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Association of HER2 amplification or overexpression with overall survival in advanced upper gastrointestinal adenocarcinomas

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BACKGROUND: Advanced esophageal (EAC), gastroesophageal junction (GEJAC) and gastric (GAC) adenocarcinomas with HER2 amplification or overexpression (HER2+) are routinely treated with trastuzumab. However, it remains unclear if HER2+ is associated with superior overall survival (OS).
METHODS: The cohort included recurrent or de novo metastatic GAC, GEJAC and EAC from Kaiser Permanente Northern California. We used Cox regression modelling to examine association between HER2+ and OS, adjusting for demographics, performance status, CCI, receipt of chemotherapy and *p53* (mutp53*)*, *KRAS* (mutKRAS), *CDKN2A*, *PIK3CA* co-mutations and *MYC* amplification.
RESULTS: Of 875 total eligible patients, 173 had EAC, 276 had GEJAC and 426 had GAC. HER2+ was associated with better OS among the full cohort (HR = 0.74, 95% CI [0.60–0.93]), among EAC (HR = 0.62; [95% CI, 0.40–0.96]) and GEJAC (HR = 0.59; [95% CI, 0.38–0.87]), but not among GAC (HR = 0.89; [95% CI, 0.59–1.35]) patients. GEJAC had better OS than EAC (HR = 0.68, [95% CI, 0.54–0.86]). Trastuzumab treatment was associated with better OS (HR = 0.40, 95% CI [0.21–0.77]). In addition, HER2+ was associated with better OS across the molecular subgroups except that of KRAS mutation (mutKRAS). Our data also show that GEJAC, EAC and GAC were differentially associated with mutp53, mutKRAS and *MYC* amplification.
CONCLUSION: HER2+ and treatment with trastuzumab in HER2+ patients were associated with superior OS in upper gastrointestinal adenocarcinomas across molecular subgroups except that of mutKRAS. These results reaffirm the importance of anti-HER2 treatment in HER2+ patients and provide insight on the prognostic and biological divergence among these anatomically linked upper gastrointestinal adenocarcinomas.

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INTRODUCTION

Esophageal (EAC), gastroesophageal junction (GEJAC) and gastric (GAC) adenocarcinoma are three anatomically linked malignancies that are a consequence of chronic inflammation and share some important risk factors, including smoking, dietary style and alcohol consumption [1–4]. They also differ in some aspects of aetiology. For example, EAC and GEJAC are often linked to Barrett's esophagus (BE) which commonly results from gastroesophageal reflux, while GAC is closely associated with gastric intestinal metaplasia caused by Helicobacter pylori (H. pylori) [2, 5–7]. GEJ is marked with a unique transitional epithelium comprising of squamous-columnar junction (SCJ) that has been shown to be the source of BE, a precursor to EAC and GEJAC through clonal evolution [8–12].

EAC, GEJAC and GAC share similar treatment approaches. For early-stage EAC and GEJAC, perioperative chemotherapy or neoadjuvant concurrent chemotherapy and radiation followed by surgery were considered standard of care, while for GAC, perioperative chemotherapy or adjuvant chemotherapy with or without radiation were considered standard of care [13–17]. The recently published ESOPEC and TOPGEAR trials have shown that perioperative chemotherapy without radiation in GAC and GAC are the current standard of care [18, 19]. In relapsed and metastatic diseases, treatment approaches for EAC, GEJAC and GAC are very similar and include systemic therapy with fluorouracil-based multiagent chemotherapy with or without immune checkpoint blockade, and with trastuzumab for HER2 amplification or overexpression (HER2+) patients [13–15, 20, 21].

Despite the routine treatment using trastuzumab in HER2+ patients, whether HER2+ is associated with better OS remains unclear. Also, despite the similarities and differences in etiologies and treatment approaches, whether these three anatomically closely linked malignancies are prognostically and biologically different remains not well understood. The goal of this study was to investigate whether HER2+ was associated with better OS among the full cohort and among the anatomic subgroups, and

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whether different genomic alterations associated differentially with OS among patients with and without HER2+.

METHODS

Study population

Beginning late 2017 next-generation sequencing (NGS) was routinely performed for patients with advanced malignancy within Kaiser Permanente Northern California (KPNC). This cohort included 875 eligible KPNC patients with relapsed or de novo metastatic EAC ($n = 173$), GEJAC ($n = 276$) or GAC ($n = 426$). We excluded esophageal squamous cell carcinoma. Molecular profiling was performed using StrataNGS (Ann Arbor, Michigan) from November 2017 to December 2023. Patient data on demographics, Charlson comorbidity index (CCI), performance status (PS), histology (Lauren's classification) and receipt of systemic chemotherapy were obtained from the electronic medical record (Epic) and cancer registry database. CCI was based on the 12 month-period prior to diagnosis of relapsed or de novo metastatic EAC, GEJAC and GAC. This study was approved by KPNC institutional review boards with waiver of consent (approval #: *1710436-3*) and was conducted according to International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS).

NGS

During the study period, StrataNGS of advanced malignancies consisted of a 429-gene, pan-solid tumour, NGS assay for formalin-fixed paraffin-embedded (FFPE) tumour tissue, performed on co-isolated DNA and RNA [22]. For the entire dataset, alterations in more than 110 individual genes were identified. The six most common genomic alterations in this cohort included HER2 overexpression or amplification (HER2+), *TP53 (mutp53), CDKN2A (mutCDKN2A), KRAS (mutKRAS), PIK3CA* (mutPIK3CA) mutation and *MYC* amplification. MYC amplification was defined as more than 10 copies of the gene identified. Majority of KRAS mutations were at codon 12 and 13. Majority of CDKN2A mutations were deletions. Majority of PI3KCA mutations were missense mutation including E542K, E545K and H1047R.

HER2+ definition

HER2 was considered positive (HER2+) if HER2 expression was 3+ in 10% or more tumour cells by immunohistochemistry, or positive by fluorescent in situ hybridisation (FISH), or positive with six copies of *ERBB2* detected by NGS [23]. There were 133 HER2+ patients, of these, 99 received trastuzumab.

Gastroesophageal junction (GEJ) definition

GEJ was based on reports by gastroenterologist who performed diagnostic upper endoscopy with tissue biopsy and was defined as the anatomic area that represents the junction between the distal esophagus and the proximal stomach (cardia) [24].

