAC in association with HER2 status and major risk factors. We found that ASCs comprised 2% of non-metastatic esophageal/GEJ adenocarcinomas, and this rare histologic subtype was associated with a statistically significant >2-fold worsening of DSS and OS after adjustment for covariates. In addition, we found that patients with ASC were statistically significantly more likely to be female, to have normal weight, and to harbor HER2-negative tumors, as compared with patients with EAC only. Together, these data indicate that ASC is a distinct aggressive subtype of EAC that warrants further investigation.

Close intermingling of SCC and adenocarcinoma cells was observed in most primary esophageal ASCs, consistent with prior pathologic descriptions of ASCs in ESCC populations.[13, 17] The percentage of squamous *vs* adenocarcinoma cells in the primary showed good agreement with the corresponding percentage in paired nodes, with minimal internodal variation in the same patient, suggesting the intratumor heterogeneity of the primary ASC is generally recapitulated in metastatic lesions, although discrepancies between primary and node were also observed. While the precise molecular characterization of this heterogeneity remains to be elucidated, it is increasingly accepted that clonal diversity in primary cancers may increase the risk of subsequent progression due to a broader assortment of subclones which allow for the selection and rapid progression of specific tumor subclones.[18]

Consistent with the theoretically greater tumor aggressiveness conferred by intratumor heterogeneity, we found that patients with esophageal ASC had a significantly worse survival compared to those with adenocarcinoma only, even after adjustment for known prognostic variables. Our data are supported by two recent reports from population-based studies in the U.S. which found that esophageal ASCs are associated with worse OS as

compared to EAC-only.[10, 11] Advantages of the current study over prior reports include the expert pathologic confirmation of ASC, as well as the availability of data on treatment, BMI, smoking, and HER2 status. Interestingly, multiple prior studies have determined that the prognosis of esophageal ASCs were not significantly different from ESCCs.
10, 11, 13, 17, 19, 20

Due to the low frequency of esophageal ASC, its clinicopathologic features are not well established. In our study patients with esophageal ASCs did not have significant differences from patients with EAC-only in terms of smoking history, depth of tumor invasion, pathologic tumor grade, or the number of malignant nodes. However, patients with esophageal ASC (*vs* EAC-only) were less likely to have excess BMI, which is a known risk factor for EAC, but not ESCC.[21] In addition, no ASC demonstrated HER2 protein expression or gene amplification, in contrast to 17% of EAC-only tumors. Interestingly, esophageal ASCs did not show a significant difference from pure EACs with regard to their tumor location or the presence of adjacent Barrett's metaplasia, suggesting that esophageal ASC may be as likely to arise from Barret's as pure EAC. Our finding is consistent with data from rat models with surgically induced gastroduodenal reflux which have shown that ASCs arise in esophageal mucosa characterized by chronic squamous esophagitis[22] and glandular metaplasia,[23] suggesting that alkaline reflux esophagitis contributes to the genesis of not only EAC, but also ASC.[22]

Esophageal or gastric ASCs are believed to develop from an unidentified common progenitor cell in some cases[24, 25] or through collision of two individual tumors in others.[26] Preliminary data indicate that EAC and ESCC components of esophageal ASC tumors share patterns of allelic loss at multiple chromosomal locations, *TP53* mutation, and/or aberrant expression of p53, p16, and RB in some tumors,[27–29] while other esophageal ASC tumors exhibit significantly divergent alterations in microsatellites and beta-catenin and EGFR expression.[28, 30] In lung xenograft models, recent data suggest that ASCs originate from cancer stem like cells that differentiate to multi-lineage structures with branching lung morphology expressing bronchial, alveolar and neuroendocrine markers in vitro. Our findings provide rationale for examining esophageal ASC as a distinct entity in ongoing and future comprehensive molecular characterizations.

Strengths of our study include the large size and extensive clinicopathologic annotation of our cohort, knowledge of treatment received, and long duration of survival follow-up. Novel clinicopathologic annotations include BMI, adjacent Barrett's, smoking history, and HER2 expression and amplification using modern disease-specific methods. In addition, unlike prior studies,[10, 13, 17, 19, 20] the presence of ASC was confirmed by expert pathologic examination including the use of new immunostains. Limitations of our study include the retrospective nature of data collection and the relatively small number of ASC cases.

