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Prognostic role of tumor *PIK3CA* mutation in colorectal cancer: a systematic review and meta-analysis

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Background: Somatic mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT pathway play a vital role in carcinogenesis. Approximately 15%–20% of colorectal cancers (CRCs) harbor activating mutations in *PIK3CA*, making it one of the most frequently mutated genes in CRC. We thus carried out a systematic review and meta-analysis investigating the prognostic significance of *PIK3CA* mutations in CRC.

Materials and methods: Electronic databases were searched from inception through May 2015. We extracted the study characteristics and prognostic data of each eligible study. The hazard ratio (HR) and 95% confidence interval (CI) were derived and pooled using the random-effects Mantel–Haenszel model.

Results: Twenty-eight studies enrolling 12 747 patients were eligible for inclusion. Data on overall survival (OS) and progression-free survival (PFS) were available from 19 and 10 studies, respectively. Comparing *PIK3CA*-mutated CRC patients with *PIK3CA*-wild-type CRC patients, the summary HRs for OS and PFS were 0.96 (95% CI 0.83–1.12) and 1.20 (95% CI 0.98–1.46), respectively. The trim-and-fill, Copas model and subgroup analyses stratified by the study characteristics confirmed the robustness of the results. Five studies reported the CRC prognosis for *PIK3CA* mutations in exons 9 and 20 separately; neither exon 9 mutation nor exon 20 mutation in *PIK3CA* was significantly associated with patient survival.

Conclusions: Our findings suggest that *PIK3CA* mutation has the neutral prognostic effects on CRC OS and PFS. Evidence was accumulating for the establishment of CRC survival between *PIK3CA* mutations and patient-specific clinical or molecular profiles.

Key words: *PIK3CA*, mutation, colorectal cancer, prognosis

introduction

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is one of the crucial kinases in the PI3K/AKT1/MTOR pathway, playing a role in the cellular growth, proliferation and survival of multiple solid tumors [1–3]. Approximately 15%–20% of colorectal cancers (CRCs) harbor activating mutations in *PIK3CA* exon 9 and/or exon 20, making *PIK3CA* one of the most frequently mutated genes in CRC [4–7].

A number of studies have examined a prognostic role of somatic *PIK3CA* mutations in CRC [8–35]. Although some studies have reported the prognostic effect of the *PIK3CA* mutation status on CRC patient survival [4, 33, 36–40], several other studies of patients in various settings have reported varying results on the association of *PIK3CA* mutation with CRC survival outcomes [8, 9, 11–14]. It has been found in several studies that *PIK3CA* mutations showed significant positive association with CRC survival [8, 38, 39], while still others reported negative [9–11, 13, 14, 20, 35] or null association [12, 21, 24, 30]. Therefore, the prognostic role of *PIK3CA* mutations in CRC remains uncertain.

We therefore conducted a systematic review and meta-analysis to assess the evidence for the association between *PIK3CA* mutation and CRC patient recurrence and survival outcomes. In our secondary analysis considering the studies [38, 39] which have reported survival benefit associated with aspirin use in *PIK3CA*-mutated CRC (but not in *PIK3CA*-wild-type CRC), we meta-analyzed the prognostic associations of aspirin use after CRC diagnosis in strata of tumor *PIK3CA* mutation status.

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materials and methods

search strategy

A computerized literature search of PubMed, Embase, the Cochrane Library Central Register of Controlled Trials and American Society of Clinical Oncology (ASCO) databases through May 2015 was conducted for all peer-reviewed studies that reported an association between CRC survival outcomes and *PIK3CA* mutations. As is presented in supplementary Appendix Table SA1–4, available at *Annals of Oncology* online, the following combinations of free-text words and Medical Subject Headings (MeSH)/EMTREE terms were used: '*PIK3CA* or Phosphoinositide-3-kinase catalytic alpha polypeptide or PIK3 catalytic alpha polypeptide', 'mutation* or mutated', and 'colorect* or colon* or rectum or rectal', 'cancer* or tumor* or tumour* or carcinom* or neoplas* or adenocarcinoma* or malignan*' and 'prognos* or survival or recurren* or mortality or predict* or outcome* or death'. In our secondary analysis concerning patients using aspirin, we also combined 'aspirin or non-steroidal anti-inflammatory drugs or NSAIDS' as searching words with the Boolean logical operator 'AND'. We also manually searched recent relevant papers (since 2004) in major journals such as *Journal of the European Society for Medical Oncology* (*Annals of Oncology*), American Society of Colon and Rectal Surgeons (*Diseases of the Colon & Rectum*) and *JNCI* (the *Journal of the National Cancer Institute*). The references of primary selected studies, reviews or meta-analyses were scrutinized for additional records that were not identified through database search. We did not apply restrictions to the date or language in our search strategy.

