Next-Generation Sequencing of 487 Esophageal Adenocarcinomas Reveals Independently Prognostic Genomic Driver Alterations and Pathways

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ABSTRACT

Purpose: To delineate recurrent oncogenic driver alterations and dysregulated pathways in esophageal adenocarcinoma and to assess their prognostic value.

Experimental Design: We analyzed a large cohort of patients with lower esophageal and junctional adenocarcinoma, prospectively sequenced by MSK-IMPACT with high-quality clinical annotation. Patients were subdivided according to treatment intent, curative versus palliative, which closely mirrored clinical staging. Genomic features, alterations, and pathways were examined for association with overall survival using Cox proportional hazard models, adjusted for relevant clinicopathologic factors knowable at the time of diagnosis.

Results: Analysis of 487 patients revealed 16 oncogenic driver alterations, mostly amplifications, present in ≥5% of patients. Patients in the palliative-intent cohort, compared with those in the curative-intent cohort, were more likely to have metastatic disease, *ERBB2* amplifications, Cell-cycle and RTK–RAS pathway alterations, as well as a higher fraction of genome altered and rate of whole-genome doubling. In multivariable analyses, *CDKN2A* alterations, *SMAD4* alterations, *KRAS* amplifications, Cell-cycle and TGFβ pathways, and overall number of oncogenic drivers were independently associated with worse overall survival. *ERBB2* amplification was associated with improved survival, presumably due to trastuzumab therapy.

Conclusions: Our study suggests that higher levels of genomic instability are associated with more advanced disease in esophageal adenocarcinoma. Furthermore, *CDKN2A, KRAS*, and *SMAD4* represent prognostic biomarkers, given their strong association with poor survival.

Introduction

Esophageal adenocarcinoma (EAC) is an aggressive malignancy with a rapidly rising incidence in the USA and a five-year survival rate of 20% or less (1). Most patients are diagnosed at the onset of symptoms, at which point the disease is usually advanced. Less than half of patients are eligible for curative therapy, of which surgical resection is the mainstay. According to National Comprehensive Care Network (NCCN) Guidelines, current treatment algorithms for tumors of the distal esophagus and esophagogastric junction involve surgery alone or in combination with chemoradiotherapy for clinical stage I–III tumors, while clinical stage IV disease is often palliated with systemic therapy only (2). First-line systemic therapy regimens comprise either a platinum and a fluoropyrimidine or a platinum and a taxane, with the addition of trastuzumab for HER2-amplified tumors or an immune-checkpoint inhibitor for tumors with microsatellite instability (MSI; ref. 3).

At present, only a few clinicopathologic characteristics of EAC are known to have meaningful prognostic value in the patient-care setting. These are effectively limited to the components of pathologic staging, which encompasses local extent of tumor (T), lymph node status (N), distant metastasis (M), and tumor grade (G; ref. 4). Pathologic stage, however, is unavailable for decision-making in patients receiving neoadjuvant therapy prior to surgery, which has become the standard in locally advanced disease treated with curative intent. Therefore, clinical staging is frequently relied upon to direct treatment decisions, though prognostication using clinical stage has been shown to be inaccurate by comparison (5). Additional prognostic factors of importance vary by clinical scenario and may include age, comorbidities, performance status, length or location of tumor, and presence of lymphovascular invasion (6, 7). However, in the era of precision oncology, both expanded prognostics and therapeutics are essential to improve outcomes and survival.

Few large-scale genomic studies on EAC have been reported (8, 9). Moreover, its genomic landscape remains incompletely annotated, largely due to the lack of clinical contextualization. Thus far, observations from these studies reveal considerable genomic heterogeneity with a relatively high background mutation burden in comparison with other solid tumors, but few recurrently mutated genes, aside from *TP53*, and even fewer actionable targets (10, 11).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2021;27:3491–8

doi: 10.1158/1078-0432.CCR-20-4707

Translational Relevance

Esophageal adenocarcinoma is an aggressive malignancy with a rapidly rising incidence in the USA and a five-year survival of less than 20%. Therefore, expanded prognostics and therapeutics are essential to improve survival. This study integrates next-generation sequencing and clinicopathologic data from 487 patients with lower esophageal and junctional adenocarcinoma to identify genomic features, alterations, and pathways associated with overall survival. Results indicate that *CDKN2A*, *SMAD4*, and *KRAS* amplification are independently prognostic of poor survival. *ERBB2* amplification, by contrast, is associated with improved survival, likely because it is already effectively targeted by trastuzumab. In addition, genomic features and pathways related to increased chromosomal instability—including overall number of oncogenic drivers, fraction of genome altered, whole-genome doubling, and Cell-cycle pathway enrichment—were all significantly associated with more advanced disease and may contribute to the dismal prognosis of this disease.

According to the Cancer Genome Atlas (TCGA) study, EACs may be classified broadly into three major subtypes: those with MSI, those with chromosomal instability (CIN), and genomically stable (GS; ref. 12). The overwhelming majority of lower esophageal and junctional adenocarcinomas were found to exhibit CIN with a high frequency of copy-number alterations and aneuploidy. As a result, biomarkers of treatment response and survival have been challenging to identify, and we have a limited understanding of which genomic events drive the development of EAC and determine its prognosis.

To address these gaps in knowledge, we performed a comprehensive analysis of 487 lower esophageal and junctional adenocarcinomas, genomically characterized by broad-panel next-generation sequencing with high-quality clinical annotation. Our objectives were to identify recurrent oncogenic driver events implicated in EAC and to examine whether these alterations—at both the individual and pathway levels—are associated with overall survival (OS) and therefore may be useful for prognostic purposes.

