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A systematic review and meta-analysis of somatic and germline DNA sequence biomarkers of esophageal cancer survival, therapy response and stage

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Introduction: There is an urgent need for biomarkers to help predict prognosis and guide management of esophageal cancer. This review identifies, evaluates and meta-analyses the evidence for reported somatic and germline DNA sequence biomarkers of outcome and stage.

Methods: A systematic review was carried out of the PubMed, EMBASE and Cochrane databases (20 August 2014), in conjunction with the ASCO Level of Evidence scale for biomarker research. Meta-analyses were carried out for all reported markers associated with outcome measures by more than one study.

Results: Four thousand and four articles were identified, 762 retrieved and 182 studies included. There were 65 reported markers of survival or recurrence 12 (18.5%) were excluded due to multiple comparisons. Following meta-analysis, significant associations were seen for six tumor variants (mutant *TP53* and *PIK3CA*, copy number gain of *ERBB2/HER2*, *CCND1* and *FGF3*, and chromosomal instability/ploidy) and seven germline polymorphisms: *ERCC1* rs3212986, *ERCC2* rs1799793, *TP53* rs1042522, *MDM2* rs2279744, *TYMS* rs34743033, *ABCB1* rs1045642 and *MTHFR* rs1801133. Twelve germline markers of treatment complications were reported; 10 were excluded. Two tumor and 15 germline markers (11 excluded) of chemo (radio)therapy response were reported. Following meta-analysis, associations were demonstrated for mutant *TP53*, *ERCC1* rs11615 and *XRCC1* rs25487. There were 41 tumor/germline reported markers of stage; 27 (65.9%) were excluded.

Conclusions: Numerous DNA markers of outcome and stage have been reported, yet few are backed by high-quality evidence. Despite this, a small number of variants appear reliable. These merit evaluation in prospective trials, within the context of high-throughput sequencing and gene expression.

Key words: biomarkers, genomic, cancer, esophageal, prognosis, cancer staging

introduction

Esophageal and gastroesophageal junctional (GEJ) carcinoma account for 3.9% of cancer diagnoses yet 5.9% of cancer deaths [1]. Worldwide, squamous cell carcinoma (SCC) predominates but, in Western countries, incidence of adenocarcinoma is increasing rapidly [2, 3]. Treatment with curative intent involves either resection with or without neoadjuvant therapy, or definitive chemoradiotherapy with or without salvage resection. More than 5000 patients undergo esophagectomy in the United States and the UK every year, with 85% receiving neoadjuvant therapy [4, 5]. However, the majority experience complications, operative mortality remains relatively high and quality of life may be significantly impaired [6–8]. Neoadjuvant, adjuvant and definitive chemo- and/or radiotherapy also carry risk [9], and while the absolute survival benefit of neoadjuvant therapy ranges from 7% to 13% at 2 years [9], 50%–60% of tumors are resistant [10].

Prognosis overall remains bleak; even following ostensibly curative treatment 5-year survival is just 35%–45% [11–13]. This highlights limitations in our biological understanding, and our urgent need for biomarkers to predict prognosis, recurrence and sensitivity to therapy, and ultimately better personalize care. Most clinical experience with esophageal biomarkers to date has largely involved protein expression with or without sequence changes; while such markers are used to select patients for early phase trials, the sole tumor marker in routine use is *ERBB2/HER2* status [14, 15]. However, rapid advances in high-throughput next-generation sequencing (NGS) have highlighted the potential role of somatic DNA sequence markers. These may function as independent markers, serve to refine or explore existing

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expression markers, or constitute novel therapeutic targets [16–18]. Similarly, advances in custom and genome-wide single nucleotide polymorphism (SNP) arrays have emphasized the role of germline variants in modulating cancer and treatment outcome [19, 20].

We therefore undertook the first systematic review of DNA sequence biomarkers of esophageal cancer, to systematically identify and evaluate all candidate somatic and germline DNA sequence markers of outcome (survival, recurrence, therapy response and treatment complications) and stage. We then performed meta-analysis for all markers with a nominally statistical association in at least one study.

methods

inclusion criteria

Studies eligible were those testing association between a DNA sequence marker (germline or somatic) and outcome (clinical, radiological or pathological) or stage of esophageal/GEJ cancer. Markers included germline SNPs, tumor mutations, copy number variants (CNVs), loss of heterozygosity (LOH), microsatellite instability (MSI) and chromosomal instability (CIN; alterations in ploidy). Clinical outcomes comprised survival (any measure), recurrence, disease progression and treatment complications. Radiological outcomes comprised response to therapy. Histopathological outcomes comprised tumor response and incomplete resection. Stage comprised radiological TNM staging and pathological tumor grading [21].

exclusion criteria

Studies using cell lines or expression data were excluded unless discrete tumor or DNA-specific data could be extracted. Non-English articles were excluded.

literature search

A search was performed on 20 August 2014 of the PubMed, EMBASE and Cochrane databases, in accordance with MOOSE (Meta-analysis Of Observational Studies in Epidemiology) and PRISMA guidelines [22]. The following term was used: (esophageal OR esophagus OR gastroesophageal) AND (cancer OR carcinoma or adenocarcinoma OR SCC) AND (genomic OR genetic OR genome OR pharmacogenetic OR pharmacogenomic OR amplification OR copy OR mutation OR polymorphism OR polymorphic OR variant OR deletion OR insertion OR locus OR loci OR allele) AND (outcome OR prognosis OR survival OR response OR stage OR surgery OR chemotherapy OR radiotherapy OR marker OR biomarker OR complication). The references cited by retrieved articles were also assessed for relevant articles.

study data

Data extracted were: methodology; the variant(s) and gene(s) assessed; outcome measures and population. Extraction was carried out independently by two authors (JMF and IT). Gene names were standardized (HUGO Gene Nomenclature Committee) [23]. Variants were mapped to reference SNP (rs) identification numbers (US National Library of Medicine dbSNP database; http://www.ncbi.nlm.nih.gov/snp) when not provided by searching referenced methodology, *in vitro* polymerase chain reaction (http://genome.ucsc.edu) with specialized SNP flank BLAST® (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov), or New England Biocutter v2.0 (NEBcutter; http://tools.neb.com/NEBcutter2). Gene function was classified using the US National Library of Medicine Gene Database (http://www.ncbi.nlm.nih.gov/gene). For all reported associations, it was determined whether statistical significance persisted following correction for multiple comparisons (Bonferroni or false discovery rate correction), or multivariate analysis of all variables including genotypes. Were none made, *post hoc* Bonferroni correction was carried out [24]. For genome-wide association studies, significance was assumed at $P < 5 \times 10^{-8}$. For reported markers assessed by a single study, for which P was <0.05 but >corrected α, effect metrics were calculated but the marker excluded and presented in supplementary Tables S1 and S2, available at *Annals of Oncology* online. Those assessed by more than one study underwent meta-analysis irrespective.

evidence quality

Quality was appraised using the revised American Society of Clinical Oncology Level of Evidence (LOE) scale for biomarker research [25].

meta-analysis

Meta-analysis was carried out for all markers with a statistically significant association (uncorrected $P < 0.05$) reported by at least one study. For SNPs, analysis was carried out using the major common allele as reference, using genotype permutations shared by all studies. In the case of A/T and C/G substitutions, the minor variant was confirmed from study allele frequencies. Where possible, separate analyses were carried out for major methodological differences such as adjusted/unadjusted hazard ratios (HRs), genotyping methods, treatment, cell type and ethnicity (as determined by the International HapMap Consortium) [26]. Natural logarithms of HR, odds ratios (ORs) and standard errors (SEs) were extracted. In studies not presenting these, these were estimated using the methods of Parmar, or extracted from magnified Kaplan–Meier survival curves: HR and SE were estimated at constant time points; censoring was assumed to be constant and starting from the minimal follow-up period, with censored patients allocated to the appropriate time interval [27]. In six meta-analyzed studies (all nonsignificant results) [28–33], it was not possible to extrapolate statistics for all variants; an lnHR of 0 (a HR of 1) and SE of the most closely matched study (regarding cell type, size and methodology) were used to minimize selection bias. When not presented, ORs were calculated from available data. Meta-analysis was carried out using RevMan v5.2 (Copenhagen: the Nordic Cochrane Centre, The Cochrane Collaboration).

study heterogeneity and bias

Heterogeneity was quantified using I^2 and χ^2 estimates; for moderate heterogeneity ($I^2 \geq 50\%$) random rather than fixed-effects models were used. Heterogeneity and bias were also assessed by funnel plot asymmetry; [34] visually for all analyses, and statistically for analyses involving at least 10 studies [34, 35] using Begg's and Egger's tests. Statistical significance was