study selection and inclusion criteria

Two reviewers (ZM and CD) independently selected and identified the appropriate studies based on the prespecified selection criteria. Discrepancies between the two reviewers were resolved by discussion or the senior author (SO). Prospective or retrospective studies if all of the following criteria were met: (i) studies published as an original article, regardless of the language; (ii) studies reporting on the outcome measures, such as overall survival (OS) and progression-free survival (PFS); and (iii) studies evaluating the *PIK3CA* mutation status in resected samples of primary CRC and providing relevant patient survival data with a hazard ratio (HR) estimate and its 95% confidence interval (CI) comparing *PIK3CA*-mutated cases with *PIK3CA*-wild-type cases. The exclusion criteria were: (i) studies having no prognostic outcomes recorded; (ii) studies including no sufficient data for analysis; and (iii) letters, comments, reviews or meta-analyses containing no original data. When more than one publication reported on the same population or overlapping populations, a study with a larger sample size (with *PIK3CA* mutation data) was selected into the meta-analysis.

data extraction and quality assessment

For each study, the following details were extracted: the full names of the first and last authors; publication year; study design; country where the study was carried out; number of hospitals involved; number of outcome events (number of *PIK3CA* mutated aspirin user); sample size; tumor site and disease stage; mutation detection assay; number of *PIK3CA* mutants; the availability of *KRAS* and *BRAF* mutation status data; survival end points; and HRs with corresponding 95% CIs and adjustment variables. For each study, we assessed the quality of the evidence on the association between the CRC survival outcomes and *PIK3CA* mutation status using a set of modified predefined criteria for evaluating the quality of the studies [41, 42].

statistical analyses

The primary outcomes of interest were OS and PFS of CRC patients with *PIK3CA* mutations compared with those with wild-type *PIK3CA*. For the quantitative aggregation of the survival outcomes, the HRs with corresponding 95% CIs were directly retrieved from the original studies and pooled with the DerSimonian and Laird random effects model [43] or using the method described by Parmar et al. [44]. We investigated the between-study heterogeneity by the Cochran's Q-test and I^2 statistic, and a P value for heterogeneity by the I^2 value $\geq$50% suggested substantial heterogeneity [45]. The source of heterogeneity was explored using subgroup analyses by examining all the possible factors that could explain the heterogeneity observed. Differences between the subgroups were assessed using the methods described by Deeks et al. [46]. We also conducted a secondary analysis based on aspirin use after CRC diagnosis (compared with non-use) in *PIK3CA*-mutated cancer patients. Further analysis was also carried out limited to those studies investigating CRC patients treated with anti-epidermal growth factor receptor (EGFR) therapy.

We assessed the evidence of publication bias by visual inspection of the contour-enhanced funnel plot symmetry as well as by Begg's regression and Egger's linear regression method [47, 48]. Duval's non-parametric trim-and-fill procedure was applied to further assess the possible effect of publication bias [49]. Moreover, the Copas model was used to conduct sensitivity analysis by considering both the effect size and sample size [50]. The statistical analyses were carried out with R software version 3.1.2. All statistical tests were two-sided, and significance was defined as a P value of <0.05.

results

search and selection of studies

We identified 1248 eligible citations in the initial literature search and 77 potentially relevant studies for further review. After removing 49 studies, a total of 28 studies met our inclusion criteria and were included in the final analysis (Figure 1 and supplementary Tables S1, S7 and S8, available at *Annals of Oncology* online) [8–35].

study characteristics

A total of 12 747 patients were included in the studies with a median sample size of 258 (inter-quartile range, 112–628). The median follow-up period ranged from 28 to 113 months. Table 1 and supplementary Table S11, available at *Annals of Oncology* online, provide the basic characteristics of each study that met our inclusion criteria. All studies were published between 2009 and 2015 in English peer-reviewed journals. For case ascertainment, 9 studies had a prospective design (7264 participants) [10, 12, 16,