Materials and Methods

Patients/samples

We used the Memorial Sloan Kettering (MSK) cBioPortal to mine our institutional database of clinical samples sequenced by MSK-IMPACT (MSK-Integrated Mutation Profiling of Actionable Cancer Targets) for all patients with esophagogastric cancer from 2014 through 2019 (13, 14). A total of 1,029 patients were identified. We excluded patients with gastric adenocarcinomas ($N = 473$), as well as esophageal squamous cell carcinomas ($N = 53$), and other histologies ($N = 16$), such that only lower esophageal and esophagogastric junction adenocarcinomas remained for analysis. Clinical annotations were then obtained via cross-referencing our manually curated, prospectively maintained institutional database. All patients provided written informed consent for targeted sequencing under clinical trial protocol NCT01775072, approved by the MSK Institutional Review Board and in accordance with the ethical guidelines of the Declaration of Helsinki. Tumor tissue for sequencing was obtained from either primary or metastatic sites at the time of biopsy or surgery. Tumor purity was assessed by histopathologic review of specimens by an expert pathologist from the MSK Molecular Diagnostics Service. For patients who had more than one sample sequenced, the sample with the higher tumor purity was selected for inclusion.

Next-generation sequencing and computational analysis

The MSK-IMPACT next-generation sequencing assay was performed as part of routine clinical assessment in a CLIA-compliant laboratory, as previously described (15). Briefly, genomic DNA was extracted from tumor tissue and patient-matched blood samples to generate barcoded libraries. After capture of exons and selected introns of the genes included in the sequencing panel, pooled libraries were sequenced on the Illumina HiSeq 2500 system. Forty-five patients were sequenced using MSK-IMPACT v1 (341 genes), 104 using MSK-IMPACT v2 (410 genes), and 338 using MSK-IMPACT v3 (468 genes).

Sequencing files were processed using stringent quality-control criteria and analyzed using an optimized informatics pipeline to identify somatic mutations, copy-number alterations, and select structural rearrangements. Full details regarding the performance and validation of the MSK-IMPACT assay, which is currently FDA authorized, have been reported (16). Utilizing OncoKB and Cancer Hotspots databases, we excluded variants of unknown significance (17, 18). Copy-number alterations were identified by comparing targeted regions of the tumor sample to the matched diploid normal sample. The log ratio coverage values for segments were calculated and compared between the tumor and normal samples. A fold-change threshold of < -2 and false discovery rate corrected $P < 0.05$ was used to determine whole gene loss, or deep deletion/homozygous deletion, while a fold-change threshold of >2 was used to determine whole gene amplification. Alterations (oncogenic mutations, copy-number alterations, structural rearrangements, or fusions) were considered for analysis only if present in at least 5% of patients in the cohort. The number of oncogenic drivers was calculated for each patient as the total number of driver alterations present.

MSI status was assessed using the MSI-sensor algorithm, which calculates the percentage of microsatellite loci covered by the MSK-IMPACT assay that are unstable in the tumor as compared with the patient's matched normal sample (19). Samples with a score of ≥ 10 were classified as MSI-high. To calculate tumor mutation burden, the total number of somatic nonsilent protein-coding mutations in the sequenced genes was determined and normalized to the exonic coverage of the respective MSK-IMPACT panel in megabases. Tumor mutation burden calculations using this panel are strongly associated with those assessed by whole-exome sequencing (20). The fraction of genome altered was defined as the fraction of $\log_2$ copy-number variation (gain or loss) >0.2, divided by the size of the genome whose copy number was profiled. Fraction of genome altered was corrected for tumor purity, ploidy, and clonal heterogeneity using the FACETS method (21). Presence or absence of whole-genome doubling was estimated using the probability model as previously described (22). Mutual exclusivity or co-occurrence of genomic alterations was analyzed using the Mutual Exclusivity Modules in cancer algorithm (23).

We evaluated 11 canonical cancer-related signaling pathways as defined by the TCGA PanCancer Atlas Project (24). The pathways analyzed were p53, cell cycle, Hippo, Myc, Notch, NRF2, PI3K, RTK (receptor tyrosine kinase)/RAS/MAPK, TGFβ, Wnt, and DDR (DNA damage response). A tumor was considered altered in a specific pathway if at least one gene belonging to that pathway was altered. Number of pathways altered was calculated for each patient as the total number of altered pathways out of the 11 pathways specified above.

Her2/ERBB2 expression

Clinical Her2 status was based on Her2 protein expression by IHC or *ERBB2* gene amplification by fluorescence *in situ* hybridization using College of American Pathologists/American Society of Clinical Oncology criteria (25). Positivity was defined as 3+ by IHC or HER2:CEP17 fluorescence *in situ* hybridization ratio ≥2.0.

Statistical analysis

Clinicopathologic characteristics were summarized using frequency and percentage for categorical variables and median and interquartile range (IQR) for continuous variables. Genomic and/or pathway alterations were counted as either present or absent. Association of somatic driver or pathway alterations with clinicopathologic factors was evaluated using the Wilcoxon rank-sum test for continuous factors or a Fisher exact test for categorical factors. Furthermore, multivariable linear or logistic regression models were used to evaluate the association of treatment intent with continuous or binary independent variables, respectively, including tumor mutation burden, whole-genome doubling, fraction of genome altered, and number of oncogenic drivers and pathways, while controlling for tumor purity and site of tissue sampling.