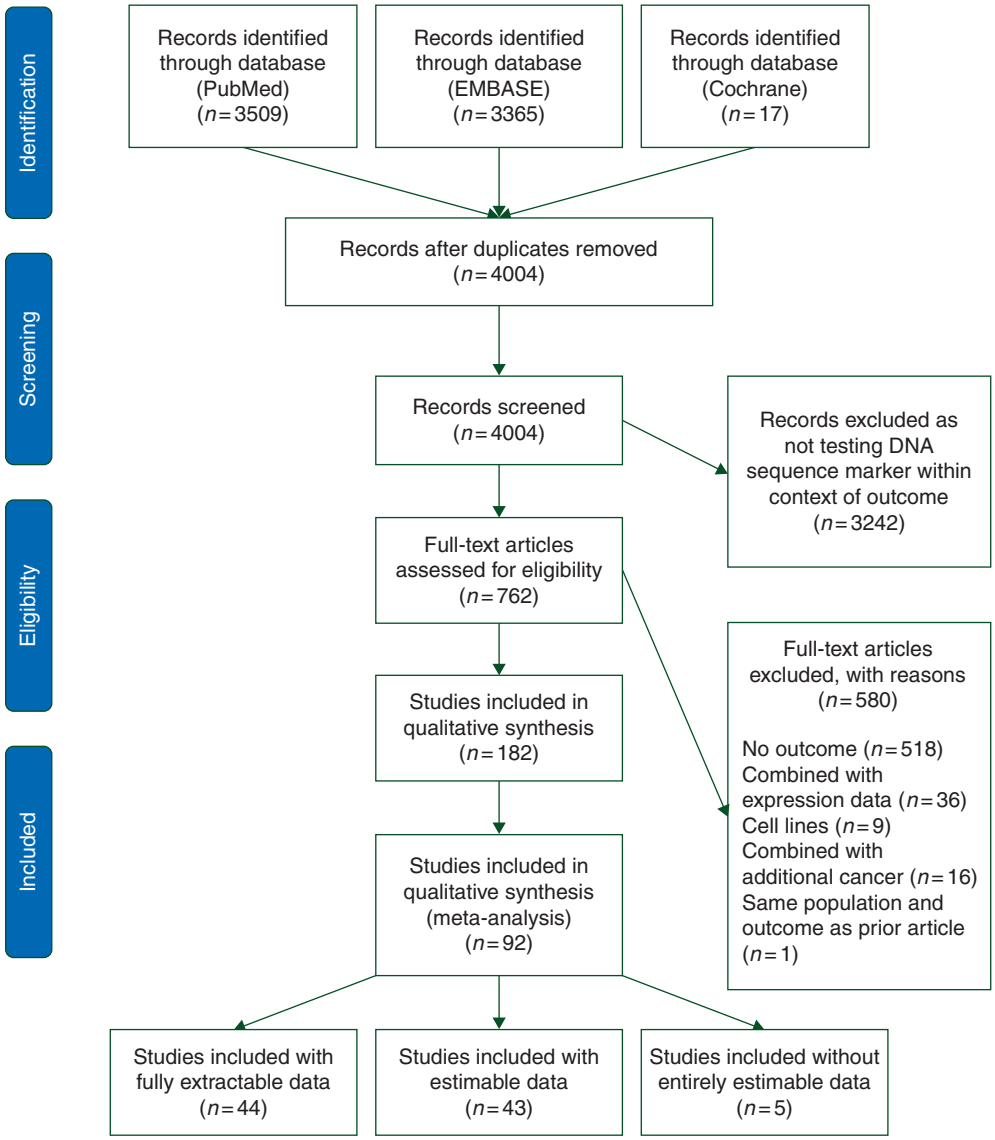

Figure 1. PRISMA flow diagram.

assumed at $P < 0.05$. Following consideration of alternative causes, probable publication bias was corrected using the 'trim and fill' method [36]. All other analysis was carried out using R (v3.0.2) [37]. Sensitivity analyses were carried out for all analyses including five studies or more, whereby studies were omitted one by one.

results

study characteristics

Four thousand and four articles were identified, 762 retrieved for evaluation, 580 excluded (Figure 1) and 184 included (supplementary Tables S3–S25, available at *Annals of Oncology* online), published between 1989 and 2014. Seventy-three assessed markers of clinical outcome, 80 clinical outcome and stage and 29 stage alone. Survival measures were overall survival (OS; $n = 133$), disease-free survival (DFS; $n = 20$), recurrence ($n = 19$), progression-free survival (PFS; $n = 4$) and disease-specific survival (DSS; $n = 4$). Twenty nine studies assessed response to therapy (chemo ± radiotherapy, or biological). Eleven assessed treatment complications. Treatment intent was curative ($n = 156$), palliative ($n = 5$), mixed ($n = 21$) and unspecified in 1. Curative modalities were resection alone ($n = 111$), resection ± neoadjuvant ($n = 33$) or adjuvant ($n = 2$) therapy, or definitive chemoradiotherapy ($n = 9$). All chemotherapy regimens involved platinum agents with or without 5-fluorouracil, except three (bleomycin, gefitinib, irinotecan). One hundred and seventy-six studies were candidate based, and 6 genome-wide (1 SNP, 5 CNV). One hundred and seventeen studies assessed tumor variants, 64 germline and 1 both. Cell types assessed were SCC ($n = 117$), adenocarcinoma (AC) ($n = 40$), both ($n = 3$) and unspecified ($n = 22$).

methodological quality

LOE was B for five studies (2.75%), C for 104 (57.1%) and D for 73 (40.1%). Median number of subjects was 90 (range 10–2932),

although 48 studies included fewer than 50. Forty-six (25.3%) studies were prospective; 135 (74.7%) were retrospective; 1 (0.55%) had both components. Multivariate adjustment of effect sizes was carried out by 57 studies (31.3%).

molecular quality

Just 37 (56.3%) of 65 studies assessing germline variants assessed Hardy–Weinberg equilibrium; 59 (90.8%) reported genotyping success rate (or provided data allowing its calculation).

markers of survival and recurrence

There were 65 reported markers of survival or recurrence: 24 tumor (Table 1; 3 mutations, 16 CNV, 2 LOH regions, 1 telomere length ratio, CIN, heterogeneous ploidy) and 40 germline polymorphisms (Table 2).

tumor mutations

Three mutant genes were reported to be associated with outcome: *TP53*, *PIK3CA* and *NRF2* (LOE IV). *TP53* ($n = 21$) and PIK3CA ($n = 3$, SCC) underwent meta-analysis.

TP53 status was variably defined and genotyped, although all studies assessed exons 5–8 as a minimum, by single-strand conformation polymorphism (SSCP) analysis with or without sequencing ($n = 13$), or Sanger sequencing alone ($n = 8$). Following correction for likely publication bias, a significant negative survival association was demonstrated for mutant *TP53* tumors: HR 1.27 (1.01–1.59; $P = 0.04$; $n = 21$ studies; supplementary Figures S1 and S2, available at *Annals of Oncology* online). Significant associations were demonstrated on subgroup meta-analysis of: genotyping technique (SCCP), AC and SCC cell types, and treatment (resection only). Directions of effect were consistent but nonsignificant for adjusted HR alone ($n = 6$) and use of neoadjuvant chemoradiotherapy ($n = 7$).

An association with DFS was demonstrated for mutant *PIK3CA* SCC tumors [HR 0.42 (0.21–0.85); $n = 2$; $P = 0.02$], but not OS.

tumor copy number variants

Sixteen tumor CNVs had previously reported associations with prognosis; three were excluded due multiple comparisons (supplementary Table S1, available at *Annals of Oncology* online). For the remaining 13, LOE was II (1), III (7) and IV (5). Four markers underwent meta-analysis: associations with worse survival were demonstrated for gains in *ERBB2 (HER2*; LOE III), *CCND1* (LOE III) and *FGF3* (LOE IV), but not *EGFR* (LOE II). For all four, there was heterogeneity regarding definition of CNV (absolute copy numbers, or ratio to normal), and genotyping technique [fluorescent/silver *in situ* hybridization (F/SISH), quantitative (q)PCR and slot/southern blot].

ERBB2/*HER2* analysis was restricted to 11 studies performing ISH; 3 (using qPCR [28], or slot [75]/southern [74] blot) were excluded. Worse OS was demonstrated for *ERBB2/HER2* gain overall: HR 1.63 (1.20–2.21; $P = 2 \times 10^{-4}$, $n = 11$). Significance persisted for adjusted HR [2.32 (1.64–2.58); $P < 1 \times 10^{-5}$; $n = 3$], and cell type (SCC; AC $P = 0.06$). Treatment regimens were resection alone for all studies, except one [61] including mixed regimens involving the c-MET-GFR inhibitor Crizotinib.

Two meta-analyses were carried out for *CCND1*: studies using qPCR ($n = 4$), and slot blot/FISH ($n = 2$). Worse OS was demonstrated for qPCR [HR = 2.09 (1.27–3.42); $P = 0.004$], with a concordant nonsignificant trend for FISH/blot. All four studies assessing *FGF3* used slot/southern blot; an association with worse OS was demonstrated [HR 1.83 (1.18–2.83); $P = 0.006$]. *EGFR* meta-analysis was carried out for FISH and blot techniques (excluding two studies using anti-EGFR therapy [62, 63], and one performing qPCR) [28]. No associations were demonstrated.

loss of heterozygosity

LOH (six markers in total) was associated with outcome by four studies; one study (6p and 13q) was excluded due to multiple comparisons; [132] for another (2p, 3p and 12p), while 3-year survival rates were significant the extrapolated HR was not [86]. *1q22–23* LOH was associated with worse OS in one study (LOE IV).

telomere length ratio

One study reported worse OS with a tumor : normal telomere length >1.17 (LOE III).

genomic instability

CIN was assessed by six studies. Following exclusion of one study including intratumoral heterogeneous ploidy [93] an association with worse OS was demonstrated [HR 1.63 (1.25–2.11); $n = 4$; $P = 0.2 \times 10^{-4}$]. One study assessed intratumoral heterogeneity alone, reporting better survival than with homogeneity [94]. There were no associations between MSI and survival.

germline polymorphisms

Twenty-nine reported associations were identified (following exclusion of 12 due to multiple comparisons) [107, 109, 112, 133]. Cumulative LOE was II ($n = 3$), III ($n = 22$) and IV ($n = 4$). Fifteen variants underwent meta-analysis (Table 2). Significant associations were demonstrated for six SNPs: *ERCC1* rs3212986 (cisplatin treatment; LOE II; Caucasian ethnicity), ERCC2 rs1799793 (cisplatin; Caucasian) *TP53* rs1042522 (Caucasian), *MDM2* rs2279744 (Caucasian), *TYMS* rs34743033 (Japanese; LOE III) *ABCB1* rs1045642 (Caucasian and Japanese; LOE IV). An association was demonstrated for *VEGFA* rs2010963, but combining two studies with East Asian ethnicities (Taiwanese and Japanese). One association with recurrence was demonstrated: *MTHFR* rs1801133 (Caucasian; LOE III).