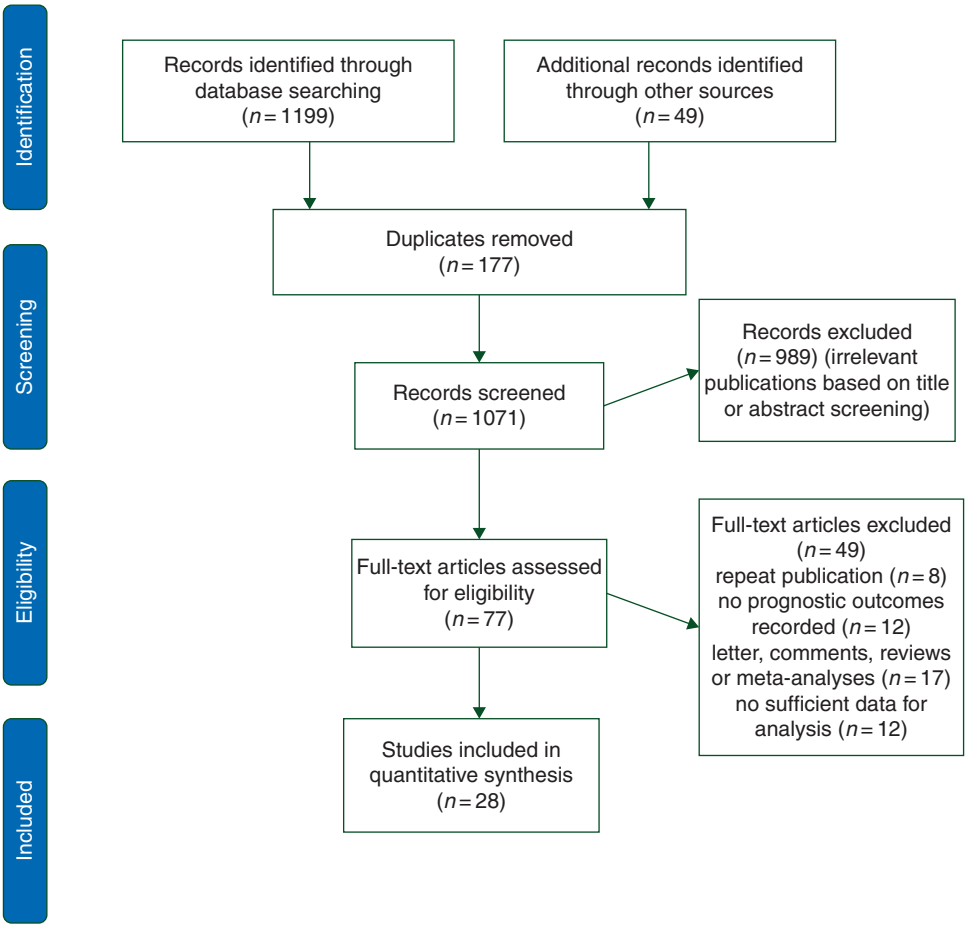

Figure 1. Flow diagram of the study selection.

18, 22, 23, 27, 34, 35], and 19 had a retrospective design (5483 participants) [8, 9, 11, 13–15, 17, 19–21, 24–26, 28–33].

Ten studies involved single-center data [9, 11, 13, 15, 17, 19, 26, 28–30], whereas 18 were multi-center studies [8, 10, 12, 14, 16, 18, 20–25, 27, 31–35]. Seven studies were conducted in the USA [12, 20, 21, 23, 26–28], nine in Europe [8, 11, 14–16, 24, 29, 34, 35], five in Asia [9, 13, 17, 19, 30], and seven covered multiple continents [10, 18, 22, 25, 31–33]. Nineteen studies investigated the association between *PIK3CA* status and OS for CRC patients [8, 10–12, 17–21, 23, 26–34], whereas 10 studies reported the PFS [11, 15, 17, 18, 26, 29, 31–34]. Most studies involved patients with both colon and rectal cancers. Five studies evaluated only colon cancer [8, 14, 16, 21, 27], and one study evaluated only rectal cancer [35]. A mixture of I–IV disease stages was included in 18 studies [8–10, 12–14, 16, 19–25, 27, 29, 32, 35], and only stage IV cancers were included in 10 studies [11, 15, 17, 18, 26, 28, 30, 31, 33, 34]. Two methods for detecting the sequence were employed in the included studies; 21 used Sanger sequencing [8, 10, 11, 13–19, 22–25, 27, 29, 31–35]; 6 used pyrosequencing [9, 12, 21, 26, 28, 30]; and 1 used pyrosequencing combined with Sanger sequencing [20]. Most of the included studies (24/28) used formalin-fixed paraffin-embedded (FFPE) CRC samples, one used fresh-frozen samples and the other three used both FFPE CRC samples and fresh-frozen samples (Table 2). Most studies investigated the prognostic impact of the overall *PIK3CA* mutation status (exon 9 or 20 mutation) on CRC survival. Five studies also separately assessed the prognostic association of *PIK3CA* exon 20 or 9 mutations with the CRC survival [12, 14, 21, 24, 31]; one study investigated the effect of concomitant *PIK3CA* exon 9 and 20 mutations with CRC survival [12]. Gender, age at diagnosis, tumor location, stage and *KRAS* and *BRAF* mutation status are commonly investigated covariates that were adjusted for in Cox's proportional-hazard model evaluation of the relationship between the *PIK3CA* mutations and survival.

relationship between *PIK3CA* mutations and CRC prognosis

As shown in Figure 2A, the summary HR for the OS comparing *PIK3CA* mutation versus wild-type *PIK3CA* was 0.96 (95% CI 0.83–1.12; $P = 0.60$), and there was moderate heterogeneity between the studies ($I^2 = 40.9\%$, $P_{\text{heterogeneity}} = 0.027$). Figure 2B summarizes the HR (1.20; 95% CI 0.98–1.46; $P = 0.079$) for PFS, and there was no heterogeneity between studies ($I^2 = 0$, $P_{\text{heterogeneity}} = 0.97$). We also found no association between the *PIK3CA* mutation status and survival in patients with CRC in terms of other outcome measures (supplementary Table S2, available at *Annals of Oncology* online).