The primary outcome of interest was OS, which was calculated from the date of diagnosis to the date of death or last follow-up. Cox proportional hazards models were used to quantify the association of clinicopathologic variables and genomic alterations or pathways with OS. Alterations that were significantly associated with OS after adjustment for age, tumor grade, treatment-intent cohort, and clinical stage were then evaluated for independent association with OS in multivariable models. Two separate multivariable models were constructed: one with individual alterations and the other with pathways; both models also included the same clinicopathologic variables listed above. We assessed the discriminatory performance of these models using a Harrell's C-index. For survival analyses stratified by a genomic alteration of interest, OS was estimated using the Kaplan–Meier method and compared using the log-rank test.

P values <0.05 were considered statistically significant, and false discovery rates (*q*-values) using the Benjamini–Hochberg procedure were reported wherever multiple hypotheses were tested. All statistical analyses were performed using R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria).

Data availability

All study data are freely available on cBioPortal (https://www.cbioportal.org/study/summary?id=egc_mskcc_2020).

Results

Comparison of palliative-intent therapy and curative-intent therapy cohorts

In total, 487 patients were included in our study. Patients were divided into two cohorts according to major treatment paradigms for lower esophageal and junctional adenocarcinomas: curative-intent therapy (CIT; $N = 230$) and palliative-intent therapy (PIT; $N = 257$; **Table 1**; **Fig. 1A**). Assignment to treatment cohort closely mirrored clinical staging, which was based on positron-emission tomography and endoscopic ultrasound or endoscopic mucosal resection. Patients in the CIT cohort had predominantly early-stage and locally advanced tumors treated with endoscopic or surgical resection, in conjunction with neoadjuvant or adjuvant chemoradiotherapy as indicated. By contrast, patients in the PIT cohort had overwhelmingly clinical stage IV disease and were treated with systemic therapy. A single clinical stage I patient was included in the PIT cohort due to multiple comorbidities that precluded curative therapy. Median OS among the entire population was 31.6 months (95% CI, 27.9–36.0), and median length of follow-up from the date of diagnosis was 39.2 months (95% CI, 32.7–47.0). As expected, OS differed significantly between CIT and PIT treatment cohorts. Median OS in the PIT cohort was 22.8 months (95% CI, 19.3–27.1), which was significantly shorter than the median OS in the CIT cohort, 42.5 months (95% CI, 35.6–48.4; $P < 0.001$; **Fig. 1B**). All deaths in both cohorts were confirmed to be esophageal cancer related (i.e., esophageal cancer was documented as either the primary or secondary cause of death based

Table 1. Patient characteristics by treatment cohort. The data represent frequency (%) or median (IQR).

Characteristic	Curative intent ($N = 230$)	Palliative intent ($N = 257$)	*P* value
Sex			1.0
Female	33 (14%)	36 (14%)	
Male	197 (86%)	221 (86%)	
Age at sequencing	64 (56–69)	61 (54–68)	0.032
Site of tissue sampling			0.153
Primary tumor	187 (81%)	195 (76%)	
Metastatic site	43 (19%)	62 (24%)	
Clinical Stage (AJCC 8th edition)			<0.001
I	20 (8.7%)	1 (0.39%)	
II	24 (10%)	0 (0%)	
III	156 (68%)	0 (0%)	
IV	26 (11%)	250 (97%)	
Unknown	4 (1.7%)	6 (2.3%)	
Systemic therapy			N/A
Platinum + taxane	150 (65%)	135 (53%)	
Platinum + fluoropyrimidine	142 (62%)	232 (90%)	
Targeted therapy	39 (17%)	163 (63%)	
Immune-checkpoint inhibitor	63 (27%)	93 (36%)	
N/A or unknown	29 (13%)	9 (3.5%)	
Pathologic stage (AJCC 8th edition)			<0.001
I	82 (36%)	0 (0%)	
II	28 (12%)	0 (0%)	
III	72 (31%)	0 (0%)	
IV	36 (16%)	250 (97%)	
Unknown	12 (5.2%)	7 (2.7%)	
Tumor grade			1.0
Well/moderate	101 (44%)	109 (42%)	
Poor	128 (56%)	139 (54%)	
Unknown	1 (0.43%)	9 (3.5%)	
Tumor purity	30 (20–40)	30 (22–50)	<0.001
Number of pathways altered	2 (1–3)	3 (2–4)	<0.001
Number of oncogenic drivers	2 (1–3)	3 (2–3)	<0.001
Fraction of genome altered	0.40 (0.23–0.53)	0.50 (0.38–0.58)	<0.001
Tumor mutation burden	4.4 (2.6–7)	4.9 (3–7)	0.590
Whole-genome doubling	59 (29%)[a]	117 (46%)	<0.001
MSI-high	9 (3.9%)	6 (2.3%)	0.432

Note: All statistical comparisons were based on available data.
Abbreviation: N/A, not applicable.
[a]Whole-genome doubling was unknown in 27 patients total: 24 in the curative-intent cohort and 3 in the palliative-intent cohort.