markers of treatment complications

Twelve reported germline associations (8 studies) were identified; 10 were excluded due to multiple comparisons. One marker (*TNFA* rs1800629) [134] underwent meta-analysis (nonsignificant). The remaining variant, *ACE* rs4646994 (LOE III), was associated with postoperative pulmonary complications by one study (Table 3).

markers of response to chemo(radio)therapy

Two tumor variants (mutant *TP53* and CIN) and 15 germline polymorphisms were reported to be associated with clinical or

Table 1. Reported tumor markers (mutations, copy number variants, and chromosomal instability) associated with survival and recurrence following treatment of esophageal cancer

LOE	Variant	Gene / function	Association – minor variant	Association – wild type	No association	Cell type	Population	LOE	Meta-HR [effect variant]	Chi	I^2	N	P
Mutations													
III	Exon mutant	*TP53*	OS and DFS Casson 2003 [38]			AC	Res	C	$OS^{(A)PB}$: 1.27 (1.01–1.59)	68.1	62	21	0.04
		Apoptotic / DNA repair	OS^{A} – Schneider 2000 [39]			AC	Res	C	$OS^{A\ PB}$: 1.19 (0.63–2.27)	34.5	80	6	0.590
		regulator	OS and DFS–Madani 2010 [40]			AC	Res	C	$AC^{(A)}$: 1.99 (1.44–2.81)	2.68	0	5	< 0.001
					OS – Puhringer 2006 [45]	AC	Res + /−NAC(RT) (CF)	B	$SCC^{(APB}$: 1.47 (1.24–1.73)	22.7	43	11	< 0.001
					OS – Soontrapornchai 1999 [46]	AC	Res	D	$US^{(A)PB}$: 0.77 (0.41–1.47)	21.3	72	5	0.440
			OS^{A} – Yamasaki 2010 [41]			SCC	Mixed	D					
			OS^{A} – Kunisaki 2006			SCC	Res + NAC	C	$DFS^{(A)}$: 2.67 (1.38–5.15)	7.44	73	3	0.003
			OS – Kobayashi 1999 [42]			SCC	Res+ NAC	D	Res $OS^{(A)PB}$: 1.35 (1.04–1.76)	27.0	56	13	0.030
					OS – Makino 2010 [47]	SCC	Res + NACRT (CF)	D	$NAC/R^{A(PB)}$: 1.23 (0.81–1.87)	32.5	66	7	0.320
			OS – Uchino 1996 [29]			SCC	Res	D					
					OS – Shimada 1997 [48]	SCC	Res	C	*SSCP analysis*				
					OS – Egashira 2011 [49]	SCC	Res	C	$OS^{(A)PB}$: 1.56 (1.33–1.82)	27.8	44	13	< 0.001
					OS – Ito 2001 [50]	SCC	DCRT	C	*Direct sequencing only*				
					OS – Lam 1997 [51]	SCC	Res	D	*$OS^{(A)PB}$: 0.96 (0.62–1.46)*	28.3	65	8	0.830
					OS – Shibagaki 1995 [52]	SCC	Res	C	*SCCP band only*				
					OS: Goan 2005 [53]	SCC	Res	D					
					OS: Cao 2004 [54]	SCC	Res	C					
					OS^{A} – Gibson 2003 [55]	US	Res + NACRT	C					
					OS^{A} – Coggi 1997 [56]	US	Res	D					
			OS and DFS – Ribeiro 1998 [43]			US	Res + NACRT (CF + IFN)	D					
			OS and DSS – Kandioler 2014 [44]			US	Res + NAC (CF)	C					
IV	Exon 9/20 mutation	*PIK3CA*		OS^{A} and DFS^{A} – Shigaki 2013 [58]	Rec – Shigaki 2013	SCC	Res + /−NACRT (CF + /−tax)	D	$OS^{(A)}$: 0.63 (0.26–1.56)	6.13	67	3	0.320
		Cell signalling kinase			OS^{A} and Rec^{Ex} – Wang 2014	SCC	Res + /−NACRT	D	$DFS^{(A)}$: 0.42 (0.21–0.85)	0.26	0	2	0.020
					OS^{Ex} and DFS^{Ex} – Hou 2014	SCC	Res	C	Rec^{Ex}: 0.64 (0.23–1.75)	2.81	64	2	0.390
IV	Exon mutation	*NRF2/BIRC2*	OS and Rec – Shibata 2011			SCC	Resection + NACRT (F) (Japan)	D	OS^{Ex}: 3.54 (1.60–7.88)	NA	NA	1	0.005
		Transcription factor							Rec—NP				0.046
Copy number variants													
II	Gain	*EGFR*	OS – Marx 2010 [59]			AC	Res	D	*Gain assessed by FISH/CISH*				
		Epidermal growth factor	OS – Kitagawa 1996 [60]			SCC	Res	D	OS: 2.43 (0.75–7.84)	23.9	92	3	0.140
		receptor			OS – Miller 2003 [28]	AC	Res	C					
					OS – Janmaat 2006 [63]	SCC	Palliative gefitinib	B	*Gain assessed by slot/Southern*				
					OS Rec – Chikuba 1995 [30]	SCC	Res + ACRT (C)	D	*blot*	18.1	89	3	0.320
					OS –Itakura 1994 [31]	SCC	Res	D	OS: 1.63 (0.63–4.22)				
			OS – Lennerz 2011 [61]			US	Mixed	D					
				OS^{A} – Luber 2011 [62]		AC	PC (OLF + cetuximab)	B	Excluding Miller 2003 (qPCR)				
					OS – Sunpaweravong 2005 [64]	SCC	Res	D					
III	Gain	*ERBB2/HER2*											
		Epidermal growth factor	OS – Prins 2013 [65]			AC	Res	D	$OS^{(A)}$: 1.63 (1.20–2.21)	31.2	62	11	0.002
		receptor			DSS^{A}, OS^{A} – Yoon 2012 [71]	AC	Res	C	OS^{A}: 2.31 (1.64–3.24)	0.72	0	3	< 0.001
			DFS^{A} – Rauser 2007 [66]		OS^{A} – Rauser 2007 [66]	AC	Res	D	$OS^{(A)}$ (AC): 1.59 (0.99–2.56)	15.4	68	6	0.060
			OS^{A} – Brien 2000 [67]			AC	Res	D	$OS^{(A)}$ (SCC): 1.92 (1.12–3.29)	27.7	82	5	0.020
					OS – Thompson 2011 [72]	AC	Res	D	DFS^{A}: 2.1 (1.06–4.26)	NA	NA	1	0.033
					OS – Miller 2003 [28]	AC	Res	C					
					OS – König 2013 [73]	Both		D	*Gain assessed via*				

			OS – Zhan 2012 [68]		SCC		D	*FISH/SISH/IHC; Miller 2003,*				
			OS – Sato-Kuwabara 2009 [69]		SCC	Res	D	*Ideka 1996, Suzuki 1997*				
			OS^{A} – Mimura 2005 [70]		SCC	Res	D	*excluded*				
				OS – Sunpawerayong 2005 [64]	SCC	Res	C					
				OS – Suzuki 1997 [74]	SCC	Res	D					
				OS and DSS Ikeda 1996 [75]	SCC	Ress	D					
				OS – Lennerz 2011 [61]	US	Mixed	D					
III	Gain	*ERBB2/HER2*	DSS^{A}, OS^{A} – Yoon 2012 [71]		AC	Res	C	OS: 2.02 (1.09–3.74)	NA	NA	1	0.026
		Epidermal growth factor receptor	(heterogeneous amplification)					DSS: 2.04 (1.09–3.79)	NA	NA	1	0.025
III	Gain	*CCND1*	OS^{A}: Wang 2012b [76]		SCC	Res	C	*Gain assessed by qPCR only*				
		Cell cycle kinase	OS^{A} – Miller 2003 [28]		AC	Res	C	OS^{A}: 2.09 (1.27–3.42)	3.17	5	4	0.004
			OS^{A} – Takeshita 2010 [77]		SCC	Res	C	*Gain assessed by FISH/IHC*				
				OS–Sunpawerayong 2005 [64]	SCC	Res	D	OS^{A}: 1.54 (0.93–2.57)	3.94	75	2	0.100
				OS – Gramlich 1994 [80]	SCC	Res	C	*Gain assessed by slot blot*				
			OS – Shimada 1997 [48]		SCC	Res	D	OS: 4.29 (2.47–7.45)	NA	NA	1	< 0.001
			OS – Shinozaki 1996 [78]		SCC	Res	D					
			Rec^{A} – Komatsu 2014 [79] (ctDNA)		SCC	Res	C					
III	Gain	*1p36.32*	OS^{A} – Carneiro 2008 [81]		SCC	Res	C	OS^{A}: HR 19.6 (2.5–153.9)	NA	NA	1	0.005
III	Gain	*19p13.3*	OS^{A} – Carneiro 2008 [81]		SCC	Res	C	OS^{A}: HR 7.0 (1.5–31.9)	NA	NA	1	0.011
III	Gain	*MDM2*	OS – Shibagaki 1995 [52]		SCC	Res	C	OS: HR^{Ex} 3.82 (1.81–8.07)	NA	NA	1	$5.3x10^{-3}$
		Ubiquitin ligase										
IV	Gain	*FGF3/INT2*		OS – Mori 1992 [82]	AC	Res	D	OS: ^{PB}HR 1.83 (1.18–2.83)	5.65	29	4	0.006
		Fibroblast growth factor	OS – Ikeda 1996 [75]		SCC	Res	D	PB – Corrected for Ikeda 1996				
				OS – Shimada 1997 [48]	SCC	Res	D					
				OS – Suzuki 1997 [74]	SCC	Res	D					
IV	Gain	*FGF4/HST1*	OS – Chikuba 1995 [30]	Rec – Chikuba 1995 [30]	SCC	Res + ACRT (C)	D	Median survival different but:	NA	NA	1	>0.05
		Fibroblast growth factor			SCC	Res	D	OS: HR^{Ex} 1.4 (0.86–2.30) Rec: HR^{Ex} 1.09 (0.659–1.80)				
IV	Gain	*TERC*	OS – Wang 2013 [83]		SCC	Res	D	OS: HR^{Ex} 7.87 (3.32–18.7)	NA	NA	1	0.010
		Telomerase										
IV	Gain	*MET*	OS – Lennerz 2011 [61]		US	Mixed	D	OS: HR^{Ex} 3.72 (2.56–5.39)	NA	NA	1	< 0.001
		Growth factor										
IV	Gain	*CPT1A*	OS^{A} – Shi 2011 [84]		SCC	Res	D	OS^{A}: 4.39 (1.34–14.14)	NA	NA	1	0.015
		Mitochondrial oxidation										
Telomere length												
III		Telomere length ratio (>1.17)	OS^{A} – Gertler 2008 [85]		AC	Res	C	OS: HR^{A} 3.40 (1.3–8.9)	NA	NA	1	< 0.02
LOH												
III		LOH at one of 2p, 3p, 17p	OS – Ikeguchi 1999 [86]		SCC	Res	C	2 loci: OS (3yr) 48% versus 75% HR^{Ex}: 1.81 (0.53–6.25)	NA	NA	1	0.048 > 0.05
IV		LOH 1q21-23	OS – Maru 2009 [87]		AC	Res	D	HR^{Ex}: 3.90 (1.13–13.5)	NA	NA	1	0.030