Histology

Squamous cell carcinoma of esophagus was excluded. For GAC patients, Lauren's classification data were extracted from pathology reports and included diffuse type, intestinal type, mixed type and unknown.

Helicobacter Pylori and Epstein Barr Virus tests

Among 875 patients, 265 patients (190 GAC and 75 EAC) had Helicobacter Pylori test performed, only 13 (11 GAC and 2 EAC) had a positive test. There were only 3 patients with Epstein Barr Virus test performed and among them one had a positive test.

Chemotherapy

Chemotherapy was defined as administered after the diagnosis of relapsed or metastatic disease and was primarily a fluorouracil-based multiagent regimen with or without a checkpoint inhibitor, or with or without Trastuzumab (for HER2+ patients).

PD-L1 expression and treatment with immune checkpoint inhibitor

There were 314 patients with PD-L1 expression test results available, among them 195 had positive PD-L1 expression and 119 patients had negative PD-L1 expression. Immunohistochemical analysis for PD-L1 was performed on archival or newly obtained pretreatment formalin-fixed, paraffin-embedded tumour specimens. Cell-surface staining for PD-L1 in 5% threshold or more tumour cells was defined as positive. Approximately 20% of all patients received treatment with an immune checkpoint inhibitor (ICI), either nivolumab or pembrolizumab as a single agent or in combination with chemotherapy (Table 1).

Definition of gain-of-function (GOF) and non-gain-of-function (non-GOF) *TP53* mutation

The mutp53 GOF in our study was defined as R175H, R248Q, R248W, R249S, R273H, R273L and R282W, all located at the DNA binding domain (DBD) and all the other *p53* alterations were grouped as non-GOF, same as in our previous study [25].

Statistical analysis

OS was measured from the date of diagnosis of relapsed or metastatic disease to the date of death or end of study follow-up (March 10th, 2024), whichever came first. Patients who were alive at the end of the study period were censored at that time. We used the Pearson's chi squared test to assess differences in distributions of demographic and clinical factors and mutations (HER2+, mutTP53, mutKRAS, mutCDKN2A, mutPIK3CA mutation and *MYC* amplification) across comparison groups. We used the one-way ANOVA test to assess differences in continuous variables. We used Kaplan-Meier plots (log rank test) to perform unadjusted (univariate) OS analysis and estimate median OS. The number of patients at risk in the Kaplan-Meier OS curves accounted for delayed entry into the cohort at the time of receipt of NGS results (i.e. left truncation, with median study entry of 0.7 months post-diagnosis) [26]. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence interval (CI) for the association between anatomic subgroups or sex and OS, adjusted for covariates. Time since diagnosis of advanced adenocarcinoma was the time scale used in the regression models, allowing for delayed entry into the cohort. Covariates included in our main regression models (and unless otherwise stated) were age (continuous), sex (male, female), ethnicity (Non-Hispanic White, Black, Asian, Hispanic, other/unknown), PS (0 to 1, 2 to 4), CCI (continuous) and chemotherapy received (yes, no). We examined the association of OS with specific genomic alterations in a model that included all six alterations simultaneously: (HER2+ [yes, no], mutp53 [yes, no], mutCDKN2A [yes, no], mutKRAS [yes, no], mutPIK3CA (yes, no) and *MYC* amplification (yes, no). Among patients with GAC, histology (Lauren's classification) was also included in the Cox model. Because patients who did not receive chemotherapy had extremely poor OS, we performed independent analysis of patients who received and patients who did not receive chemotherapy. The statistical analysis was performed using SAS software version 9.4, R (R Core Team, 2020).

All methods were performed in accordance with the relevant guidelines and regulations.

RESULTS

Demographic and clinical characteristics of study population

There were 173 patients with EAC, 276 with GEJAC and 426 with GAC. EAC patients were slightly older than GEJAC and GAC patients (Table 1). Among 772 patients with microsatellite instability (MSI) test performed, 26 (3.4%) had MSI-high. Patients with EAC and GEJAC were predominantly male (87% and 81% respectively), while there were only slightly more males (52.6%) among patients with GAC. Over 70% of patients with EAC and GEJAC were White, whereas nearly 70% of patients with GAC were non-White. A higher percent of patients with GAC than with EAC or GEJAC were Asian. PS, CCI and receipt of chemotherapy were similar among the three anatomic subgroups. A higher percent of patients with EAC and GEJAC than with GAC had tumours that were HER2+ or had mutp53 or mutCDKN2A. In contrast, a higher percent of patients with GEJAC than EAC and GAC had tumours that had mutKRAS. A similar percent of patients among the three anatomic subgroups had tumours with mutPIK3CA or *MYC* amplification (Table 1). There were 43 (24.9%), 52 (18.8%) and 38 (8.9%) HER2+ patients among the EAC, GEJAC and GAC anatomic subgroups, respectively (Table 2). Thirty-one (72.1%) of

Table 1. Demographics of esophageal (EAC), GE junction (GEJAC) and gastric (GAC) adenocarcinoma.