Table 2 presents the results of the subgroup analyses by potential sources of heterogeneity among certain major clinical characteristics of the included studies for the OS and PFS. The

Table 1. Characteristics of the studies on survival outcomes of colorectal cancer patients according to *PIK3CA* mutation status

Authors [ref.]	Study design	Country	No. of hospitals involved	No. of events	Sample size	Tumor site	Disease stage	Specimens used/mutation detection assay	No. of *PIK3CA* mutants		*KRAS* data	*BRAF* data	Survival end points	Adjusted variables
									Exon 9	Exon 20				
Chen et al. [19]	Retrospective cohort	China	1	88	214	CRC	I–IV	FFPE/SS	12	14	Y	Y	OS	Age, sex, differentiation grade, tumor diameter, number of lymph nodes examined, TNM stage and *KRAS/BRAF* genotype
Day et al. [25]	Retrospective cohort	Australia	Multiple	NR	589	CRC	II, III	Fresh-frozen or FFPE/SS	49	19	Y	Y	DFS	Age at diagnosis, gender, tumor location, stage, differentiation, MSI status and adjuvant treatment
De Roock W et al. [31]	Retrospective cohort	11 centers in seven European countries	Multiple	NR	743	CRC	IV	Fresh-frozen or FFPE/SS	74	22	Y	Y	OS, PFS	Age, sex, number of previous chemotherapy lines and center
Eklof et al. [24]	Retrospective cohorts	Sweden	Multiple	NR	611	CRC	I–IV	FFPE/SS	NR	13	Y	Y	CS	Sex, age, tumor site and tumor stage.
Farina Sarasqueta et al. [14]	Retrospective cohort	The Netherlands	Multiple	183	616	Colon cancer	I–III	FFPE/SS	66	17	Y	Y	CS	Age, gender, tumor location, adjuvant chemotherapy, T stage, MMR status, tumor differentiation
Garrido-Laguna I, et al. [28]	Retrospective cohort	USA	1	25	168	CRC	IV	FFPE/PS	9	5	Y	Y	OS	Sex, tumor stage, *KRAS* and *BRAF* status
Gavin et al. [27]	Prospective cohort	USA	Multiple	NR	2299	Colon cancer	II, III	FFPE/SS	NR	NR	Y	Y	OS, RFS	*BRAF, KRAS, KRAS, NRAS, MET* and *PIK3CA* mutations
He et al. [35]	Prospective cohort	The Netherlands	Multiple	84	240	Rectal cancer	I–III	Fresh-frozen/SS	12	7	Y	Y	RFS	TNM stage, CRM
Iida et al. [13]	Retrospective cohort	Japan	1	26	165	CRC	I–IV	FFPE/SS	NR	NR	Y	N	CS	Sex, age, tumor location, stage, differentiation, methylation etc.
Kang et al. [23]	Prospective population-based cohort	USA	Multiple	59	304	CRC	I–IV	FFPE/SS	NR	NR	Y	Y	OS	Age, sex, tumor location, chemotherapy, *MSI* status etc.

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Table 1. *Continued*

Authors [ref.]	Study design	Country	No. of hospitals involved	No. of events	Sample size	Tumor site	Disease stage	Specimens used/mutation detection assay	No. of *PIK3CA* mutants		*KRAS* data	*BRAF* data	Survival end points	Adjusted variables
									Exon 9	Exon 20				
Karapetis et al. [18]	Prospective cohort	Canada, Australia, New Zealand	Multiple	NR	572	CRC	IV	FFPE/SS	44	6	Y	Y	OS, PFS	ECOG performance status, gender, age, baseline lactate dehydrogenase level, baseline alkaline phosphatase, baseline hemoglobin, number of disease sites, number of previous chemotherapy drug classes, primary tumor site, presence of liver metastases, treatment, *BRAF*/*PTEN* status
Kishiki et al. [17]	Retrospective cohort	Japan	1	6	84	CRC	IV	FFPE/SS	NR	NR	Y	Y	OS, PFS	*KRAS, BRAF* mutation status; *PTEN* or *MET* expression
Liao et al. [30]	Retrospective cohort	China	1	NR	61	CRC	IV	FFPE/PS	NR	NR	Y	Y	OS	Treatment regimens, mutation status, metastatic location, number of metastatic lesions
Liao et al. [12]	Prospective cohort	USA	Multiple	552	1170	CRC	I–IV	FFPE/PS	116	80	Y	Y	OS, CS	Age at diagnosis, sex, tumor location, CIMP status, MSI status, LINE-1 methylation, *BRAF* mutation, *KRAS* mutation
Manceau et al. [8]	Retrospective cohort	France	Multiple	99	693	Colon cancer	I–III	Fresh-frozen/SS	66	43	Y	Y	RFS, OS	Sex, age, tumor location, stage, *KRAS, BRAF* mutation status, CIMP status
Mouradov et al. [22]	VICTOR and community prospective cohort	Australia UK,	Multiple	99	1197	CRC	II, III	FFPE/SS	NR	NR	Y	Y	DFS	Age, gender, cancer location, tumor stage and grade, use of radiochemotherapy, randomization to rofecoxib treatment, MSI, CIN, measures of LOH and specific gene mutations