Continued

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Table 1. *Continued*

LOE	Variant	Gene / function	Association – minor variant	Association – wild type	No association	Cell type	Population	LOE	Meta-HR [effect variant]	Chi	I^2	N	P
Chromosomal instability													
III		Aneuploid / polyploidy	OS – Doki 1993 [88]			SCC	Res	D	$^{PB}OS^{(A)}$*: 1.63 (1.25–2.11)	2.99	25	4	$2x10^{-4}$
			Rec – Tsutsui 1992 [89]			SCC	Res	D	Rec: 5.41 (0.87–33.8)	2.11	53	2	0.070
			Rec – Kaketani 1989 [90]			SCC	Res	D					
			OS – Ohno 1989 [91]			SCC	Res + NACRT	C					
			OS – Kuwano 1995 [92]			SCC	Res	C					
					OS^{A} – Wang 1999 [32]	SCC	Res	D					
					OS Edwards 1989 [93]	SCC	Res	D					
IV		Ploidy heterogeneity		(Homogeneity)	DFS- Deguchi 1993 [94]	SCC	Res	D	OS: 0.10 (0.03–0.36)	NA	NA	1	< 0.05
				OS – Deguchi 1993 [94]					DFS: 0.34 (0.08–1.42)	NA			

OS = overall survival; DFS = disease-free survival; rec = recurrence; A = adjusted; (A) = including adjusted; PB = adjusted for publication bias; Ex = extrapolated; NP = not presented; AC = adenocarcinoma; SCC = squamous cell carcinoma; US = unspecified; NAC = neoadjuvant chemotherapy; NACRT = neoadjuvant chemoradiotherapy; DCRT = definitive chemoradiotherapy; PC = palliative chemotherapy; Res = resection; C = cisplatin; F = 5FU; CFL = cisplatin-5FU-leucovorin; OFL = cisplatin-5FU-leucovorin;; tax = taxane; SSCP = single strand conformation polymorphism; ctDNA = circulating tumor DNA; NA = not applicable

pathological response to chemo ± radiotherapy (Table 3); 11 polymorphisms were excluded due to multiple comparisons).

Mutant *TP53* was assessed by six studies; three for pathological and three for clinical response. A lower OR of pathological response was demonstrated [OR 0.24 (0.06–0.95), $n = 3$, $P = 0.04$]; effect direction for clinical response was concordant but non-significant [OR 0.43 (0.08–2.24); $P = 0.32$].

Following meta-analysis, two polymorphisms were associated with a major pathological response to platinum-based chemo/radiotherapy in Caucasians: wild-type *XRCC1* rs25487 [GG genotype, LOE III; OR 1.91 (1.30–2.81), $n = 3$, $P = 0.001$], and variant *ERCC1* rs11615 [TT/CT; OR 4.57 (3.01–6.94); $n = 3$; $P < 1 \times 10^{-5}$]. The AA variant of *ERCC1* rs3212986 (LOE III) was associated with radiological response to palliative cisplatin-based chemotherapy in one study (Chinese ethnicity), but not major pathological response to neoadjuvant chemotherapy in two (Caucasian).

markers of stage

Twenty-four tumor markers were reported: 2 mutations, 12 CNV, 7 LOH, 2 MSI and CIN; 15 were excluded.

tumor mutations

Following exclusion of *PIK3CA*, the sole tumor mutation with a reported association was *TP53* ($n = 19$; LOE III; Table 4). Following meta-analysis, mutant *TP53* tumors were associated with more advanced T (T3/T4) and N (≥N1) stages, but not overall TNM stage (III/IV), grade (G3/4) or positive resection margin (R1).

copy number variants

Twelve tumor CNVs were identified; 8 were excluded due to multiple comparisons (Table 4). Meta-analysis was possible for two markers, with one significant association demonstrated: *EGFR* (T1/2 stage; LOE IV).

loss of heterozygosity

Seven LOH variants were identified; five were excluded (Table 4). Meta-analysis was possible for one marker. A significant association was demonstrated for LOH *13p* and ≥N1 stage (Table 4).

genomic instability

Following meta-analysis CIN (LOE III; Table 4) was associated with overall stage [III/IV: OR 2.68 (1.10–6.54); $P = 0.03$; $n = 2$ studies] and nodal stage [OR 2.18 (1.06–4.47); $P = 0.03$; $n = 7$ studies], but not T stage or grade. MSI (using the 5 Bethesda markers, and 10 at 17q24–25) was associated with more advanced stage; another measure was excluded.

germline polymorphisms

Seventeen polymorphisms were identified; 12 were excluded due to multiple comparisons. One marker (*GNAS1* rs7172) underwent meta-analysis, without significance.

Table 2. Reported germline markers (polymorphisms) associated with survival and recurrence following treatment of esophageal cancer

LOE	SNP (major/minor allele)	Gene / function	Association–minor variant	Association–wild type	Non-significant	Cell type	Population		Meta-HR [variant allele / genotype] versus wild-type' [Ethnicity]	Chi	I^2	N	P
II	rs3212986 (C/A)	*ERCC1* *DNA NER*		OS^A–Bradbury 2009b [95] DFS^A–Wang 2011 [96] OS^A–Rumiato 2013 [97]		US SCC US US	Res + NAC (CF) PC (CF) Res +/− NAC (CF) Res + NACRT (CF)	B C C	$OS^{(A/Ex)}$: 0.63 (0.42–0.93) [TT/CT, cisplatin, Caucasian]	4.12	51	3	0.02
					OS–Warnecke 2009 [33]			C	$DFS^{(A)}$: 1.98 (1.19–3.03) [AA/CA + cis,,Chinese]	NA	NA	1	0.001
II	rs1799793 (G/A)	*ERCC2* *DNA NER*		OS^A–Bradbury 2009b [95]		US	Res + NAC (CF)	B	$OS^{(A/Ex)}$: 0.71 (0.54–0.94)	3.88	48	3	0.020
					OS and Rec – Ott 2011 [98]	AC	Res + NAC (C/OF +/−tax)	B	[GA/AA; cisplatin; Caucasian]				
					OS–Rumiato 2013 [97]	US	Res +/−NAC (CF)	C	DFS: 0.33 (0.20–0.07) [AA]	NA	NA	1	0.002
II	rs13181 (T/G)	*ERCC2* *DNA NER*		OS^A and DFS^A–Bradbury 2009b [95]		US	Res + NAC (CF)	B	$OS^{(A/Ex)}$: 0.82 (0.65–1.05)	5.14	42	4	0.110
					OS and Rec - Ott 2011 [98]	AC	Res + NAC (C/OF +/−tax)	B	[TG/GG, Caucasian]				
					OS–Rumiato 2013 [97]	US	Res +/−NAC (CF)	C	DFS^A: 0.32 (0.20–0.60)	NA	NA	1	0.002
					OS^A, Rec^A–Wu 2006	US	Res + NACRT (CF +/−tax)	D	Rec^A: 0.94 (0.30–2.81)	0.03	0	2	0.91
III	rs11614913	*MIR196A2* *Micro RNA*		OS^A–Wu 2014 [99]		SCC	PC	C	OS^A: 1.26 (0.72–2.21)	2.17	54	2	0.420
								C	[TT; Chinese/Taiwanese]				
					OS^A–Yang 2014b [100]	SCC	Mixed		PFS^A: 1.01 (0.54–1.88)	NA	NA	1	0.972
III	CA-SSR-1 (DNR)	*EGFR* *Epidermal growth factor receptor*	OS and Rec–Vashist 2014 [101]			Both	Res	C	OS (AC): 1.70 (1.20–2.80) [LL; Caucasian]	NA	NA	1	0.010
									Rec (AC): 2.70 (1.70–4.30)				< 0.001
									OS (SCC): 3.50 (2.10–6.00)				< 0.001
									Rec (SCC): 2.50 (1.30–4.80)				0.005
III	rs1800796 (C/G)	*IL6* *Interleukin*	OS–Motoyama 2012b [102]		DSS–Motoyama 2012b [102]	SCC	Res +/− AC	C	OS: HR^A 3.40 (CI NP) [GG/GC; Japanese]	NA	NA	1	$5.9x10^{-3}$
									OS: HR^{AEx} 3.49 (1.28–9.45)				< 0.05
III	rs238406 (G/T)	*ERCC2* *NER repair*		OS^A and DFS^A Lee 2011 [103]		SCC	Res + NACRT (CF/C + tax)	C	OS^A: 0.61 (0.40–0.93) [CC; Taiwanese]	NA	NA	1	0.02
									DFS^A: 0.57 (0.38–0.85)				0.007
III	rs1800975 (G/A)	*XPA* *DNA NER repair*	OS^A–Yang 2013 [104]		DFS–Yang 2013 [104]	SCC	Mixed	C	OS^A: 1.36 (1.06–1.76) [AG; [Taiwanese]	NA	NA	1	0.014
									DFS: 1.20 (0.95–1.51) [AG]				0.126
III	rs34743033 (STR 2/3/4)	*TYMS* *DNA repair/ replication*	OS^A–Kaneko 2011 [105]			SCC	DCRT (CF)	C	OS^A: 1.54 (1.00–2.38)	5.78	65	3	0.61
					OS–Okuno 2007 [106]	SCC	Res + NACRT (CF)	C	[≥2/3; Caucasian/Japanese]				
					OS–Sarbia 2006 [107]	SCC	Res + NACRT (CF + E)	C	$OS^{(A)}$: 2.47(1.20, 5.06) [Japanese]	0.45	0	2	0.010
					OS–Rumiato 2013	US	Res + NAC (CF)	C	OS^A: 1.18 (0.69, 2.03) [Caucasian]	0.18	0	2	0.550