	EAC ($n = 173$)	GEJAC ($n = 276$)	GAC ($n = 426$)	Total ($N = 875$)	p value
Age					0.01
- Median	68.0	65.0	65.0	66.0	
- Mean	66.6	64.2	63.2	64.2	
- Range	39.0–90.0	24.0–89.0	21.0–93.0	21.0–93.0	
- SD	10.50	11.35	14.42	12.84	
Sex					<1e-05
- F	22 (12.7%)	51 (18.5%)	202 (47.4%)	275 (31.4%)	
- M	151 (87.3%)	225 (81.5%)	224 (52.6%)	600 (68.6%)	
Ethnicity					<1e-05
- Asian	16 (9.2%)	39 (14.1%)	122 (28.6%)	177 (20.2%)	
- Black	5 (2.9%)	7 (2.5%)	35 (8.2%)	47 (5.4%)	
- Hispanic	11 (6.4%)	13 (4.7%)	55 (12.9%)	79 (9.0%)	
- Other	11 (6.4%)	22 (8.0%)	78 (18.3%)	111 (12.7%)	
- White	130 (75.1%)	195 (70.7%)	136 (31.9%)	461 (52.7%)	
PS					0.43
- ECOG 0–1	86 (49.7%)	157 (56.9%)	212 (49.8%)	455 (52.0%)	
- ECOG 2–4	32 (18.5%)	44 (15.9%)	79 (18.5%)	155 (17.7%)	
- Unknown	55 (31.8%)	75 (27.2%)	135 (31.7%)	265 (30.3%)	
CCI					0.33
- Median	1.00	1.00	1.00	1.00	
- Mean	1.53	1.29	1.37	1.38	
- Range	0.00–6.00	0.00–8.00	0.00–10.00	0.00–10.00	
- SD	1.59	1.47	1.77	1.65	
Chemo					0.69
- N	32 (18.5%)	45 (16.3%)	80 (18.8%)	157 (17.9%)	
- Y	141 (81.5%)	231 (83.7%)	346 (81.2%)	718 (82.1%)	
Immunotherapy					
-N	146 (84.4%)	232 (84.1%)	326 (76.5%)	704 (80.5%)	0.017
-Y	27 (15.6%)	44 (15.9%)	100 (23.5%)	171 (19.5%)	
HER2					<1e-05
- neg	130 (75.1%)	224 (81.2%)	388 (91.1%)	742 (84.8%)	
- pos	43 (24.9%)	52 (18.8%)	38 (8.9%)	133 (15.2%)	
Trastuzumab(For HER2+)					0.79
-N	12 (26.2%)	12 (23.1%)	10 (26.3%)	133 (26.1%)	
-Y	31 (73.8%)	40 (76.9%)	28 (73.7%)	99 (73.9%)	
p53					<1e-05
- wt	19 (11.0%)	63 (22.8%)	184 (43.2%)	266 (30.4%)	
- mut	154 (89.0%)	213 (77.2%)	242 (56.8%)	609 (69.6%)	
Mutp53					0.80
- Non-GOF	119 (68.8%)	170 (61.6%)	188 (44.1%)	477 (54.5%)	
- GOF	35 (20.2%)	43 (15.6%)	54 (12.7%)	132 (15.1%)	
KRAS					0.01
- wt	148 (85.5%)	218 (79.0%)	372 (87.3%)	738 (84.3%)	
- mut	25 (14.5%)	58 (21.0%)	54 (12.7%)	137 (15.7%)	
CDKN2A					<1e-05
- wt	96 (55.5%)	165 (59.8%)	345 (81.0%)	606 (69.3%)	
- mut	77 (44.5%)	111 (40.2%)	81 (19.0%)	269 (30.7%)	
PIK3CA					0.09
- wt	160 (92.5%)	255 (92.4%)	375 (88.0%)	790 (90.3%)	
- mut	13 (7.5%)	21 (7.6%)	51 (12.0%)	85 (9.7%)	

Table 1. continued

	EAC ($n = 173$)	GEJAC ($n = 276$)	GAC ($n = 426$)	Total ($N = 875$)	p value
MYC					0.28
- Amplification neg	158 (91.3%)	255 (92.4%)	403 (94.6%)	816 (93.3%)	
- Amplification pos	15 (8.7%)	21 (7.6%)	23 (5.4%)	59 (6.7%)	

CCI Charlson comorbidity index, *Wt* wild-type, *Mut* mutation, *GOF* gain-of-function, *Non-GOF* non-gain-of-function.

the 43 HER2+EAC patients, forty (76.9%) of the 52 HER2 + GEJAC patients, twenty-eight (73.7%) of the 38 HER2 + GAC patients received Trastuzumab.

Association of HER2 and other molecular markers with OS among the full cohort

The median OS for the full cohort was 9.1 months. In fully adjusted Cox regression models, HER2+ patients had substantially better OS than HER2 negative patients (HR = 0.74, [95% CI, 0.60–0.93]) (Fig. 1a), with median OS of 15.4 versus 8.3 months (Fig. 1b). In contrast, Mutp53, mutKRAS, mutCDKN2A, mutPIK3CA and *MYC* amplification were not associated with an OS difference (Fig. 1a). We also observed that chemotherapy was associated with substantially better OS among the entire cohort (HR = 0.31; [95% CI, 0.25–0.37]) (Fig. 1a) and among all three anatomic subgroups (Supplementary Fig. 1). The median OS for patients who received chemotherapy was 12.2 months, but 1.7 months for patients who did not receive chemotherapy (Fig. 1c). After excluding patients who did not receive chemotherapy (Supplementary Table 1), the association between HER2 status and OS was largely unchanged (HR = 0.73, [95% CI, 0.51–0.92]) (Supplementary Fig. 2), although median OS was longer (17.4 versus 11.4 months, $p = 0.006$) (Supplementary Fig. 3). Among 133 HER2+ patients, 99 received trastuzumab (Table 1). Trastuzumab was associated with dramatically better OS (HR = 0.40; [95% CI, 0.21–0.77]) in HER2+ patients (Fig. 1d).

Association of anatomic subgroup and OS

In adjusted Cox regression models, patients with GEJAC had better OS than patients with EAC (HR = 0.68, [95% CI, 0.54–0.86]), modestly better OS than patients with GAC (HR = 0.85; [95% CI, 0.69–1.04]) (Fig. 1e). Median OS was 11.3, 7.7 and 8.2 months respectively for GEJAC, EAC and GAC (Fig. 1f; $P = 0.004$ for GEJAC vs EAC, and $P = 0.37$ for GEJAC vs GAC, by log-rank tests). Results were largely unchanged after restricting the analysis to patients who received chemotherapy (Supplementary Figs. 4 and 5).

Association of HER2+ with OS in anatomic subgroups

HER2+ patients had substantially better OS than HER2 negative patients among EAC (HR = 0.62, [95% CI, 0.40–0.96]) and GEJAC (HR = 0.59, [95% CI, 0.38–0.87]), but not among GAC patients (HR = 0.89; [95% CI, 0.59–1.35]) (Fig. 2a). For the full cohort, HER2+ patients had better OS than HER2 negative patients across molecular subgroups except mutKRAS (HR = 1.44, [95% CI, 0.69–2.85]) (Fig. 2b). For example, HER2+ versus HER2 negative for patients with wtp53, HR was 0.49 (95% CI, [0.27–0.89]), and HR was 0.82 (95% CI, [0.64–1.04]) for patients with mutp53, 0.69 (95% CI, [0.55–0.88]) for patients with wtKRAS, 0.67 (95% CI, [0.56–0.89]) for patients with wtCDKN2A, 0.78 (95% CI, [0.53–1.14]) for patients with mutCDKN2A, 0.76 (95% CI, [0.61–0.95]) for patients with wtPIK3CA, 0.73 (95% CI, [0.58–0.91]) for patients with MYC amplification negative.