Ogino et al. [21]	Retrospective cohort	USA	Multiple	502	627	Colon cancer	III	FFPE/PS	48	25	Y	Y	OS, RFS, DFS	Age, sex, baseline body mass index, family history of colorectal cancer in first-degree relatives, baseline performance status, presence of bowel perforation or obstruction at the time of surgery, treatment arm, tumor location, stage, *KRAS, BRAF* and MSI status
Perrone et al. [15]	Retrospective cohort	Italy	1	NR	32	CRC	IV	FFPE/SS	NR	NR	Y	Y	PFS	NR
Phipps et al. [20]	Retrospective woman cohort	USA	Multiple	97	275	CRC	I–IV	FFPE/PS, SS	NR	NR	Y	Y	OS, CS	Age and stage at diagnosis
Prenen et al. [34]	Prospective cohort	Belgium	Multiple	NR	200	CRC	IV	FFPE/SS	NR	NR	Y	N	OS, PFS	NR
Reimers et al. [16]	VICTOR and community prospective cohort	The Netherlands	Multiple	298	631	Colon cancer	I–IV	FFPE/SS	NR	NR	N	N	OS	Sex, age, comorbidity, year of incidence, histologic grade, stage and chemotherapy
Rosty et al. [10]	Prospective cohort	Australia, New Zealand	Multiple	261	651	CRC	I–IV	FFPE/SS	81	27	Y	Y	OS	Sex, age at diagnosis, tumor location, histologic grade, MSI status, MGMT expression, *KRAS* and *BRAF* status
Saridaki et al. [29]	Retrospective cohort	Greece	1	NR	112	CRC	I–IV	FFPE/SS	8	3	Y	Y	OS, PFS	*KRAS, BRAF* mutation, EREG mRNA expression, skin rash, tumor differentiation
Sartore-Bianchi et al. [33]	Retrospective cohort	Italy, Switzerland	Multiple	88	110	CRC	IV	FFPE/SS	4	11	N	Y	OS, PFS	Score of cutaneous toxicity and number of previous chemotherapy line
Sood et al. [26]	Retrospective cohort	USA	1	NR	76	CRC	IV	FFPE/PS	NR	NR	Y	Y	OS, PFS	NR
Souglakos et al. [32]	Retrospective cohort	Greece, USA	Multiple	43	92	CRC	I–IV	FFPE/SS	18	8	Y	Y	OS, PFS	Age, differentiation, tumor location, solitary metastasis and *BRAF* mutations

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Table 1. *Continued*

Authors [ref.]	Study design	Country	No. of hospitals involved	No. of events	Sample size	Tumor site	Disease stage	Specimens used/mutation detection assay	No. of *PIK3CA* mutants		*KRAS* data	*BRAF* data	Survival end points	Adjusted variables
									Exon 9	Exon 20				
Ulivi et al. [11]	Retrospective cohort	Italy	1	NR	67	CRC	IV	FFPE/SS	7	2	Y	Y	OS, PFS	ECOG performance status, cutaneous toxicity, and number of previous chemotherapy lines
Zhu et al. [9]	Retrospective cohort	China	1	21	148	CRC	I–III	FFPE/PS	NR	NR	Y	Y	RFS	*KRAS* and *BRAF* mutation status, stage, distance to anal verge, CRM

CIMP, CpG island methylator phenotype; CIN, chromosomal instability; CRC, colorectal cancer; CRM, cumferential resection margin; CS, cancer-specific survival; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; EREG, epiregulin; FFPE, formalin-fixed paraffin-embedded; LINE-1, long interspersed nucleotide element-1; LOH, loss of heterozygosity; MMR, mismatch repair; MSI, microsatellite instability; N, no; NR, not reported; OS, overall survival; PFS, progression-free survival; PS, pyrosequencing; RFS, recurrence-free survival; SS, Sanger sequencing; Y, yes.

summary HR estimates in most subgroups were not significantly altered by the study characteristics, including the study design, research country, the number of centers involved, sample size and mutation detection assay. A possible interaction was noted in two features (stage of disease and tumor location) for OS, although there was multiple hypothesis testing by seven features. Results of analyses limited to stage I–III and stage IV are presented in Table 2. For patients with colon cancer, a possible prognostic association of *PIK3CA* mutation was noted. However, due to the small number of studies in each of these subgroups, further studies should investigate the prognostic association of *PIK3CA* mutation in these subgroups.

Five studies reported on the prognostic association of *PIK3CA* exon 9 mutation, and that of *PIK3CA* exon 20 mutation, separately [12, 14, 21, 24, 31]. Nevertheless, neither exon 9 nor exon 20 *PIK3CA* mutations was significantly associated with survival (supplementary Table S3 and supplementary Figure S1, available at *Annals of Oncology* online). We assessed a difference in frequencies of *PIK3CA* mutations in exon 9 versus exon 20 among the included studies. A difference in studies that showed varying frequencies of mutations in exon 9 and exon 20 did not appear to influence our main conclusion of no substantial prognostic role of *PIK3CA* mutation in CRC (supplementary Table S10, available at *Annals of Oncology* online).

prognostic association of post-diagnosis aspirin use according to *PIK3CA* mutation status in CRC

In our secondary analysis, we identified three studies investigating the prognostic association of post-diagnosis aspirin use according to *PIK3CA* mutation status [16, 38, 39] (supplementary Table S11, available at *Annals of Oncology* online). Compared with aspirin non-users, regular aspirin use after CRC diagnosis was associated with longer OS in *PIK3CA*-mutated CRC patients (HR 0.55, 95% CI 0.42–0.72; $P = 0.015$), but not in *PIK3CA*-wild-type CRC patients (HR 0.77, 95% CI 0.50–1.18; $P = 0.20$; supplementary Table S4, available at *Annals of Oncology* online and Figure 3). Furthermore, we did not observe statistically significant association of tumor *PIK3CA* mutations with PFS (HR 1.20, 95% CI 0.98–1.46; $P = 0.079$) or OS (HR 1.16, 95% CI 0.97–1.39; $P = 0.138$) in metastatic CRC patients treated with anti-EGFR monoclonal antibodies.