Continued

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Table 2. *Continued*

LOE	SNP (major/minor allele)	Gene / function	Association–minor variant	Association–wild type	Non-significant	Cell type	Population		Meta-HR [variant allele / genotype] versus wild-type' [Ethnicity]	Chi	I^2	N	P
III	rs2279744 (T/G)	*MDM2* *Ubiquitin ligase*		DFS^{A} Boonstra 2011 [109]		Both	Res	D	(SCC) DFS^{A}: 2.78 (0.24–31.9) [GG; Caucasian]	9.36	89	2	0.410
				OS^{A} and DFS^{A} Cescon 2009 [110]		Both	Mixed	C	(AC) DFS^{A}: 0.92 (0.65–1.29) [GG]				
			OS^{A}–Renouf 2013 [108]			AC			(AC) OS^{A}: 2.01 (1.38–2.95)	0.04	0	2	0.620
									(SCC) OS^{A}: 7.89 (2.40–26.0)	0.48	0	2	< 0.001
										NA	NA	1	< 0.001
III	rs2273535 (A/T)	*AURKA* *Cell cycle kinase*	OS and DFS^{A}–Pan 2012 [111]			US	Res + NACRT (CF + tax)	C	OS^{Ex}: 0.30 (0.10–0.92) [TT; Caucasian]	NA	NA	1	< 0.05
					DFS^{A}–Boonstra 2011 [109]	Both	Res	D	DFS^{A}: 0.55 (0.17–1.74)	2.92	66	2	0.310
III	rs2010963 (G/C)	*VEGFA* *Epithelial mitogen*		OS–Tamura 2012 [112]		SCC	DCRT (CF)	C	OS HR$^{(Ex)}$: 0.68 (0.50–0.92) [CC]	0.16	0	2	0.01
				OS - Yang 2014 [113]		SCC	Res +/−NACRT (CF)						
III	rs3025039 (C/T)	*VEGFA Epithelial mitogen*	DFS^{A}–Lorenzen 2011 [114]			AC	Res + NAC (CF)	C	OS$^{(A)}$: 0.75 (0.54–1.03) [CT; Caucasian/Japanese]	0.79	0	2	0.080
				OS^{A}–Bradbury 2009 [115]		AC	Mixed	C	DFS^{A}: 1.8 (1.04–3.09) [CT/TT; Caucasian]				
					OS–Tamura 2012 [112]	SCC	DCRT (CF)	C		NA	NA	1	0.040
III	rs1042522 (C/G)	*TP53* *Apoptotic / DNA repair regulator*	OS^{A} and PFS^{A}–Renouf 2013 [108]			AC	Mixed	C	OS^{A}: 1,84 (1.34–2.53) [GG; Caucasian]	0.44	0	3	< 0.001
			OS^{A}, DFS^{A}–Cescon 2009 [110]			AC	Mixed	C	DFS^{A}: 2.03 (1.29–3.18) [GG]	NA	NA	1	0.002
					OS^{A}, Rec^{A}–Wu 2006	US	Res + NACRT (CF +/−tax)	D	Rec^{A}: 1.29 (0.24–7.14) [GG]	NA	NA	1	> 0.05
III	rs2069762 (A/C)	*IL2* *Cytokine*	DSS^{A}–Motoyama 2011 [116]			SCC	Res	C	DSS: 3.54 1(1.69–7.39) [C; Japanese]	NA	NA	1	0.0231
									DSS^{A}: 3.36 (NA)				0.0136
III	rs1800471 (C/G)	*TGFB1* *Growth factor regulator*	OS–Tang 2013 [117]			SCC	Mixed	C	OS: 3.51 (2.18–5.67) [CG/GG; Chinese]	NA	NA	1	< 0.001
III	rs1050631 (G/A)	*SLC39A6* *Zinc transporter*	OS^{A}–Wu 2013 [118]			SCC	Mixed	C	OS^{A}: 1.3 (1.19–1.43) [AA; Chinese]	NA	NA	1	3.77×10^{-8}
III	rs41458645 (C/T)	Mitochondrial D loop	OS^{A}–Zhang 2010 [119]			SCC	Mixed	C	OS^{A}: 3.00 (1.03–8.76) [CT; Chinese]	NA	NA	1	0.044
III	rs139001869 (A/G)	Mitochondrial D loop	OS^{A}–Zhang 2010 [119]			SCC	Mixed	C	OS^{A}: 3.48 (1.07–11.36) [AG; Chinese]	NA	NA	1	0.039
III	rs3769818 (G/A)	*CASP8* *Caspase*	OS^{A} –Umar 2011 [120]			SCC	Mixed	C	OS^{A}: 3.36 (1.07–10.61) [AA; Indian]	NA	NA	1	0.039
III	rs1695 (A/G)	*GSTP1* *Detoxification enxyme*	OS^{A}–Lee 2005 [121]			SCC	Res + NAC (C + F/tax)	C	OS^{A}: 1.29 (1.03–1.61) [TG/GG; Caucasian/ Taiwanese/Japanese]	2.36	0	5	0.030
					OS–Okuno 2007 [106]	SCC	Res + NACRT (CF)	C	OS^{A} 1.15 (0.78–1.70)				
					OS–Warnecke 2009 [33]	US	Res + NACRT (CF)	C	[TG/GG; Caucasian]	1.84	0	3	0.490
					OS^{A} and Rec^{A} – Wu 2006 [122]	US	Res + NACRT (CF +/−tax)	D	Rec^{A}: 0.50 (0.16–1.58) [Caucasian]				
					OS^{A} – Rumiato 2013	US	Res + NAC (CF)	C		NA	NA	1	> 0.05
III	rs72214039 (CA ins)	*EGFR* *Epidermal growth factor receptor*	OS^{A}–Lee 2011b [123]			SCC	Res NACRT (CF/ C + tax)	C	OS^{A}: 1.88 (1.02–3.49) [Short/Short; Taiwanese]	NA	NA	1	0.045
III	rs7121 (T/C)	*GNAS* *G protein subunit*		OS^{A} – Alakus 2014 [124]		US	Res	D	OS^{A}: 0.73 (0.46–1.16) [CC; Caucasian]	9.08	67	3	0.180
				OS^{A} and DFS^{A}–Vashist 2011 [125]		US	Res	C	DFS^{A}: 0.55 (0.34–0.89				
					OS – Alakus 2009 [126]	US	Res + NACRT (CF)	B		NA	NA	1	2.50×10^{-3}