Association of mutp53 status and OS in anatomic subgroups

Mutp53 versus wild-type p53 (wtp53) was not associated with an OS difference among all three anatomic subgroups (Fig. 3a). Intriguingly, mutp53 GOF versus non-GOF was associated with worse OS among GAC (HR = 1.46; [95% CI, 1.03–2.12]) but not among EAC and GEJAC (Fig. 3a). The median OS for GAC patients with mutp53 GOF versus non-GOF was 5.5 versus 8.0 months (log-rank test, $p = 0.05$, Fig. 3b). After excluding patients who did not receive chemotherapy from the analysis, the results remained similar with median OS of 10.1 months for GAC patients with a mutp53 GOF versus 12.4 months for GAC patients with a non-GOF ($p = 0.05$, Supplementary Fig. 6).

Association of mutKRAS status and OS in anatomic subgroups

MutKRAS versus wild-type KRAS (wtKRAS) was associated with worse OS among EAC (HR = 1.81, [95% CI, 1.10–2.99]), modestly worse OS among GEJAC (HR = 1.29, [95% CI, 0.93–1.79]), and no OS difference among GAC (HR = 0.85, [95% CI, 0.60–1.20]) (Fig. 4a). Among the full cohort with HER2 negative patients, mutKRAS versus wtKRAS was not associated with an OS difference, (HR = 1.06; [95% CI, 0.85–1.32]) (Fig. 4b). However, mutKRAS versus wtKRAS was associated with substantially worse OS among HER2+ patients of the full cohort (HR = 2.36, [95% CI, 1.14–4.80]) (Fig. 4b). MutCDKN2A and mutPIK3CA were not associated with an OS difference (Supplementary Figs. 7 and 8).

Association of *MYC* amplification status and OS in anatomic subgroups

MYC amplification positive versus *MYC* amplification negative was associated with a dramatically better OS among patients with EAC (HR = 0.19, [95% CI, 0.08–0.42]) (Fig. 4c). However, *MYC* amplification positive versus *MYC* amplification negative was associated with worse OS among patients with GEJAC (HR = 2.00, [95% CI, 1.15–3.40]) and GAC (HR = 1.76 [95% CI, 1.10–2.81]) (Fig. 4c). Of note, the sample size with *MYC* amplification was relatively small ($n = 15$ to 23, Table 1).

Summary of data

Figure 4d summarises the OS differences among the three anatomic subgroups by molecular features. HER2+ patients had substantially better OS than HER2 negative patients among EAC and GEJAC but not GAC. Chemotherapy was associated with a dramatic OS benefit for all patients regardless of anatomic location. Mutp53 GOF versus non-GOF was inversely associated with sex-dependent OS among GAC but not among EAC or GEJAC, while mutKRAS was associated with worse OS among EAC and among HER2+ patients, but not among GEJAC and GAC. Most surprisingly, *MYC* amplification was associated with dramatically better OS among EAC but worse OS among GEJAC and GAC.

DISCUSSION

In this study using a large dataset from a tertiary multi-centre healthcare organisation we have shown that HER2+ patients had better OS than HER2 negative patients with advanced upper gastrointestinal adenocarcinomas and that this OS difference was persevered across the molecular subgroups except that of mutKRAS. Also, patients with advanced GEJAC appeared to have better OS than patients with advanced EAC. Despite overall mutp53 not being associated with OS difference among all three anatomic subgroups, mutp53 GOF versus non-GOF was associated

Table 2. Demographics of HER2 negative (HER2 neg) and HER2+(HER2 pos) patients.

	HER2 neg ($n = 742$)	HER2 pos ($n = 133$)	Total ($N = 875$)	*p* value
Age				0.21
- Median	66.0	64.0	66.0	
- Mean	64.4	62.9	64.2	
- Range	21.00–93.00	31.00–86.00	21.00–93.00	
- SD	13.1	11.4	12.8	
Sex				0.02
- F	245 (89.1%)	30 (10.9%)	275 (100.0%)	
- M	497 (82.8%)	103 (17.2%)	600 (100.0%)	
Race				0.45
- Asian	158 (89.3%)	19 (10.7%)	177 (100.0%)	
- Black	40 (85.1%)	7 (14.9%)	47 (100.0%)	
- Hispanic	67 (84.8%)	12 (15.2%)	79 (100.0%)	
- Other	93 (83.8%)	18 (16.2%)	111 (100.0%)	
- White	384 (83.3%)	77 (16.7%)	461 (100.0%)	
Anatomic subgroup				<1e-05
- EAC	130 (75.1%)	43 (24.9%)	173 (100.0%)	
- GEJ	224 (81.2%)	52 (18.8%)	276 (100.0%)	
- GAC	388 (91.1%)	38 (8.9%)	426 (100.0%)	
PS				0.04
- ECOG 0–1	373 (82.0%)	82 (18.0%)	455 (100.0%)	
- ECOG 2–4	139 (89.7%)	16 (10.3%)	155 (100.0%)	
- Unknown	230 (86.8%)	35 (13.2%)	265 (100.0%)	
CCI				0.11
- Median	1.00	1.00	1.00	
- Mean	1.42	1.17	1.38	
- Range	0.00–10.00	0.00–7.00	0.00–10.00	
- SD	1.66	1.57	1.65	
Chemo				0.09
- N	140 (89.2%)	17 (10.8%)	157 (100.0%)	
- Y	602 (83.8%)	116 (16.2%)	718 (100.0%)	
Immunotherapy				0.34
-N	593 (84.2%)	111 (15.8%)	704 (100.0%)	
-Y	149 (87.1%)	22 (12.9%)	171 (100.0%)	
Trastuzumab				<1e-05
- N	741 (95.5%)	35 (4.5%)	776 (100.0%)	
- Y	1 (1.0%)	98 (99.0%)	99 (100.0%)	
p53				3e-05
- wt	246 (92.5%)	20 (7.5%)	266 (100.0%)	
- mut	496 (81.4%)	113 (18.6%)	609 (100.0%)	
Mutp53				0.0001
- Non-GOF	386 (80.9%)	91 (19.1%)	477 (100.0%)	
- GOF	110 (83.3%)	22 (16.7%)	132 (100.0%)	
KRAS				0.02
- wt	617 (83.6%)	121 (16.4%)	738 (100.0%)	
- mut	125 (91.2%)	12 (8.8%)	137 (100.0%)	
CDKN2A				0.21
- wt	520 (85.8%)	86 (14.2%)	606 (100.0%)	
- mut	222 (82.5%)	47 (17.5%)	269 (100.0%)	
PIK3CA				0.01
- wt	662 (83.8%)	128 (16.2%)	790 (100.0%)	
- mut	80 (94.1%)	5 (5.9%)	85 (100.0%)	