In conclusion, our study identifies esophageal ASC as a rare aggressive HER2-negative subtype with divergent clinicopathologic characteristics from pure EAC, highlighting the importance of further elucidating the molecular landscape of ASC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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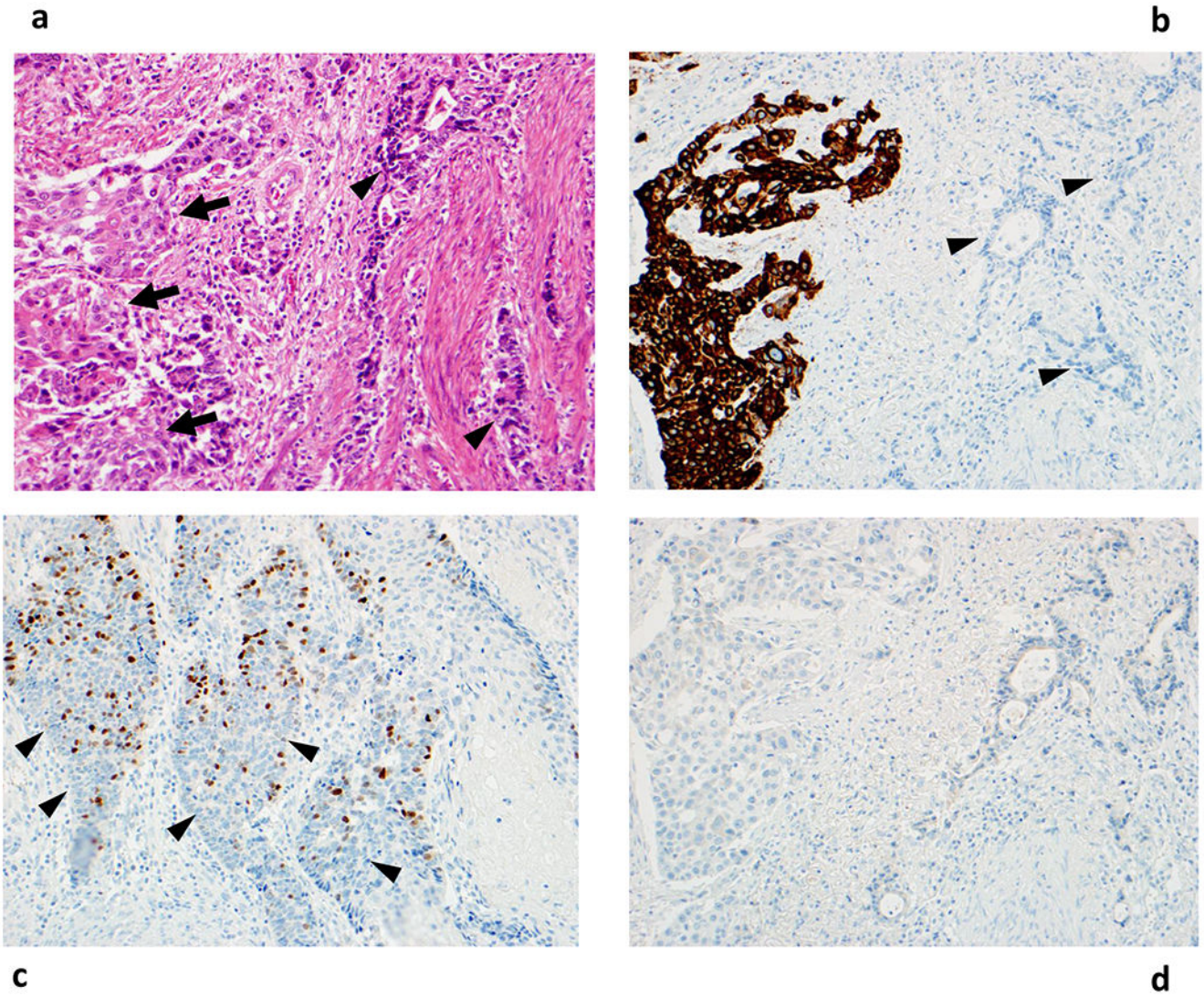

Figure 1:

Histologic features of adenosquamous carcinoma. Panel A shows discrete subpopulations of squamous cell carcinoma and adenocarcinoma in a hematoxylin and eosin-stained image. Panel B confirms the presence of squamous carcinoma cells through cytokeratin 5/6 staining in the same tumor. By contrast, Panel C shows close intermingling of squamous cell carcinoma (demonstrated by p63 staining) and adenocarcinoma cells from a different tumor. Panel D shows that the adenosquamous carcinoma is negative for chromogranin stain. Arrows denote squamous cell carcinoma cells, and arrowheads denote adenocarcinoma cells. All images are 200×.