III	rs111509018 (STR)	*ECRG2/SPINK7* *Serpin-inhibitor*	OS^A and DFS^A Kaifi 2007 [127]			US	Res	C	OS^A: 2.56 (1.53–4.29) [TCA_4/TCA_4; Caucasian] DFS^A: 2.30 (1.37–3.87)	NA	NA	1	< 0.001
												1	< 0.001
III	rs9344 (G/A)	*CCND1* *Cell cycle kinase*	OS^A–Izzo 2007 [57]			AC	Res	D	OS^A: 3.48 (1.94–6.23) [AA]	NA	NA	1	< 0.001
IV	rs1801133 (G/A)	*MTHFR* *Folate metabolism*		Rec^A–Wu 2006 [122]	OS^A–Wu 2006 [122]	US	Res + NACRT (CF +/ −tax)	D	OS^A: 0.93 (0.67–1.29)[AA; Caucasian/Chinese/ Indian]	3.06	0	6	0.660
					OS^A and Rec^A Ott 2011 [98]	AC	Res + NAC +(C/OF +/ −tax)	B	OS^A: 0.92 (0.63–1.33) [Caucasian] Rec^A: 0.40 (0.21–0.78) [Caucasian]				
					OS – Lu 2011 [128]	SCC	Res only	C		0.69	0	4	0.640
					OS–Umar 2010 [129]	SCC	Mixed	C		1.54	35	2	0.007
					OS–Sarbia 2006 [107]	SCC	Res + NACRT (CF, E)	C					
					OS–Warnecke 2009 [33]	SCC	Res + NACRT (CF)	C					
IV	rs1801131 (A/C)	*MTHFR* *Folate metabolism*		OS^A–Wu 2006 [122]	Rec^A–Wu 2006 [122]	US	Res + NACRT (CF +/ −tax)	D	OS^A: 0.99 (0.64–1.55) [AA; Caucasian]	0.67	0	3	0.970
					OS and rec–Ott 2011 [98]	AC	Res + NAC (C/OF +/ −tax)	B	Rec^A: 0.80 (0.22–2.95)				
					OS–Warnecke 2009 [33]	SCC	Res + NACRT (CF)	C		2.38	54	2	0.740
IV	rs1045642 (C/T)	*ABCB1* *Drug efflux*			OS – Narumiya 2011 [130]	US	Res + NACRT (CF)	D	OS^A: 0.57 (0.37–0.87) [TT; Caucasian/Japanese]	1.87	0	4	0.009
					OS – Okuno 2007 [106]	SCC	Res + NACRT (CF)	C	OS^A: 0.51 (0.32–0.81) [Caucasian]				
					OS – Warnecke 2009 [33]	US	Res + NACRT (CF)	C	Rec: 0.26 (0.09–0.81)	0.68	0	3	0.004
				OS^A and Rec^A – Wu 2006 [122]		US	Res + NACRT (CF +/ −tax)	D		NA	NA	1	< 0.05
IV	rs11267092 (DEL/INS)	*F2R* *Angiogensis*	OS^A and Rec^A–Lurje 2011 [131]			AC	Res	D	OS^{Ex}: 1.70 (1.16–2.48) [INS/INS / INS/DEL; Caucasian]	NA	NA	1	< 0.001
									Rec: 2.41 (1.25–4.65) [INS/INS]			1	0.003

OS = overall survival; DFS = disease-free survival; rec = recurrence; A = adjusted; (A) = including adjusted; PB = adjusted for publication bias; Ex = extrapolated; NP = not presented; AC = adenocarcinoma; SCC = squamous cell carcinoma; US = unspecified; Res = resection; NAC = neoadjuvant chemotherapy; NACRT = neoadjuvantchemoradiotherapy; DCRT = definitive chemoradiotherapy; C/Cis = cisplatin; O = oxaliplatin; tax = taxane; F = 5FU; E = etoposide; Res = resection; Ex = Extraplotated; INS = insertion; DEL = deletion; NA = not applicable

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Table 3. Reported tumor variants and germline polymorphisms associated with treatment complications and response to chemo(radio)therapy

LOE	Variant	Gene	Association – mutant	Association – wild type	No association	Cell type	Population	LOE	Meta OR [effect allele / genotype / haplotype]	Chi	I^2	N	P
Treatment complications													
III	rs4646994	*ACE*	Post-op pulmonary^A Lee 2005b [135]			US	Res	C	OR^A: 3.12 (1.01–9.65)	NA	NA	1	0.049
	(INS/DEL)	*Vasodilator*							[DEL/DEL; Taiwanese]				
III	rs1800629	*TNFA*	Post-op infection^A – Azim 2007 [134]			US	Res +/− NAC(RT)	C	OR^A: 4.02 (0.00–18347)	0	0	2	0.750
	(G/A)	*Cytokine*			Motoyama 2009 [136]	SCC	Res	D	[GG; Caucasian/Japanese]				
Response to chemo(radio)therapy													
Tumor													
III	High DNA ploidy	-	mPR – HCR versus CR – Ohno 1989 [91]			SCC	Res + NACRT versus HNACRT (bleomycin)	C	mPR 13.18 (5.30–32.7) [High ploidy]	NA	NA	1	< 0.001
IV	Mutant (exon)	*TP53*		mPR – Ribeiro 1998 [43]		US	Res, NACRT (CF + IFN)	D	mPR 0.24 (0.06–0.95) [mutant]	2.87	30	3	0.040
		Apoptosis / DNA repair regulator		mCR –Yamasaki 2010 [41]		SCC	Res + NAC	D	mCR 0.43 (0.08–2.24)	14.2	86	3	0.320
				cPR – Makino 2010 [47]		SCC	Res + NACRT / DCRT (CF)	D					
				mCR – Kunisaki 2006		SCC	DCRT	C					
					cCR – Ito 2001 [50]	SCC	Res + NACRT (CF)	C					
					cCR – Gibson 2003 [55]	US	Res + NACRT (CF)	C					
Germline													
II	rs7121	*GNAS1*	mPR^A – Alakus 2009 [126]			US	Res + NACRT (CF)	B	mPR^A 7.25 (1.30–40.62)	NA	NA	1	< 0.05
	(T/C)	*G protein subunit*							[CC; Caucasian]				
III	rs3212986	*ERCC1*	mCR^A–Wang 2011 [96]			SCC	PC (CF)	C	mCR OR^A: 2.62 (1.11–6.23)	NA	NA	1	< 0.05
	(C/A)	*DNA NER*			mPR–Warnecke 2009 [33]	US	Res + NAC (CF)	B	[AA/CA; Chinese]	0.76	0	2	0.260
					mPR–Rumiato 2013 [97]	US	Res + NAC (CF)	C	mPR OR: 1.52 (0.73–3.20)				
									[CT/CC; Caucasian]				
III	rs11615	*ERCC1*	mPR–Metzger 2012 [137]			AC	Res + NACRT (CF)	D	mPR OR: 4.57 (3.01–6.94) [TT/	3.48	43	3	< 1x10^{-5}
	(A/G)	*DNA NER*	mPR–Warnecke 2009 [33]			US	Res + NACRT (CF)	C	CT; Caucasian]				
					mPR-Rumiato 2013 [97]	US	Res + NAC (CF)	C					
IV	rs25487	*XRCC1*	cPR–Wu 2006 [122]			US	Res + NACRT (CF +/−tax)	D	m/cPR 1.91 (1.30–2.81) [GG;	3.13	36	3	0.001
	(G/C)	*DNA repair*			cPR - Ott 2011 [98]	AC	Res + NAC (C/OF +/−tax))	B	Caucasian]				
					mPR–Warnecke 2009 [33]	US	Res + NACRT(CF)	C					

OS = overall survival; DFS = disease-free survival; rec = recurrence; A = adjusted; AC = adenocarcinoma; SCC = squamous cell carcinoma; US = unspecified; NAC = neoadjuvant chemotherapy; NACRT = neoadjuvantchemoradiotherapy; DCRT = definitive chemoradiotherapy; HNACRT = hyperthermicneoadjuvant chemoradiotherapy; PC = palliative chemotherapy; CF = cisplatin-5FU; DEL = deletion; OF = cisplatin-5FU; Res = resection mPR = major pathological response; cPR = complete pathological response; mCR = major clinical response; cCR = complete clinical response; OR = odds ratio; IFN = interferon; LOE = level of evidence; NA = not applicable

Table 4. Reported tumor markers (mutations, copy number variants, genomic and chromosomal instability) associated with stage of esophageal cancer