Table 2. continued

	HER2 neg ($n = 742$)	HER2 pos ($n = 133$)	Total ($N = 875$)	*p* value
MYC				1.00
- Amplification neg	692 (84.8%)	124 (15.2%)	816 (100.0%)	
- Amplification pos	50 (84.7%)	9 (15.3%)	59 (100.0%)	

CCI Charlson comorbidity index, *Wt* wild-type, *Mut* mutation, *GOF* gain-of-function mutation, *Non-GOF* non-gain-of-function mutation.

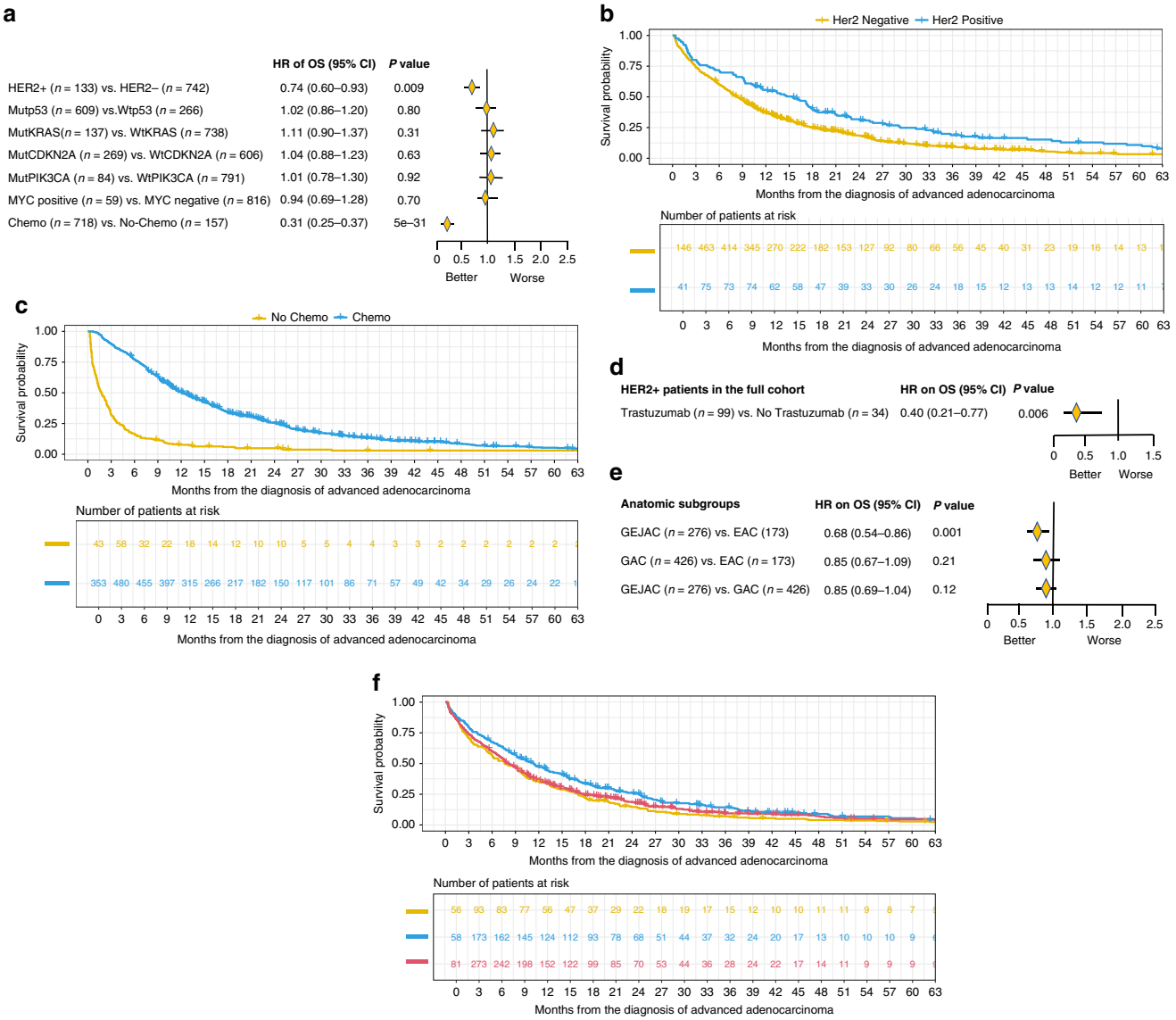


Fig. 1 Association of HER2 and other molecular markers as well as anatomic location with OS. a *Forest plots of HRs for the association of chemotherapy or molecular subgroup with OS in the full cohort*. Mutp53 p53 mutation, MutKRAS KRAS mutation, MutCDKN2A CDKN2A mutation, MutPIK3CA PIK3CA mutation, MYC positive MYC amplification positive, MYC negative MYC amplification negative. HR hazardous ratio. CI confidence interval. **b** *Kaplan-Meier OS curves by HER2 status in the entire cohort*. Median OS was 15.4 and 8.3 months respectively ($P = 0.001$). The number of patients at risk accounted for left-truncation. Patients who were still alive by the end of study period were censored. **c** *Kaplan-Meier OS curves of patients who received and patients who did not receive chemotherapy in the entire cohort*. Median OS was 12.2 and 1.7 months respectively ($P = 1e\text{-}32$). The number of patients at risk accounted for left-truncation. Patients who were still alive by the end of study period were censored. **d** *Forest plot of HR for the association of trastuzumab with OS among HER2+ (+) patients in the full cohort*. HR hazardous ratio, CI confidence interval. **e** *Forest plots of HRs for the association of anatomic subgroup and OS*. HR hazardous ratio, EAC esophageal adenocarcinoma, GEJAC GE junction adenocarcinoma, GAC gastric adenocarcinoma, HR hazardous ratio, CI confidence interval. **f** *Kaplan-Meier OS curves by EAC, GEJAC and GAC subgroups. EAC, esophageal adenocarcinoma. GEJAC, GE junction adenocarcinoma, GAC, gastric adenocarcinoma*. Median OS was 7.7, 11.3 and 8.2 months respectively. GEJAC vs EAC, $P = 0.004$; GEJAC vs GAC, $P = 0.037$; GAC vs EAC, $P = 0.20$. The number of patients at risk accounted for left-truncation. Patients who were still alive by the end of study period were censored.