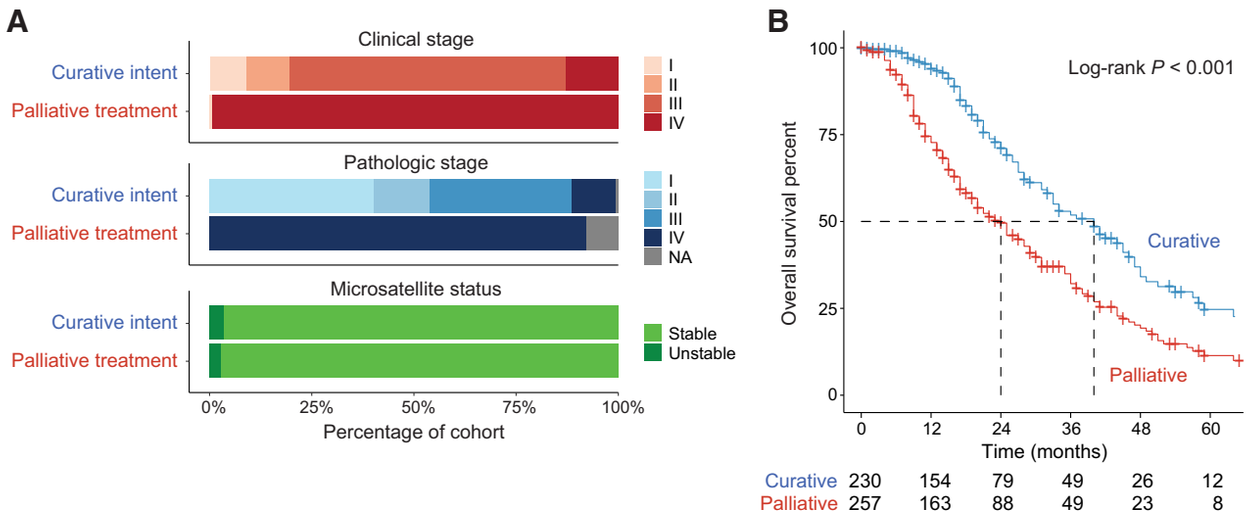

Figure 1.
Comparison of (**A**) clinical and pathologic stage and microsatellite status and (**B**) OS by curative-intent treatment and palliative-intent treatment cohorts.

on our institutional records or those obtained from the National Death Index), and thus OS approximated cancer-specific survival in our study population.

Driver alterations in EAC

Targeted sequence analysis of our study group identified 16 genes harboring recurrent oncogenic driver alterations (cross-validated using OncoKB, TCGA, and Cancer Hotspot databases) with a ≥5% prevalence (**Fig. 2A**). All 16 genes were present on each of the three versions of MSK-IMPACT used here. Ten of these 16 oncogenic drivers were gene amplifications, affecting *ERBB2, KRAS, CCNE1, MYC, CCND1, MDM2, VEGFA, EGFR, CDK6*, and *CCND3*. Frequencies of individual oncogenic driver alterations were similar across treatment cohorts, with the exception of *ERBB2* amplification, which was significantly enriched in the PIT (30%) versus CIT (13%) cohort ($P < 0.001$, $q < 0.05$). At the pathway level, both the Cell-cycle (56% vs. 40%, $P < 0.001$, $q < 0.05$) and RTK-RAS (65% vs. 46%, $P < 0.001$, $q < 0.05$) pathways were significantly enriched in the PIT cohort (**Fig. 2B**). Furthermore, patients in the PIT cohort, compared with the CIT cohort, had a significantly greater fraction of genome altered [median 0.50 (IQR 0.38–0.58) vs. 0.40 (IQR 0.23–0.53); $P < 0.001$], rate of whole-genome doubling (46% vs. 29%; $P < 0.001$; **Fig. 2C**), number of pathways altered [median 3 (IQR 2–4) vs. 2 (IQR 2–3); $P < 0.001$], and number of oncogenic drivers overall [median 3 (IQR 2–3) vs. 2 (IQR 1–3); $P = 0.001$]. After adjusting for differences in tumor purity and site of tissue sampling, fraction of genome altered (coefficient 0.06; 95% CI, 0.03–0.09; $P < 0.001$), rate of whole-genome doubling (coefficient 1.86; 95% CI, 1.24–2.80; $P = 0.003$), and number of oncogenic drivers (coefficient 0.28; 95% CI, 0.05–0.50; $P = 0.017$) remained significantly higher in the PIT cohort (see Supplementary Table S1). The difference in number of oncogenic drivers was particularly pronounced for amplifications. Of note, 191 patients (84 in the PIT cohort and 107 in the CIT cohort) had received some form of treatment, including systemic therapy and/or radiotherapy, prior to sequencing. However, we did not observe any significant differences in either tumor purity or the number of oncogenic drivers detected between patients who did or did not undergo any prior treatment (data not shown). Median tumor mutation burden overall was 4.5 mutations/Mb. Although tumor mutation burden did not vary by treatment cohort, it was negatively correlated with fraction of genome altered (Spearman correlation coefficient 0.113; $P = 0.017$), confirming the distinction between hypermutated MSI-high and CIN subtypes. Only 15 cases (3.1%) were designated as MSI-high in our patient population, and this was consistent across treatment cohorts.

Eighty percent of patients in each cohort harbored an oncogenic driver mutation in the p53 gene (*TP53*). Moreover, the majority of *TP53* wild-type cases had clear evidence of alternative driver alterations, the most common of which were *MDM2* amplifications (found in 24% of *TP53* wild-type tumors). Furthermore, as members of the p53 pathway with functionally overlapping effects, alterations in *TP53* and *MDM2* were found to be mutually exclusive ($P < 0.001$, $q < 0.05$; **Fig. 2D**), which has been previously reported (8). Other driver alterations in *TP53* wild-type patients were *CDKN2A* (23%), *ARID1A* (17%), and *ERBB2* amplifications (16%). However, unlike *MDM2*, none of these exhibited significant mutual exclusivity with *TP53* mutations.