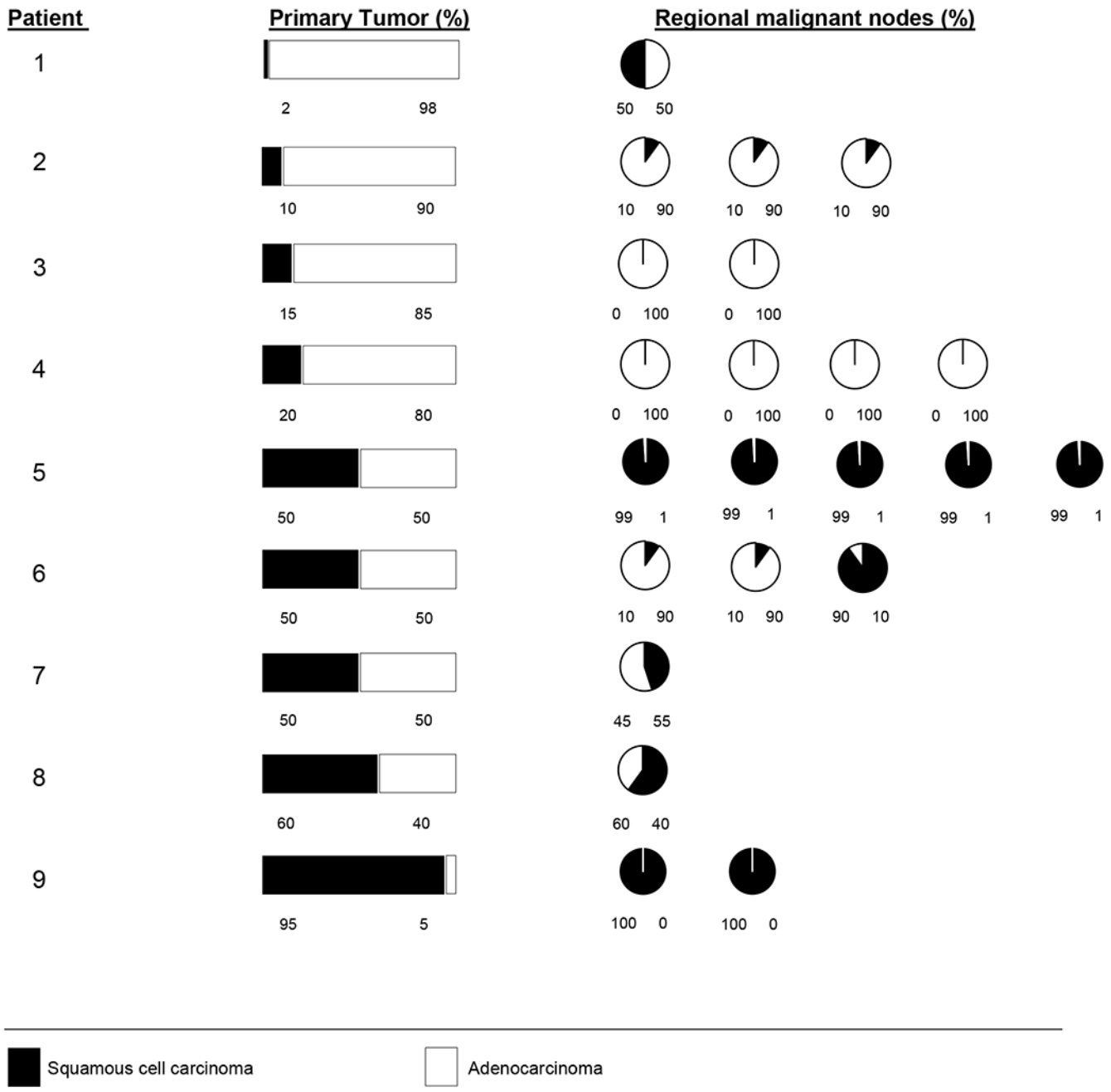

Figure 2:
Proportion of squamous carcinoma cells in primary adenosquamous carcinomas and paired regional malignant nodes.