study quality and publication bias

The methodological quality score of the prognosis studies was moderate to high in 86% (24/28) of the included studies according to the quality score (supplementary Table S5, available at *Annals of Oncology* online); most studies had adequate follow-up and prognostic factor measurement, had a sufficient measurement of outcomes, carried out appropriate covariate measurements and used appropriate statistical analysis, but most studies used hospital-based convenience sample, and only rare studies [10, 12, 16, 23, 24] did use a population-representative sample (supplementary Figure S2, available at *Annals of Oncology* online).

We also carried out a subgroup analysis according to the study quality, which showed no significant difference among the low-, moderate- and high-quality studies for OS ($P = 0.22$) or

Table 2. Subgroup analyses of the associations between *PIK3CA* mutation and overall survival or progression-free survival

Comparison variables	Overall survival			Progression-free survival		
	Number of studies (I^2 statistics %; P_{het})	HR (95% CI)	$P_{interaction}$	Number of studies (I^2 statistics %; P_{het})	HR (95% CI)	$P_{interaction}$
Total	19 (40.9; 0.027)	0.96 (0.83–1.12)	NA	10 (0; 0.93)	1.2 (0.98–1.46)	NA
Study design			0.77			0.27
Prospective	7 (54.3; 0.041)	0.95 (0.79–1.15)		2 (0; 0.93)	1.08 (0.82–1.41)	
Retrospective	12 (37.1; 0.080)	1.00 (0.77–1.29)		8 (0; 0.97)	1.35 (1.01–1.81)	
Research country			0.33			0.75
USA	7 (0; 0.96)	0.93 (0.81–1.06)		1 (—)	2.31 (1.07–5.01)	
Europe	6 (73.1; 0.001)	0.90 (0.63–1.30)		6 (0; 0.96)	1.17 (0.93–1.46)	
Asia	3 (0, 0.82)	1.57 (0.73–3.35)		1 (—)	2.22 (1.07–3.86)	
Cross-continent	3 (0, 0.75)	1.16 (0.86–1.57)		2 (0; 0.38)	1.18 (0.75–1.86)	
Centers involved			0.13			0.16
Single	7 (0; 0.97)	1.33 (0.86–2.05)		5 (0; 0.99)	2.00 (0.95–4.22)	
Multiple	12 (56.2; 0.005)	0.93 (0.78–1.10)		5 (0; 0.93)	1.15 (0.94–1.41)	
Stage of disease			0.033			0.29
I–III	10 (56.5; 0.011)	0.86 (0.70–1.06)		2 (0; 0.92)	2.00 (0.76–5.29)	
IV	9 (0; 0.99)	1.16 (0.97–1.39)		8 (0; 0.97)	1.17 (0.95–1.43)	
Sample size			0.14			0.081
<200	8 (0; 0.95)	1.24 (0.87–1.76)		7 (0; 0.99)	1.99 (1.09–3.64)	
≥200	11 (58.5; 0.004)	0.92 (0.77–1.10)		3 (0; 0.97)	1.12 (0.91–1.39)	
Tumor location			0.018			NA
Colon	3 (78.4; 0.003)	0.70 (0.50–0.98)		–	–	–
Rectum	–	–		–	–	
Colorectum	16 (0; 0.97)	1.08 (0.95–1.22)		10 (0; 0.93)	1.2 (0.98–1.46)	
Mutation detection assay			0.18			0.51
Pyrosequencing	6 (64.8; 0.009)	0.79 (0.59–1.06)		1 (—)	2.31 (0.32–16.6)	
Sanger sequencing	12 (0; 0.87)	1.05 (0.92–1.19)		9 (0; 0.96)	1.19 (0.97–1.45)	

CI, confidence interval; het, heterogeneity; HR, hazard ratio; NA, not available.

PFS ($P = 0.68$). The summary HRs for the OS were similar for studies with low quality ($n = 3$; HR 1.20, 95% CI 0.92–1.57; $I^2 = 0\%$, $P_{heterogeneity} = 0.85$), moderate quality ($n = 10$; 0.99 95% CI 0.85–1.14; $I^2 = 0\%$, $P_{heterogeneity} = 0.95$) and high quality ($n = 6$; 0.84, 95% CI 0.61–1.15; $I^2 = 71.8\%$, $P_{heterogeneity} = 0.002$). Similar results were obtained stratified by some of the clinical, pathological and molecular features for the OS and PFS panels (supplementary Tables S6 and S9, available at *Annals of Oncology* online).