Prognostic alterations and pathways

Next, we investigated the association between OS and genomic alterations at both the individual and pathway levels. Because of their robust responses to immune-checkpoint inhibitors and improved survival (26), we eliminated the 15 MSI-high tumors from this analysis. Using Kaplan–Meier methods, *KRAS* amplifications (<0.001), *SMAD4* alterations ($P = 0.028$), and *CDKN2A* alterations ($P < 0.001$) were found to be associated with significantly shorter OS, whereas *ERBB2* amplifications ($P = 0.022$) were associated with a significantly longer OS (**Fig. 3A**). The longer survival associated with *ERBB2* amplification may be attributable to the use of trastuzumab therapy in these patients. Of the 108 tumors that harbored *ERBB2* amplifications across the entire study cohort, 88 (81%) exhibited HER2 overexpression on IHC or HER2 amplification on fluorescence *in situ* hybridization when available, and 86 were treated with trastuzumab as either first-line (64) or second-line (22) therapy. Of the 86 patients who received trastuzumab therapy, 79 (94%) had stage IV disease, as this treatment is FDA approved in the metastatic setting. Thus, given that only 22 patients did not receive trastuzumab, and these were primarily patients with early- or intermediate-stage disease, we were unable to assess whether the

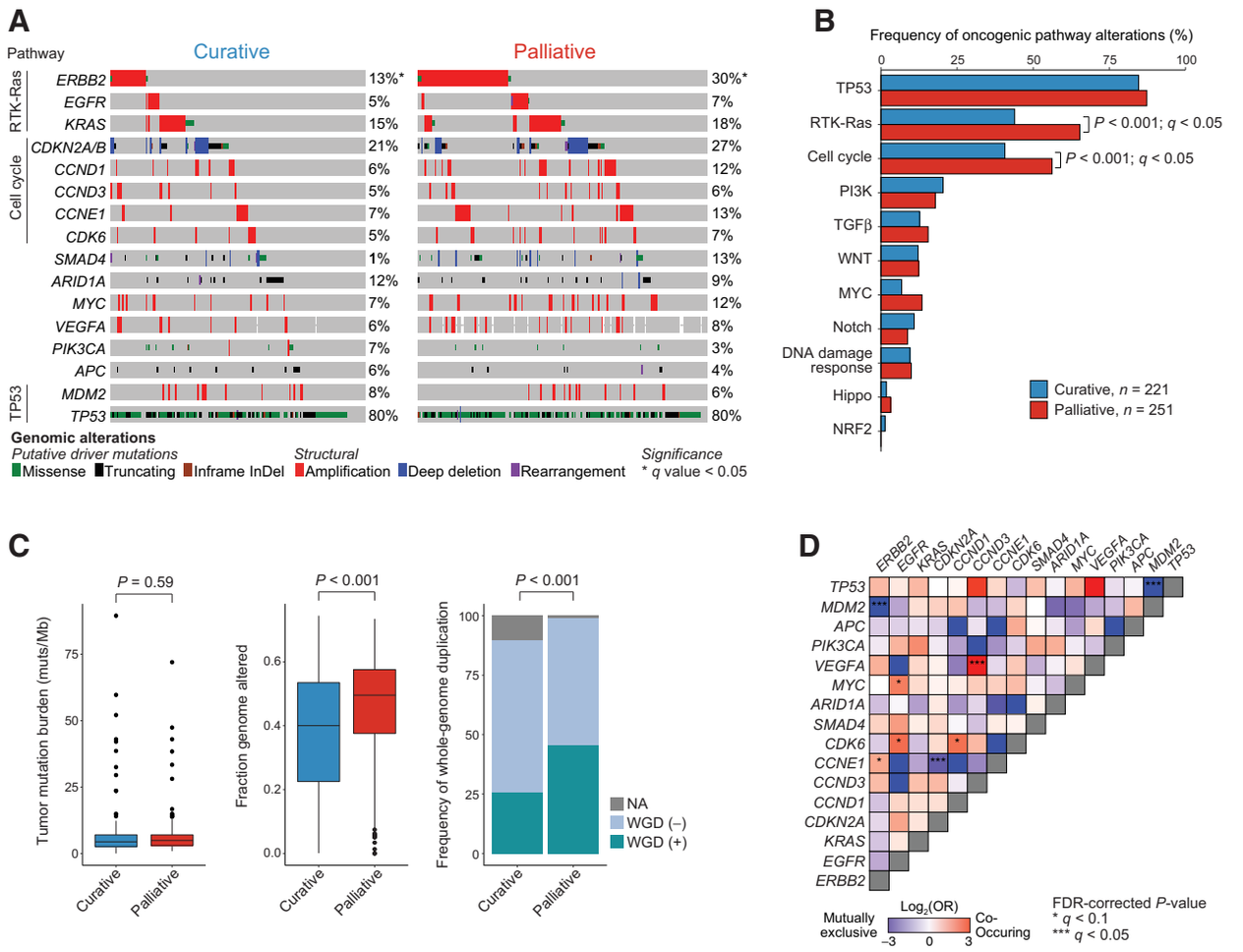

Figure 2.