LOE	Variant	Gene	Association – mutant	No association	Cell type	Population	LOE	Meta OR [effect allele / genotype / haplotype]	Chi	I^2	N	P
Mutations												
III	Mutant	*TP53*	T, N – Madani 2010 [40]	R – Madani 2010 [40]	AC	Res	C	O^{PB}: 1.28 (0.71–2.31)	32.4	63	12	0.410
		Apoptosis / DNA repair	N – Cao 2004 [54]	T – Cao 2004 [54]	SCC	Res	D	T: 1.40 (1.12–1.74)	17.5	20	15	0.003
		regulator	N – Hattori 2003 [138]		SCC	Res	C	N: 1.39 (1.07–1.81)	18.1	17	15	0.010
			O Ribeiro 1998		US	Res + NACRT	D	M: 1.21 (0.72–2.03)	2.99	0	5	0.480
				O, T, N – Schneider 2000 [39]	AC	Res	C	G: 1.46 (0.83–2.58)	19.1	53	10	0.190
				T, N, G – Soontrapornchai 1999	AC	Mixed	D	R: 2.10 (0.470–9.35)	3.45	71	2	0.330
				T, N, G – Egashira 2011 [49]	SCC	Res	C					
				T, N, M, O – Yamasaki 2010 [41]	SCC	NAC (CF) +/– Res	D	O: B p = 0.540; E p = 0.275				
				T, M, G – Ito 2001 [50]	SCC	Res + NACRT (CF)	C	T: B p = 0.393; E p = 0.071				
				O – Kobayashi 1999 [42]	SCC	Res	D	N: B p = 0.765; E p = 0.443				
				T, N, M, G – Uchino 1996 [29]	SCC	Res	D	M: B p = 0.207; E = 0.492				
				O – Coggi 1997 [56]	US	Res	D	G: B p = 0.719; p = 0.543				
			O, T, N, G-Casson 2003 [38]		AC	Res	C					
				O, T, N – Goan 2005 [53]	SCC	Res	D	^{PB} Corrected for Ribeiro 1998				
				O, G – Lam 1997 [51]	SCC	Res	D					
				O, T, N – Shibagaki 1995 [52]	SCC	Res	C					
				T, N – Puhringer 2006 [45]	AC	Res +/– NACRT	B					
			N - Makino 2010 [47]	T, M, G – Makino 2010 [47]	SCC	(CF)	D					
						Res +/– NACRT /DCRT (CF)						
Copy number variants												
III	Gain	*SPK2*	N,O – Wang 2009 [139]		SCC	Res	C	O: 8.00 (2.25–28.5)	NA	NA	1	$1.30x10^{-3}$
		Protein kinase						N: 8.10 (2.28–28.8)			1	$1.20x10^{-2}$
III	Gain	*PRKC1*	T – Yang 2008 [140]	N, O, G – NS – Yang 2008 [140]	SCC	Res	C	O: 4.64 (1.71–12.4)	NA	NA	1	0.002
		Serpin						N: 3.12 (1.21–8.02)	NA	NA	1	0.019
								T: 2.63 (0.78–8.81)	NA	NA	1	0.118
III	Gain	*HER2 (ERBB2)*	T, G – Yoon 2012 [71]		AC	Res	C	O: 1.13 (0.83–1.54)	14.02	43	10	0.510
		Epidermal growth factor	G,O – Zhan 2012 [68]	T, M, L – Zhan 2012 [68]	SCC	Res	D	T: 0.84 (0.55–1.27)	17.5	48	10	0.400
		receptor	O, G – Lennerz 2011 [61]		US	Mixed	D	N^{PB}: 0.96 (0.69–1.35)	20.2	31	11	0.510
				O, T, N – Suzuki 1997 [74]	SCC	Res	C	M: 1.77 (0.69–4.56)	3.85	22	4	0.240
			N – Ikeda 1996 [75]		SCC	Res	D	G^{PB}: 0.61 (0.34–1.09)	40.0	72	10	0.100
				O, T, N, M, R, G – Reichelt 2007 [141]	US	Res	C					
				O, N - Brien 2000 [67]	AC	Res	D	^{PB} – Corrected for Ikeda 1996, Al-Kasspooles 1993, Suzuki 1997, Minmura 2005				
				O, N, G - Mimura 2005 [70]	SCC	Res	D	^{PB} – Corrected for Sunpaweravong 2005, Zhan 2012				
				O, T, N, G, R – Sato-Kuwabara 2009 [69]	SCC	Res	D					
				O, G – Sunpaweravong 2005 [64]	SCC	Res	D					
				O, T, N, G, M, R – Thompson 2011 [72]	AC	Res	D					
				O, T, N, G, M – Al-Kasspooles [142]	AC	Res	C					
				T, N, G – Prins 2013 [65]	AC	Res	D					
IV	Gain	*EGFR*	T,N – Marx 2010 [59]	M, G – Marx 2010 [59]	AC	Res	D	O^{PB}: 0.96 (0.6–1.53)	9.27	35	6	0.850
		Epidermal growth factor	G – Lennerz 2011 [61]	O – Lennerz 2011 [61]	US	Mixed	D	T: 0.51 (0.37–0.71)	2.31	0	5	< 0.001
		receptor	N – Kitagawa 1996 [60]	O, T – Kitagawa [60]	SCC	Res	D	N: 0.95 (0.41–2.16)	8.44	41	6	0.890
			N – Yang 2012 [143]	O,G – Yang 2012 [143]	SCC	Res	D	M: 0.91 (0.29–2.87)	1.38	0	3	0.870
				O, T,N,M – Miller 2003 [28]	AC	Res	C	G: 1.24 (0.70–2.20)	3.36	8	3	0.460
				O, T,N,M,G – Al-Kasspooles 1993 [142]	AC	Res	C					
				O,T,N,G – Itakura 1994 [31]	SCC	Res	D	PB – Corrected for Kitagawa 1996				

Continued

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Table 4. *Continued*

LOE	Variant	Gene	Association – mutant	No association	Cell type	Population	LOE	Meta OR [effect allele / genotype / haplotype]	Chi	I^2	*N*	*P*
Loss of heterozygosity												
III	LOH	*3p14.2*	T^A – Qin 2008 [144]	N, M, G – Qin 2008 [144]	SCC	Res	C	T: 5.67 (1.77–18.2)	NA	NA	1	0.003
III	LOH	*13q*	T, G – Huang 2002 [145]	N – Huang 2002 [145]	SCC	Res	C	T: 3.08 (0.17–60.8)	5.04	80	2	0.440
			N – Harada 1999 [146]	T,M,H,O – Harada 1999 [146]	SCC	Res	C	N: 4.17 (1.84–9.47)	3.06	35	3	$6x10^{-4}$
			N – Shibagaki 1994 [132]		SCC	Res	C					
Chromosomal instability												
III	CIN		T,N,G Yu 1989 [147]		SCC	Res	C	O: 2.68 (1.10–6.54)	2.01	0	2	0.030
			G – Doki 1993 [88]	T,/N – Doki 1993 [88]	SCC	Res	D	T: 1.41 (0.97–2.05)	7.21	0	9	0.070
			T,N - Kuwano 1995 [92]	N,V,G – Kuwano 1995 [92]	SCC	Res	C	N: 2.18 (1.06–4.47)	18.2	57	7	0.030
			N, Ohno 1989 [91]	O,T - Ohno 1989 [91]	SCC	Res +/– NACRT	C	G: 1.51 (0.99–2.31)	5.29	5	6	0.060
				T,N,G – Tsutsui 1992 [89]	SCC	Res	D					
				T,L,G – Edwards 1989 [93]	SCC	Res	D					
				O, T, N G – Wang 1999	SCC	Res +/– AR	D					
				T – Kaketani 1989 [90]	SCC	Res	D					
					SCC	Res	D					
Microsatellite instability												
IV	MSI	*17q24-25* + Bethesda markers	T – Matsumoto 2007 [148]	N,G,O – Matsumoto 2007 [148]	SCC	Res	D	T: 0.325 (0.11–0.96)	NA	NA	1	0.043

AC = adenocarcinoma; SCC = squamous cell carcinoma; US = unspecified carcinoma; NAC = neoadjuvant chemotherapy; NACRT = neoadjuvant chemoradiotherapy; CF = cisplatin and 5-fluoruracil; T = T stage (III/IV versus I/II); N = nodal stage(N0 versus ≥N1); M = metastatic stage (M0 versus M1); G = cell grade (III/IV versus I/II); O = overall stage (III/IV versus I/II); R = resection stage (R1 versus R0); L = L stage (L1 versus L0); V = venous invasion (V1 versus V0); Res = resection; PB = corrected for publication bias; LOE = level of evidence; LOH = Loss of Heterozygosity; CIN = chromosomal instability; NA = not applicable

Table 5. Reported germline markers (polymorphisms) associated with stage of esophageal cancer

LOE	Variant	Gene	Association—variant	Association—wild type	No association	Cell type	Population	LOE	Meta OR [effect allele/genotype/haplotype]	Chi	I^2	N	P
II	rs6573 (C/A)	*RAP1A* *RAS oncogene*		O^A—Wang 2012 [149]		SCC	Mixed	B	O^A: 1.89 (1.06–3.36) [CA/AA; Chinese]	NA	NA	1	0.030
II	rs1800471 (G/C)	*TGFB1* *Growth factor regulator*	O, G—Tang 2013 [117]			SCC	Mixed	B	O: OR^A 2.71 (1.44–5.09) [GC/CC; Chinese]	NA	NA	1	<0.001
									G: OR^A 2.65 (1.44–4.87)	NA	NA	1	0.002
III	rs353163 (T/C)	*TMPRSS11A* *Serine peptidase*	N—Umar 2013b [150]			SCC	RT/DCRT (CF)	C	N: 3.27 (1.68–6.39) [CC; Indian]	NA	NA	1	<0.001
III	rs2273535 (A/T)	*AURKA* *Cell cycle kinase*	O—Miao 2004 [151]			SCC	Res	C	O: 2.13 (1.04–4.39 [TT; Chinese]	NA	NA	1	<0.05
III	rs7121 (C/T)	*GNAS1*	O, N—Vashist 2011 [125]		T, M, G—Vashist 2011	US	Res	C	O: 2.10 (1.17–3.76)	NA	NA	1	0.013
		G protein subunit			T, N, R—Alakus 2009 [126]	US	Res + NACRT (CF)	B	[T; Caucasian]				
									N: 1.16 (0.76–1.77) [T]	3.94	49	3	0.500

AC, adenocarcinoma; SCC, squamous cell carcinoma; US, unspecified carcinoma; NACRT, neoadjuvant chemoradiotherapy; RT, radiotherapy; DCRT, definitive chemoradiotherapy; T, T stage (III/IV versus I/II); N, nodal stage (≥N1 versus N0); M, metastatic stage (M0 versus M1); G, cell grade (III/IV versus I/II); O, overall stage (III/IV versus I/II); R, resection stage; L, L stage; CF, cisplatin–5FU; Res, resection; LOE, level of evidence; NA, not applicable.

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funnel plot asymmetry, heterogeneity and publication bias

Begg's and Egger's tests were nonsignificant for all meta-analyses (supplementary Table S26, available at *Annals of Oncology* online). Visual inspection of plots identified asymmetry for nine outcome analyses: mutant *TP53* (OS overall, adjusted HR, SCC and unspecified cell types, neoadjuvant therapy and SSCP/direct sequencing analyses; supplementary Tables S2 and S3, available at *Annals of Oncology* online), *ERRBB2*/*HER2* (OS) and *FGF3* (OS), and three stage analyses: *EGFR* (overall) and ERBB2/*HER2* (N and grade). These were interpreted as likely publication bias and corrected (without affecting any conclusions). All sensitivity analyses were negative.

conclusions

We identified 182 studies, which assessed a total of 165 candidate genomic markers. Overall, 91 markers were reported to have significant associations with esophageal cancer outcome, and 41 with stage. Overall study quality was poor: most studies were retrospective with small sample sizes, and all except 5 (2.75%) were of level C or D quality. There was considerable heterogeneity in patient selection, treatment approach, genotyping techniques and definitions used. Common areas of weakness were failure to perform subgroup analysis for AC and SCC; failure of quality control such as reporting call rates and Hardy–Weinberg equilibrium; failure to perform/report multivariate adjustment of HRs; and failure to adjust for multiple comparisons. Furthermore, just 30.2% of reported markers subsequently had attempted validation data published.

Despite these limitations, sufficient data were available for appropriate meta-analyses. These demonstrated a small number of associations of DNA sequence markers with worse survival (mutant *TP53*, *HER2*, *CCND1* and *FGF3* copy number gain and CIN) and resistance to chemo–radio ± therapy (*TP53*).