For OS, the contour-enhanced funnel plot demonstrated asymmetry, indicating the presence of publication bias (Figure 4A). The hollow circles show the eight missing studies that lay in the non-significant regions of the plot, suggesting that the asymmetry was attributed mainly to publication bias, which was further confirmed with the Begg's rank correlation test ($P = 0.016$). The adjusted random effects summary HRs of 0.89 (95% CI 0.78–1.03) obtained using the trim-and-fill method and 0.90 (95% CI 0.76–1.07) using the Copas model were consistent with our primary analysis. For the PFS, there was also evidence of asymmetry (Figure 4B). The results did not significantly change after applying the trim-and-fill method when including five missing studies, with the adjusted random effects summary HR of 1.14 (95% CI 0.94–1.38), similar to the summary HR of 1.16 (95% CI 0.95–1.42) using the Copas model (supplementary Table S2, available at *Annals of Oncology* online).

discussion

We conducted this study to test the hypothesis that *PIK3CA* mutation in CRC might be associated with patient survival. Studies have demonstrated that *PIK3CA* mutations in CRC are related to various clinical and tumor molecular features, including associations with *KRAS* mutations and proximal tumor location, which may be due to varying biogeographical influence of the host–microbiota–tumor interaction along the colorectal axis [10, 51]. As *PIK3CA* has been known as one of the major driver oncogenes in CRC, the prognostic significance of *PIK3CA* mutations in CRC needs to be elucidated.

Our current systematic review and meta-analysis have demonstrated that the *PIK3CA* mutation status is not significantly associated with CRC patient survival. *PIK3CA* mutation status did not appear to have a substantial prognostic role in most of the subgroups examined according to certain relevant study characteristics, including study design, country, hospital, sample size and mutation detection assay. The survival association remained similar when the results were adjusted by the trim-and-fill method, or Copas model, considering the publication bias. There are a limited number of studies that examined a prognostic role of *PIK3CA* exon 9 mutations and exon 20 mutations, separately [12, 14, 21, 24, 31], and there is only one study that examined a prognostic role of coexisting mutations in both exons 9 and 20 in

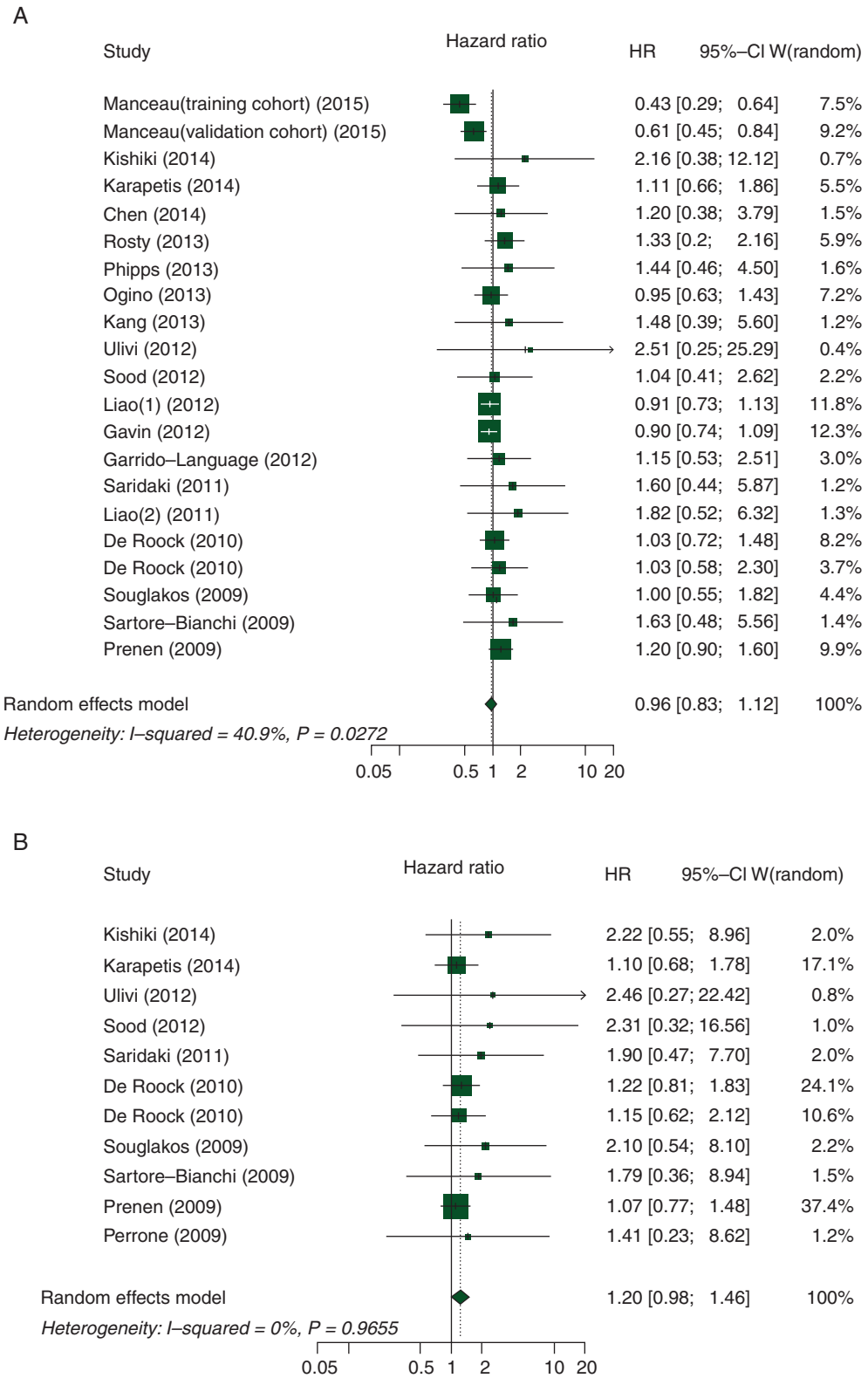

Figure 2. Association between *PIK3CA* mutation status and (A) overall survival and (B) progression-free survival.