Genomic characteristics of esophageal adenocarcinoma patients. **A,** Oncoprint of 16 recurrent oncogenic driver mutations identified in our patient population, comparing alteration frequencies by treatment cohort. **B,** Frequency of alteration of canonical cancer-related pathways compared by treatment cohort. **C,** Tumor mutation burden, fraction of genome altered, and whole-genome doubling, also compared by treatment cohort. **D,** Mutual co-occurrence/exclusivity plot of 16 recurrent oncogenic drivers from panel **A**.

trastuzumab treatment fully accounts for the better survival of patients with *ERBB2*-amplified tumors. Of note, *ERBB2* amplification was also found to be significantly associated with well/moderate versus poor tumor differentiation (38% vs. 11%; $P < 0.001$).

Using a Cox proportional hazards model that adjusted for relevant clinicopathologic variables that are knowable at the time of diagnosis (e.g., age, clinical stage, tumor grade, and treatment-intent cohort), genomic alterations associated with OS were *KRAS* amplifications (adjusted HR 2.05; 95% CI, 1.48–2.85; $P < 0.001$), *ERBB2* amplifications (adjusted HR 0.62; 95% CI, 0.45–0.85; $P = 0.003$), *CDKN2A* alterations (adjusted HR 1.65; 95% CI, 1.26–2.16; $P < 0.001$), and *SMAD4* alterations (adjusted HR 1.60; 95% CI, 1.14–2.26; $P = 0.007$). Moreover, the Cell-cycle pathway (adjusted HR, 1.32; 95% CI, 1.03–1.68; $P = 0.029$) and TGFβ pathway (adjusted HR, 1.45; 95% CI, 1.05–2.01; $P = 0.026$) were also significantly associated with OS on univariable analysis adjusted for relevant clinicopathologic variables (see Supplementary Table S2 for full results of adjusted univariable analyses). We then generated a multivariable model including both clinicopathologic variables and genomic alterations, and found that palliative treatment intent (HR, 2.63; 95% CI, 1.53–4.51; $P < 0.001$), poor tumor differentiation (HR, 1.54; 95% CI, 1.18–2.01; $P = 0.002$), *KRAS* amplification (HR, 1.83; 95% CI, 1.31–2.55; $P < 0.001$), *SMAD4* alteration (HR, 1.61; 95% CI, 1.14–2.28; $P = 0.007$), and *CDKN2A* alteration (HR, 1.50; 95% CI, 1.14–1.97; $P = 0.004$) were independently associated with a higher risk of death, whereas *ERBB2* amplification (HR, 0.65; 95% CI, 0.48–0.90; $P = 0.009$) was associated with a lower risk of death (**Fig. 3B**). The inclusion of genomic alterations enhanced the prognostic power of our multivariable model (Harrell's C-index 0.71) when compared with a multivariable model based on clinicopathologic variables alone (Harrell's C-index 0.68). A higher number of oncogenic drivers (HR, 1.11; 95% CI, 1.01–1.22; $P = 0.030$) was also independently associated with worse OS. Similarly, at the pathway level, both the Cell-cycle (HR, 1.30; 95% CI, 1.02–1.66; $P = 0.036$) and TGFβ (HR, 1.46; 95% CI, 1.05–2.02; $P = 0.024$) pathways were found to be independently associated with an increased risk of death (**Fig. 3B**). Of note, neither *TP53* alteration nor pathway wild-type status was associated with OS in our analysis, as previously suggested (27).