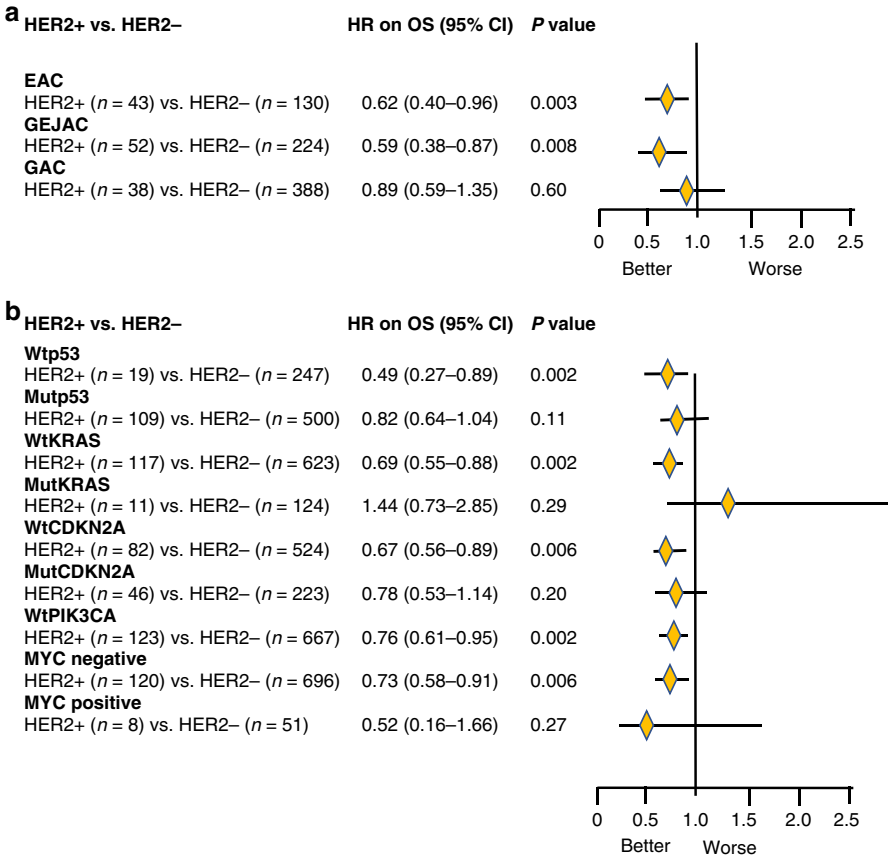


Fig. 2 Association of HER2 with OS among anatomic and molecular subgroups. a *Forest plots of HRs for the association of HER2 status with OS among anatomic subgroups*. HR hazardous ratio, CI confidence interval, EAC esophageal adenocarcinoma, GEJAC GE junction adenocarcinoma, GAC gastric adenocarcinoma. **b** *Forest plots of HRs for the association of HER2 status and OS among molecular subgroups*. HR hazardous ratio, CI confidence interval, Wtp53 wild-type p53, Mutp53 p53 mutation, WtKRAS wild-type KRAS, MutKRAS KRAS mutation, WtCDKN2A wild-type CDKN2A, MutCDKN2A CDKN2A mutation, WtPIK3CA wild-type PIK3CA, MutPIK3CA PIK3CA mutation, MYC- MYC amplification negative, MYC+ MYC amplification positive.

with worse OS among GAC but not among EAC or GEJAC. MutKRAS versus wtKRAS was associated with worse OS among EAC, modestly worse OS among GEJAC, but no OS difference among GAC. Most surprisingly, *MYC* amplification was associated with dramatically better OS among EAC, but worse OS among GEJAC and GAC.

Unlike breast cancer, where it has been known that HER2+ was associated with better prognosis because of the routine use of Trastuzumab, it has not been clear if HER2 status is similarly predictive of prognosis in upper gastrointestinal cancer [13, 14]. HER2+ was previously shown to be associated with poor prognosis in EAC, GEJ and GAC [23, 27–30]. These studies were conducted mostly with patients who did not receive Trastuzumab prior to the results of the ToGA trial that showed significant benefit of Trastuzumab in advanced disease [21]. To our knowledge our study is the first to show that HER2+ versus HER2 negative status is associated with better OS. This finding reenforces the importance of incorporating Trastuzumab into the routine treatment for advanced disease. Our data showed that HER2+ among GAC patients did not appear to be associated with a better OS compared to HER2 negative, however the HER2+ sample size was relatively small. In the subset analysis of ToGA trial, 51 participants with GAC whose histology was diffuse type did not show benefit from Trastuzumab [21].

The origin of BE which is a precancerous lesion of EAC was traced to gastric cardia stem cells by lineage tracing of $LGR5^+$ cells using a transgenic mouse model with overexpression of interleukin-1β (IL-1β) under the control of an Epstein-Barr virus promotor (ED-L2) [9, 31]. Another study using *p63*-deficient mice traced the origin of BE to the remnant embryonic stem cells at the SCJ [32]. Overexpression of IL-1β targeted specifically to the stomach caused spontaneous inflammation of gastric epithelium with early recruitment of myeloid-derived suppressive cells (MDSCs) mediated by IL-1β receptor signalling through NF-κB pathway [33]. These and other findings led to a notion that EAC, GEJAC and GAC are more similar than different in biology and a proposal to treat these three anatomically closely linked malignancies as one entity [11, 12, 34, 35]. The Cancer Genome Atlas (TCGA) data of EAC and GAC were not able to provide a signature for distinguishing EAC/GEJAC versus GAC, but were able to separate them into four different molecular subtypes with distinct prognosis which was confirmed by the Asian Cancer Research Group study (ACRG) [36–41]. The ACRG study used a two-gene signature (CDKN2A and MDM2) to measure p53 activity and found that p53-active GAC had better prognosis [37].