As far as we are aware, this is the first attempt to collate and evaluate all evidence of DNA sequence markers and esophageal cancer, and to demonstrate the above associations by meta-analysis. As such it has a number of generic and specific strengths and weaknesses. A comprehensive search strategy was used to minimize identification and selection bias (requiring detailed appraisal of studies including gastric cancer, cell lines and expression data), it is possible that studies were not identified. For those included, methodological heterogeneity and small sample sizes introduce potential for bias. Although there was no statistical evidence of funnel plot asymmetry using Begg's and Egger's tests, these are underpowered in meta-analyses of fewer than 25 studies [152]; we therefore inspected all funnel plots, explored the reasons for any apparent asymmetry, and corrected eight analyses for likely publication bias (without altering overall effects). While the small number of studies involved in each analysis precluded meaningful meta-regression to explore additional potential confounding factors [153], we sought to address potential bias by performing subgroup analyses, including cell type genotyping techniques and ethnicity. There are also limitations to the revised American Society of Clinical Oncology guidelines in this context; firstly, regarding capture of the complexity inherent in data quality, and secondly determination of LOE: evidence can be upgraded by validation studies, yet disagreement of effect size and direction between studies is not always reflected in the ultimate LOE.

The strongest evidence we found for an outcome marker was tumor *TP53* mutation. Association with worse survival was demonstrated for both AC and SCC. Whether this is truly independent of the association demonstrated with T and N stage (independent pathological markers of outcome) [154] was not conclusively demonstrated, and indeed only assessed by six studies. Although four reported significant adjusted HR, the resultant meta-analyzed direction of effect was concordant but not significant due to the use of a random-effects model. We also found *TP53* mutant tumors to be less chemo(radio)sensitive.

As other recent meta-analyses have reported similar findings in breast and colorectal carcinoma [155, 156], this is of particular translatable relevance. *TP53* is one of the most frequently mutated and studied genes in human cancer [157], with resultant attempts to develop targeted therapies [158]. Ninety-five percent of functional mutations occur within exons 5–9, which encode the DNA binding domain, and typically cause loss of efficacy either directly by disrupting DNA contact, or indirectly by aberrant protein folding [159]. Subsequently, cell cycle, DNA repair and apoptotic regulation may fail [160], although oncogenic gains of function are occasionally seen [161]. The most characterized variant is the germline rs1042522 G > C substitution, itself conferring a worse HR for both OS and DFS in this meta-analysis.

TP53 as an esophageal tumor biomarker is often considered in terms of *TP53* status: aberrant expression, with or without mutation. An association with expression alone and worse outcome has been demonstrated on meta-analysis for SCC [162], as has aberrant status [increased expression (28 studies) with or without mutation (3 studies)] and reduced likelihood of response to chemotherapy [163]. However, *TP53* mutational and expression statuses may be discordant [164] particularly in the case of high-impact mutations precluding expression, or dramatically reducing half-life. The ability to predict this from sequencing data reinforces the need to explore the interaction of these aspects of status in parallel [165, 166].

Typically, resection specimens are used to assess associations between tumor markers and pathological response to chemotherapy. However, by definition these comprise clonal populations selected for chemo/radio-resistance. While such tumors appear to be disproportionately *TP53* mutated, deep re-sequencing and clonal studies comparing the prevalence and associations of pre- and post-treatment tumor are required to establish the true pre-treatment predictive utility of *TP53* mutations in this regard.

Three associations between tumor copy number gain (albeit variably quantified) were demonstrated by meta-analysis: *ERBB2/HER2*, *CCND1* and *FGF3* gain. *ERBB2/HER2* is particularly relevant; a proto-oncogene, it is the sole molecular marker in clinical use for gastroesophageal cancer, guiding the use of targeted therapies [14]. Our findings build on a recent meta-analysis of HER2 status, defining positivity by overexpression or amplification, including six of the studies included in this meta-analysis [15]. We found gain to confer a worse prognosis for both AC and SCC, independent of stage. Interestingly, all patients in 10 of the 11 studies underwent radical treatment with resection; while palliative monoclonal antibody therapy for

HER2-positive gastroesophageal AC is effective in prolonging survival [167], an urgent unanswered question is therefore whether it has a role in curative treatment.

Similarly, regarding the cell cycle regulator *CCND1*, phase I and II data have suggested a possible role for cyclin-dependent kinase inhibitors in nongastroesophgageal cancer [168, 169]. Our findings therefore suggest the need to assess their effect in esophageal tumors with *CCND1* gain. *EGFR*, a tyrosine kinase receptor, has also been extensively investigated within gastroesophageal cancer; phase II data support targeted therapy (antibodies and tyrosine kinase inhibitors) for metastatic disease [170, 171], although not yet neoadjuvant regimens [172, 173]. While we found no association with outcome using the requisite random-effects model, significant effects were evident with a fixed model; consequently, there may be an undetected association. We also found CIN to be associated with worse outcome, in keeping with a previous colorectal cancer meta-analysis [174], although whether it modulates chemo-sensitivity is unclear.

We also demonstrated survival associations for six common germline polymorphisms by meta-analysis: *ERCC1* rs3212986 (for cisplatin treatment and Caucasian ethnicity), ERCC2 rs1799793 (cisplatin and Caucasian), *TP53* rs1042522 (Caucasian), *MDM2* rs2279744 (Caucasian), *TYMS* rs34743033 (Japanese) *ABCB1* rs1045642 (both Caucasian and Japanese). The association of *VEGFA* rs2010963 was evident only on combining Taiwanese and Japanese study populations. *MTHFR* rs1801133 was associated with recurrence in Caucasians. *XRCC1* rs25487 and *ERCC1* rs11615 were associated with response to chemotherapy in Caucasians. These associations are likely to be due to aberrant protein expression or function.

rs3212986 modifies *ERCC1* mRNA stability [175], a component of the nucleotide excision repair (NER) pathway, variants of which are associated with platinum sensitivity and survival in pancreatic, gastric, colorectal and lung cancers [95–177]. The missense rs1799793 SNP results in an aspartate–asparagine substitution at codon 312 of the *ERCC2* component of the NER pathway, and has been similarly associated with survival in gastric and other cancers [178]. The rs10456402 SNP in exon 26 of *ABCB1* (*Multi Drug Resistance 1*) reduces expression (and consequent platinum-analogue membrane transportation) [179], and is similarly associated with colorectal cancer prognosis [180]. rs2279744 increases mRNA expression of *MDM2*, which suppresses TP53 activity [181], and is associated with increased susceptibility to a number of cancers (including gastric) [182]. rs34743033 is a 28-bp variable number tandem repeat in *TYMS* (thymidylate synthase), with enhancer function correlating with increased *TYMS* expression [183], and survival in platinum-treatment nonsmall-cell lung carcinoma [184]. The rs1801133 missense SNP induces an alanine–valine substitution at codon 222, with reduced activity of methylenetetrahydrofolate reductase [185], and increased susceptibility to gastric cancer [186]. rs25487 induces a glutamine-arginine substation in codon 399, with resultant reduction in function of the DNA repair gene *XRCC1* [187], and an association with survival of lung cancer [188]. rs11615 reduces *ERCC1* expression [189], and increases likelihood of response to platinum chemotherapy in gastric and colorectal cancer [176].

Biomarkers themselves carry a number of limitations. Typically, they are classified as 'prognostic' or 'predictive'; however, in reality, these are not mutually exclusive, and we therefore did not attempt classification. Biomarker development culminates in demonstration of clinical, requiring at least multicenter prospective validation for prognosis, and incorporation into interaction randomized controlled trials for prediction. These challenges reinforce the utility of retrospectively analyzing samples archived during prospective trials. Other challenges include the use of pretreatment biopsies. First, analysis may be impaired by inclusion of noncancerous tissue; while this can be mitigated by techniques such as laser-capture microdissection and higher depth sequencing, these are time and cost-intensive. More profound is the challenge of intratumoral heterogeneity and clonality: a single biopsy is representative of just 34% of the mutational burden of a 'single' cancer [190], and will not include metastatic subclones. How to surmount this is not yet clear.

Finally, while it may be pragmatic to consider DNA sequence variants in isolation, their effects (and therefore utility) are subject to complicated modulation by the other 'omics', (epigenomics, transcriptomics, metabolomics and proteomics), genes and clinical and environmental covariates [191, 192]. While a discrete variable might provide useful complementary information of itself, this complexity at present precludes its use to dichotomize decision making. Consequently, a robust approach to personalized cancer medicine must incorporate parallel processing of DNA, RNA, proteins and metabolites.

In conclusion, numerous DNA sequence markers have been described for esophageal cancer. However, as with complementary fields within personalized cancer research, the underlying research is largely poor in quality and disparate in methodology, with a lack of robust validation of markers and incorporation into trials. While a number of promising candidates have been identified the data required to incorporate these into prognostic/predictive models do not yet exist; future validation will require larger studies, with improvements in the standardized collection of samples for analysis, parallel assessment of expression and the incorporation of parallel biomarkers within high-quality clinical trials, robust adjustment for confounding variables and sharing of resultant data with multicenter collaboration.

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Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis

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Background: A wide variety of follow-up strategies are used for patients with colorectal cancer (CRC) after curative surgery. The aim of this study is to review the evidence of the impact of different follow-up strategies in patients with non-metastatic CRC after curative surgery, in relation to overall survival and other outcomes.

Patients and methods: A systematic search of PubMed, EMBASE, SCOPUS and ISI Web of Knowledge up to June 2014 was carried out. Eligible studies were all randomized clinical trials comparing the effectiveness of different follow-up strategies after curative resection in nonmetastatic CRC.

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