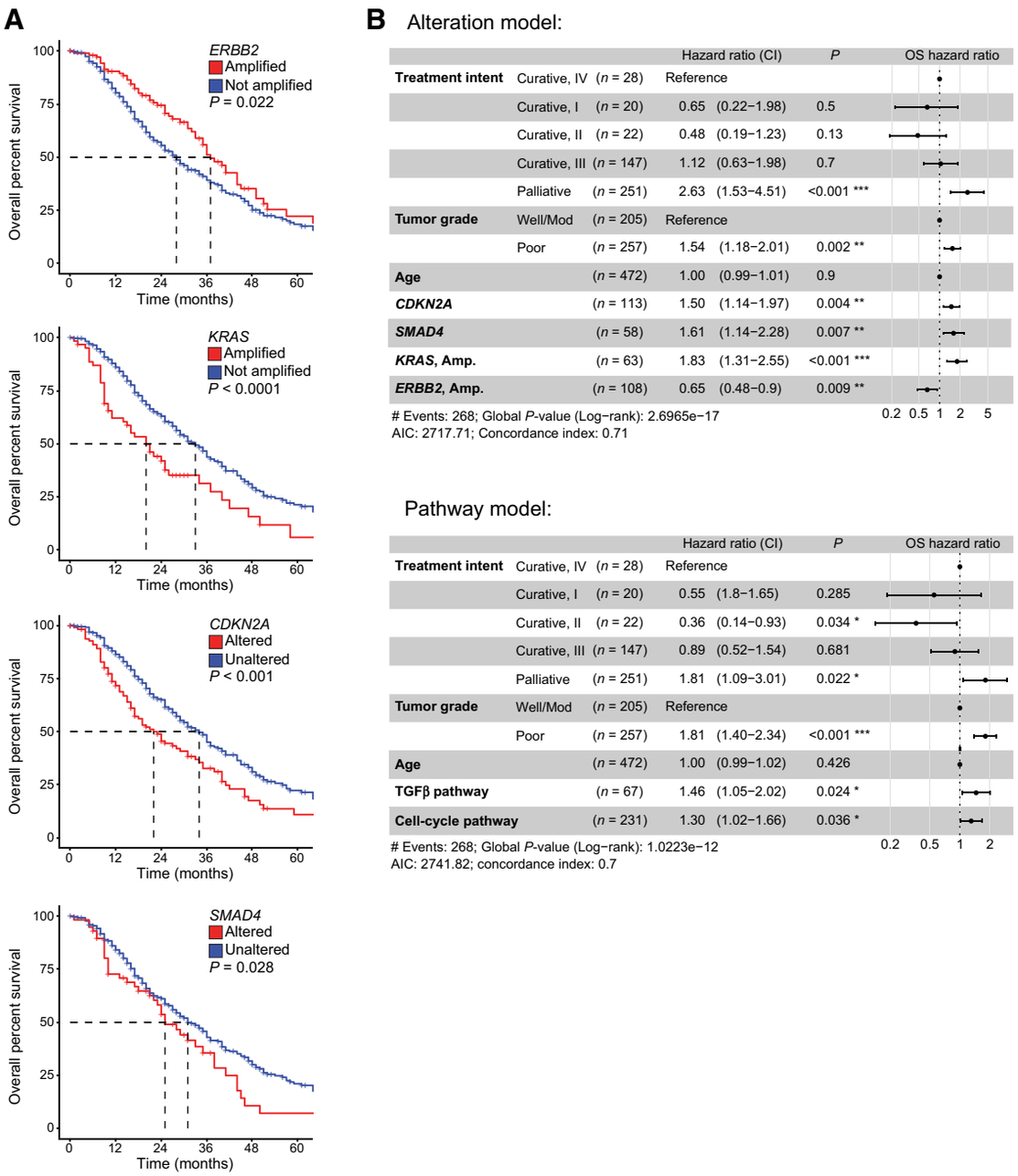

Figure 3.

Genomic and clinicopathologic factors associated with OS. **A,** Kaplan–Meier OS curves of key genomic alterations. **B,** Multivariable Cox proportional hazard models of OS that include clinicopathologic factors along with individual alterations (top) and pathways (bottom). The curative-intent cohort was subdivided by clinical stage.

Discussion

Using prospective broad-panel clinical next-generation sequencing, we identified recurrent oncogenic driver alterations characterizing EAC, and further delineated which of these may have an impact on tumor progression and clinical outcomes. The two main observations from our genomic analyses are: (i) most recurrent oncogenic drivers in EAC are copy-number alterations, leading to higher levels of genomic instability and (ii) higher levels of genomic instability are associated with more advanced disease. Furthermore, *CDKN2A* alteration, *SMAD4* alteration, *KRAS* amplification, and overall number of oncogenic drivers were all associated with worse OS, independent of clinicopathologic predictors, whereas *ERBB2* amplification was associated with better OS. To our knowledge, this is one of the first and largest studies to put forth multiple independently prognostic alterations and pathways in EAC using a robust model combining clinicopathologic and genomic data.

In our analysis, 10 of 16 recurrent oncogenic driver alterations were characterized by amplifications. Evaluation of chromosomal position and cross-referencing with RNA-seq data from the TCGA EAC cohort demonstrated expected focality and gene-expression changes (see Supplementary Fig. S1). Furthermore, data from the TCGA offer validation of driver alterations and dysregulated pathways involved in EAC, as frequencies of each are consistent with our data (see Supplementary Fig. S1). Our findings are also consistent with a whole-genome sequencing analysis of the International Cancer Genome Consortium (ICGC) cohort of patients (8). In fact, many of the oncogenic driver alterations identified in our study were designated high-confidence drivers by their analysis, using orthogonal computational methodologies. At present, to the authors' knowledge, no other large-scale clinical or genomic cohorts are available for further validation of our results.

Although our data indicate that few individual alterations differentiate treatment cohorts, alterations in both the Cell-cycle and RTK–RAS pathways were significantly enriched in the PIT cohort. These differences were determined primarily by *ERBB2* and *KRAS* in the RTK–RAS pathway, and *CDKN2A/B, CCNE1*, and *CCND1* in the cell-cycle pathway (see Supplementary Fig. 1C). Furthermore, the overall number of oncogenic drivers, the fraction of genome altered, and the rate of whole-genome doubling were all significantly higher in the PIT cohort, after adjusting for both tumor purity and site of tissue acquisition. Of these genomic characteristics and pathways, enrichment in Cell-cycle pathway genes and the number of oncogenic drivers were also independently associated with worse OS. In their totality, these findings suggest higher levels of chromosomal instability in later stages of EAC. Recent work by Noorani and colleagues examining clonal evolution during progression of EAC likewise showed that more advanced disease harbored a higher rate of structural variants, such as L1 retrotransposon activity (28). This concept fits easily within the clinical framework of EAC, where metastatic disease is often refractory to treatment and five-year survival is less than 5%. In other cancer types, increasing genomic instability and intratumoral heterogeneity have also been found to correlate with poor treatment responses (29, 30). Thus, greater levels of chromosomal instability of advanced-stage EAC may account, in part, for the treatment resistance and dismal prognosis.

Our multivariable models of OS accounted for relevant clinicopathologic prognostic factors that are knowable at the time of diagnosis, including age, clinical stage, tumor grade, and CIT versus PIT. Of the four driver alterations independently associated with OS, two (*SMAD4* alteration and *CDKN2A* alteration) included high proportions of deletions. However, neither *CDKN2A* deletions alone nor *SMAD4* deletions alone were prognostic on univariable analysis. To some extent, the frequency of deletions reported here may reflect decreased sensitivity of panel-based sequencing to detect these at lower tumor purity levels. By contrast, the other two genomic factors independently associated with OS, *KRAS* and *ERBB2* alterations, overwhelmingly consisted of oncogenic amplifications, and these were both strongly associated with prognosis.