Our findings suggest that EAC, GEJAC and GAC may be biologically distinct despite common cancer stem cell origin. There is evidence that GEJAC and EAC may use different tumorigenesis pathways based on a study using biopsy specimens from patients [42]. GEJAC was routinely grouped into clinical trials with EAC or GAC with few trials conducted with GEJAC alone [24, 43, 44]. Our data suggest that it could be helpful in future clinical trials to stratify GEJAC. Demographically, more patients with EAC and GEJAC than GAC carried mutp53, mutCDKN2A and HER2 overexpression, however, these differences cannot explain the OS differences among them. Intriguingly, only among GAC but

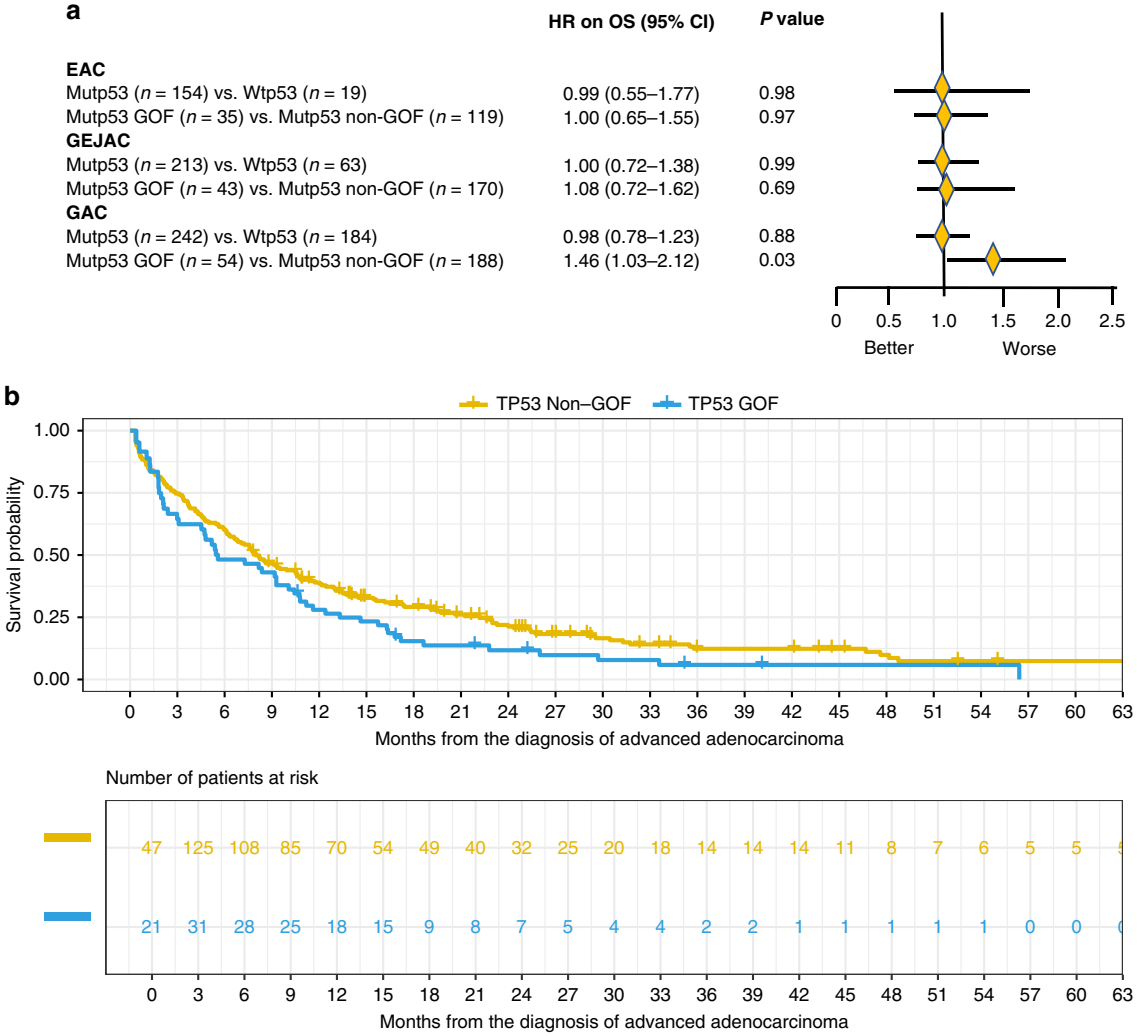


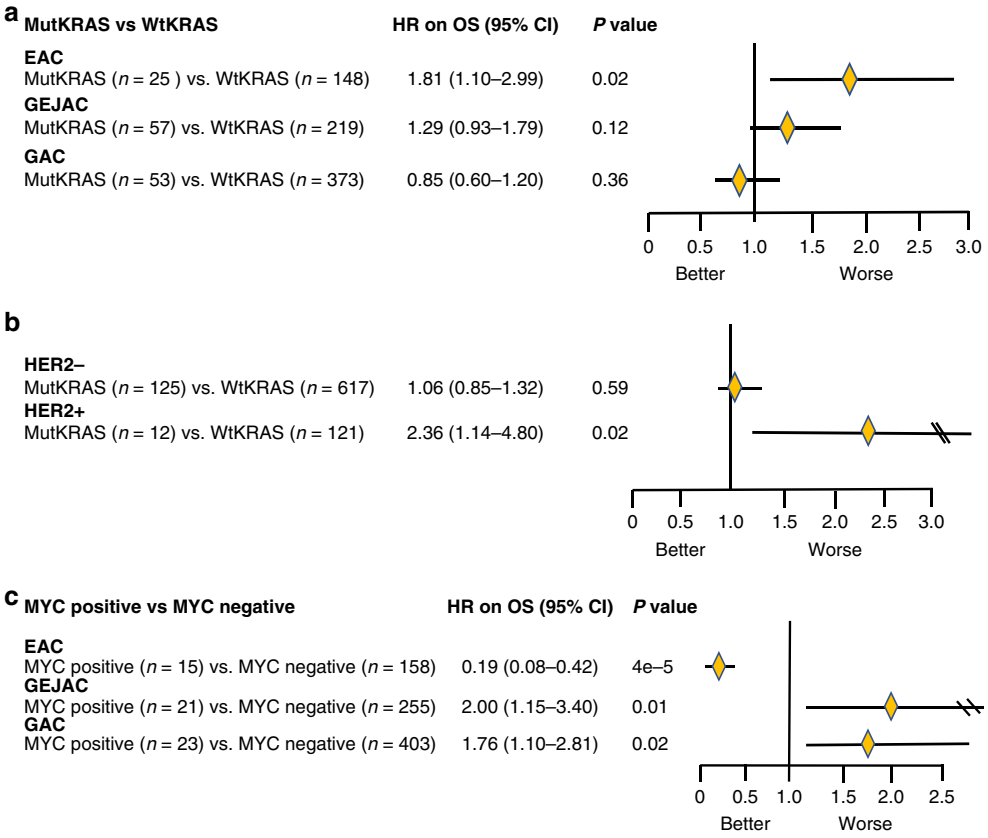
Fig. 3 Association of GOF and Non-GOF p53 mutations with OS. a *Forest plots of HRs for the association of mutp53 among anatomic subgroups*. HR hazardous ratio, CI confidence interval, Mutp53 p53 mutation, EAC esophageal adenocarcinoma, GEJAC GE junction adenocarcinoma, GAC gastric adenocarcinoma. **b** *Kaplan-Meyer curves of GAC patients with mutp53 GOF versus non-GOF*. GAC gastric adenocarcinoma, Mutp53 GOF p53 gain-of-function mutation, mutp53 non-GOF p53 non-gain-function mutation. Median OS was 5.5 and 8.0 respectively ($P = 0.05$). The number of patients at risk accounted for left-truncation. Patients who were still alive by the end of study period were censored.