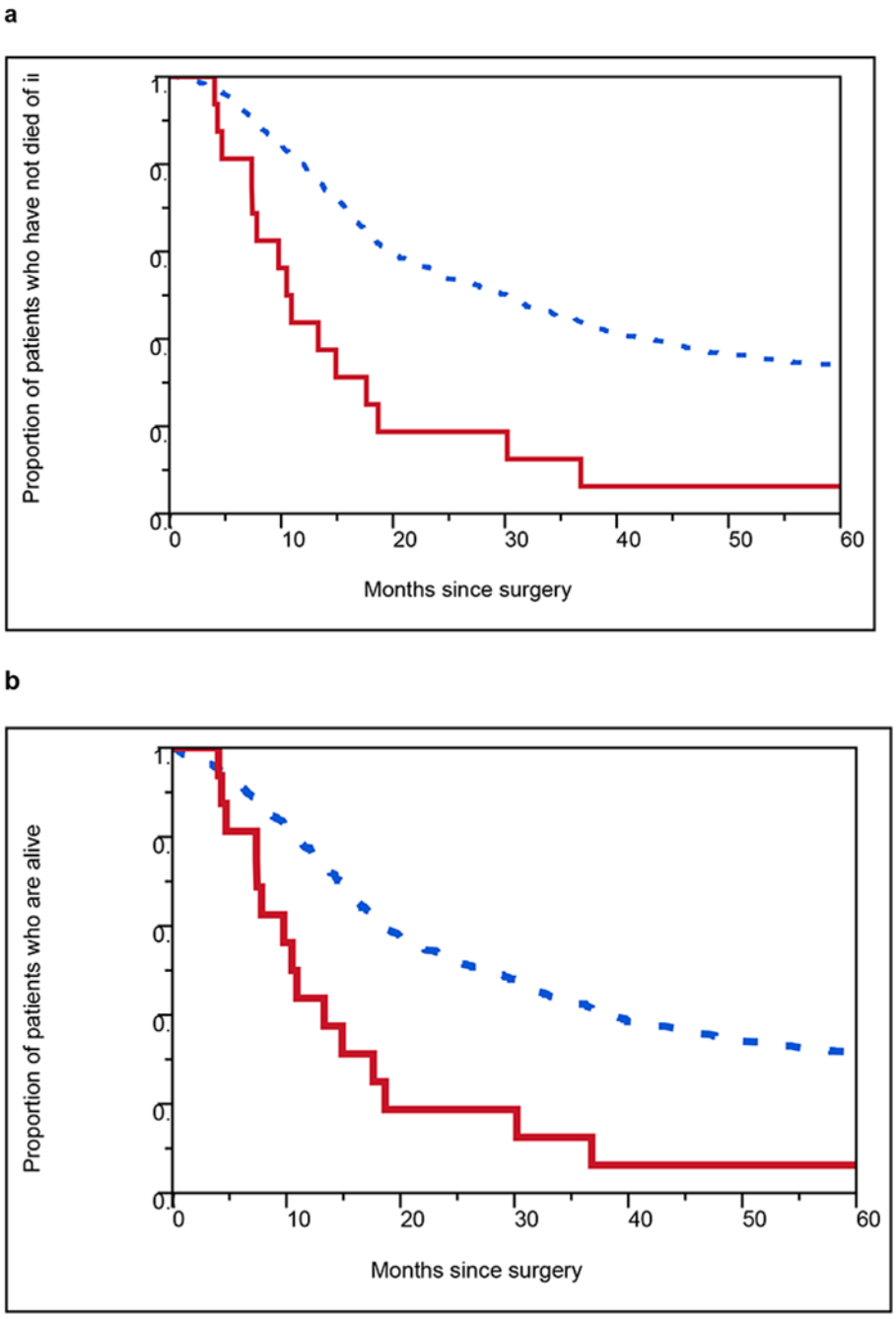

Figure 3.