Among these independently prognostic alterations, *SMAD4* loss has previously been linked to poor prognosis and a higher propensity for disease recurrence in EAC (8, 31). Furthermore, *KRAS* activation has been widely recognized in EAC, along with dramatically increased *KRAS* mRNA expression, and Wong and colleagues have reported worse OS with *KRAS* amplification in a Japanese cohort of EAC by Kaplan–Meier analysis (32). The survival benefit associated with *ERBB2* amplification was unsurprising given that 84 of the 108 patients 78%)) with *ERBB2*-amplified tumors received trastuzumab, which represents one of the few targeted therapies approved for this disease. Trastuzumab has been shown to improve progression-free survival of patients with metastatic disease and high-level Her2 expression (33, 34). Moreover, we found *ERBB2* amplification to be associated with better tumor differentiation and trended toward mutual exclusivity with *MDM2* ($P < 0.05$, $q < 0.05$) and other RTK–RAS–PIK3 pathway alterations, which have been shown to confer resistance to chemotherapy and trastuzumab therapy, respectively (35, 36). Therefore, while it was not possible to quantify the individual contributions of each of these factors, our results suggest that the survival benefit seen with *ERBB2* amplification may be multifactorial.

In summary, our study of a large cohort of lower esophageal and junctional adenocarcinomas identified 16 genes harboring recurrent oncogenic driver alterations, the majority of which are copy-number alterations. Among these, *SMAD4, CDKN2A*, and *KRAS* amplification are independently prognostic of worse survival in a multivariable model including relevant clinicopathologic factors. Amplification of *ERBB2* portends improved survival, as it represents an actionable therapeutic target with trastuzumab. Our results further demonstrate the potential value of prospective broad-panel next-generation sequencing in EAC, regardless of clinical stage, to guide treatment selection and goals of therapy. At present, panel-based sequencing is predominantly applied to patients with advanced disease, but we believe this tool is markedly underutilized in EAC given the wealth of prognostic information that it may provide to enhance clinical decision-making.

Authors' Disclosures

M. Hsu reports grants from NIH/NCI Cancer Center Support Grant P30CA008748 during the conduct of the study. W.K. Chatila reports personal fees from ImmPACT Bio USA Inc. outside the submitted work. Y.Y. Janjigian reports other from Bristol-Myers Squibb, RGENIX, Eli Lilly, Merck, Bayer, NCI, Department of Defense, Cycle for Survival, Fred's Team, Genentech/Roche, Merck Serono, Daiichi-Sankyo, Pfizer, Imugene, Zymeworks, Seattle Genetics, Basilea Pharmaceutica, and AstraZeneca outside the submitted work. S. Maron reports non-financial support from Merck, Bayer, Guardant Health, and Genentech; personal fees from Natera, Basilea, and Daiichi Sankyo; and other from Calithera outside the submitted work. A.J. Wu reports grants from CivaTech Oncology, Inc., other from Simphotek, Inc., non-financial support from AlphaTau Medical, and personal fees from AstraZeneca and MORE Health outside the submitted work. D.R. Jones reports other from Merck and AstraZeneca outside the submitted work. D. Molena reports other from Johnson & Johnson, Intuitive, Boston Scientific, AstraZeneca, Urogen, and BMS outside the submitted work. D.B. Solit reports personal fees from Pfizer, Loxo Oncology at Lilly, BridgeBio, Scorpion Therapeutics, and Vivideon Therapeutics outside the submitted work. M.F. Berger reports personal fees from Roche and grants from Grail outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

S. Sihag: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing. **S.C. Nussenzweig:** Data curation, formal analysis, visualization, and methodology. **H.S. Walch:** Software, formal analysis, visualization, and methodology. **M. Hsu:** Formal analysis, validation, and methodology. **K.S. Tan:** Formal analysis, validation, and methodology. **F. Sanchez-Vega:** Conceptualization, formal analysis, and methodology. **W.K. Chatila:** Conceptualization, formal analysis, and methodology. **S.A. De La Torre:** Data curation. **A. Patel:** Data curation. **Y.Y. Janjigian:** Supervision and investigation. **S. Maron:** Supervision, investigation, writing–review and editing. **G.Y. Ku:** Supervision, investigation, writing–review and editing. **L.H. Tang:** Visualization, methodology, writing–review and editing. **J. Hechtman:** Visualization, methodology, writing–review and editing. **P.M. Shah:** Investigation, writing–review and editing. **A.J. Wu:** Investigation, writing–review and editing. **D.R. Jones:** Resources, funding acquisition, project administration, writing–review and editing. **D. Molena:** Project administration, writing–review and editing. **D.B. Solit:** Resources, supervision, project administration,

writing–review and editing. **N. Schultz:** Formal analysis, supervision, validation, and methodology. **M.F. Berger:** Conceptualization, resources, supervision, funding acquisition, writing–review and editing.

Acknowledgments

We thank Janet Novak, PhD, of Memorial Sloan Kettering Cancer Center for substantively editing the manuscript. This work was supported, in part, by the NIH/NCI Cancer Center Support Grant P30 CA008748. S. Sihag is supported by a grant from the Fiona and Stanley Druckenmiller Center for Lung Cancer Research at Memorial Sloan Kettering Cancer Center and the Association of Women Surgeons Ethicon Research Fellowship.

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Received December 3, 2020; revised February 5, 2021; accepted March 29, 2021; published first April 1, 2021.

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