not among EAC or GEJAC, mutp53 GOF versus non-GOF was associated with worse OS. This finding is reminiscent of our previous studies in advanced colorectal cancer (CRC) in which mutp53 GOF versus non-GOF was associated with worse OS only with left-sided CRC but not right-sided CRC, while right-sided CRC had worse OS than left-sided CRC only if the tumour carried mutp53 non-GOF but not GOF [25]. We had also found in our previous study with pancreatic ductal adenocarcinoma that mutp53 GOF versus non-GOF was associated with worse prognosis across all molecular subgroups [45]. The mechanism of such an anatomically and mutp53 dependent OS difference remains unclear.

The biological divergence among EAC, GEJAC and GAC is also suggested by our finding that mutKRAS was associated with worse OS among EAC, borderline worse OS among GEJAC and no OS difference among GAC. MutKRAS also appears to be associated with worse OS in HER2+ but not HER2 negative patients, suggesting that mutKRAS could be a Trastuzumab resistance mechanism. A recent clinical study with a small sample size and an *in vitro* study appear to support our clinical findings [27, 46].

Our finding that *MYC* amplification was associated with better OS among EAC but worse OS among GEJAC and GAC was surprising. *MYC* amplification is generally considered to be associated with poor prognosis in solid tumours such as esophageal squamous cell carcinoma and osteosarcoma [47–51]. Our data showing completely opposite prognostic associations of *MYC* amplification in these three anatomically linked malignancies suggests that MYC may possess the functional versatility that was previously unappreicated [25]. The sample size in our cohort with MYC amplification was relatively small. Ourfinding requires confirmation with a larger dataset.

Our study has limitations. It is a retrospective study and the number of patients with some subgroups was relatively small. Less than 50% of the patients had PD-L1 expression result available and ~50% of the patients in the full cohort received treatment with an ICI which reflects the evolution of the integration of ICI into chemotherapy as clinical trials were being conducted. Also, the follow-up for some of the patients were short. Our study has several strengths. Our dataset is relatively large with 875 patients who received comprehensive primary and specialty services from a large integrated healthcare system that consists of 21 medical centres. In addition, the diverse membership is relatively stable, and electronic records capture virtually all encounters, diagnoses and procedures. Our study was restricted to de novo metastatic



d

	EAC	GEJAC	GAC
HER2 positive vs. negative	Better OS	Better OS	No OS difference
Chemo vs. no-chemo	Better OS	Better OS	Better OS
Mutp53 vs. Wtp53	No OS difference	No OS difference	No OS difference
Mutp53 GOF vs. non-GOF	No OS difference	No OS difference	Worse OS
MutKRAS vs. WtKRAS	Worse OS	Modestly worse OS	No OS difference
MYC positive vs. negative	Better OS	Worse OS	Worse OS

Fig. 4 Association of KRAS mutation and MYC amplification with OS among anatomic subgroups. a *Forest plots of HRs for the association of mutKRAS with OS among anatomic subgroups*. HR hazardous ratio, CI confidence interval, EAC esophageal adenocarcinoma, GEJAC GE junction adenocarcinoma, GAC gastric adenocarcinoma. **b** *Forest plots of HRs for the association of mutKRAS with OS among HER2 subgroups*. HR hazardous ratio, CI confidence interval, MutKRAS KRAS mutation. **c** *Forest plots of HRs for the association of MYC amplification status with OS among anatomic subgroups*. HR hazardous ratio, CI confidence interval, EAC esophageal adenocarcinoma, GEJAC GE junction adenocarcinoma, GAC gastric adenocarcinoma. **d** *Summary of OS differences among three anatomic subgroups by molecular features*. Mutp53 p53 mutation, Mutp53 GOF p53 gain-of-function mutation, Mutp53 non-GOF p53 non-gain-of-function mutation, MutKRAS KRAS mutation, WtKRAS KRAS wild-type, MYC positive, MYC amplification positive, MYC negative, MYC amplification negative.

and relapsed disease and included six most common genomic alterations in the Cox regression modelling, in addition to the demographics and other important determinants.

In summary, our study demonstrates that HER2+ was associated with better OS among patients with advanced upper gastrointestinal adenocarcinomas and reveals a previously unappreciated prognostic divergence among EAC, GEJAC and GAC. Our data reaffirms the importance of anti-HER therapy in patients whose tumour is HER2+.

DATA AVAILABILITY

Kaiser Permanente Northern California (KPNC) Institutional Review Board has not provided approval for StrataNGS data on individual patients used in this study to be placed in a public access repository. However, researchers can request access to use this study data by contacting the DOR Data Sharing Workgroup at DOR-DataSharingWorkgroup@kp.org.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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