Kaplan-Meier curves showing (a) disease-specific survival and (b) overall survival after surgery in patients with adenosquamous carcinoma *vs* adenocarcinoma of the esophagus or gastroesophageal junction after surgery. HRs from univariate Cox models are shown with log-rank *P* values. CI, confidence interval; HR, hazard ratio.

For both graphs:

- Red solid line should be labeled as “Adenosquamous Carcinoma” inside the graph.

- Black dotted line should be labeled as “Adenocarcinoma” inside the graph.

For a (DSS), insert this table as non-outlined textbox within graph:

	N (events)	5-year event-free rate	HR	95% CI	P log-rank
Adenosquamous Carcinoma	16 (15)	6%	2.94	1.68 to 4.74	<.0001
Adenocarcinoma	780 (491)	34%		Reference	

For b (OS), insert this table as non-outlined textbox within graph:

	N (events)	5-year event-free rate	HR	95% CI	P log-rank
Adenosquamous Carcinoma	16 (15)	6%	2.66	1.52 to 4.29	.0001
Adenocarcinoma	780 (529)	31%		Reference	

Table 1.

Baseline characteristics (N = 796)

Characteristic	Adenocarcinoma n = 780	Adenosquamous carcinoma n = 16	
	N (%)	N (%)	*P*
Host characteristics			
Age			
Median, y	65	61	0.1826
Gender			
Male	695 (89%)	10 (63%)	**0.0061**
Female	85 (11%)	6 (37%)	
Body mass index			
Normal	263 (34%)	10 (63%)	**0.0203**
Excess	517 (66%)	6 (37%)	
Smoking			
Ever	193 (25%)	4 (25%)	0.9813
Never	587 (75%)	12 (75%)	
Tumor characteristics			
T stage			
T1-T2	288 (37%)	4 (25%)	0.3027
T3-T4	486 (63%)	12 (75%)	
Missing	4	0	
No. metastatic LNs			
Median	2	2.5	0.1731
Histologic grade			
1 to 3	469 (61%)	8 (50%)	0.3785
4	300 (39%)	8 (50%)	
Missing	11	0	
Adjacent Barrett's			
Yes	277 (36%)	5 (31%)	0.7216
No	503 (64%)	11 (69%)	
Tumor location			
Esophagus	277 (36%)	6 (38%)	0.8729
GEJ or cardia	502 (64%)	10 (63%)	
HER2 status [α]			
Positive	121 (17%)	0	**0.0175**

Characteristic	Adenocarcinoma n = 780	Adenosquamous carcinoma n = 16	
	N (%)	N (%)	*P*
Negative	577 (83%)	15 (100%)	
Missing	82	1	

[a] HER2-positive defined as strong protein expression (IHC 3+) or equivocal expression (IHC 2+) with gene amplification

P <.05 is bolded.

IHC, immunohistochemistry.

Table 2.

Multivariable Cox Proportional Hazards Models Examining Esophageal Adenosquamous Carcinoma in Relation to Patient Survival (N = 796)[a]

Variable	Disease-specific survival			Overall survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Cancer Subtype						
Adenosquamous carcinoma	**2.87**	**1.59, 4.76**	**.0010**	**2.57**	**1.43, 4.26**	**.0027**
Adenocarcinoma		ref			ref	
Age, y						
Each additional year	1.01	1.00, 1.023	.0030	1.02	1.01, 1.03	<.0001
Gender						
Male	1.26	0.94, 1.73	.1221	1.30	0.97, 1.76	0.0751
Female		ref			ref	
T stage						
T3-4	1.87	1.50, 2.35	<.0001	1.80	1.46, 2.24	<.0001
T1-2		ref			ref	
No. metastatic nodes						
Each additional node	1.10	1.09, 1.13	<.0001	1.10	1.08, 1.12	<.0001
Tumor Grade						
4	1.30	1.08, 1.57	.0066	1.30	1.08, 1.55	0.0052
1 to 3		ref			ref	
HER2 status						
Positive	1.00	0.77, 1.28	1.00	0.96	0.74, 1.22	0.7205
Negative		ref			ref	

Abbreviations: CI, confidence interval; HR, hazard ratio; ref; reference.

[a]HRs are adjusted for all variables shown. Values >1 denote a higher risk of adverse survival compared to the reference level.