PIK3CA in CRC [12]. Interestingly, our secondary analysis has provided evidence for a prognostic role of regular aspirin use after CRC diagnosis (compared with non-use) in *PIK3CA*-mutated CRC patients, but not in *PIK3CA*-wild-type CRC patients.

PIK3CA mutation has no substantial prognostic role, in that some tumors have PIK3CA mutation as a driver mutation plus other driver mutations, while other tumors do not carry *PIK3CA* mutation but have another set of driver mutations.

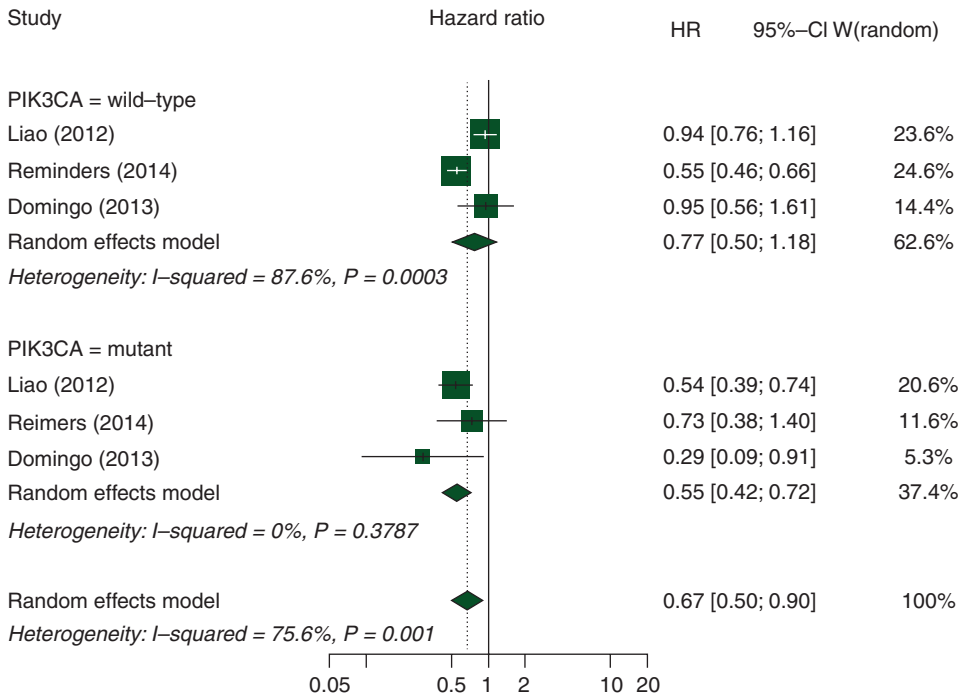

Figure 3. Summary estimates of the hazard ratio (and 95% confidence interval) for the overall survival of colorectal cancer (CRC) patients with mutated versus wild-type *PIK3CA* in patients who regularly ingested aspirin after a CRC diagnosis.

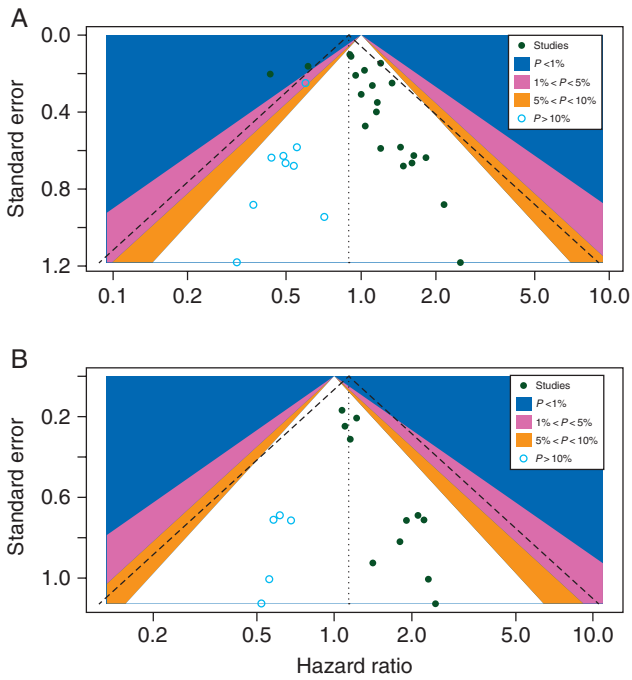

Figure 4. (A) Contour-enhanced funnel plot for meta-analysis of the association between the *PIK3CA* mutation status and overall survival. The left blank area represents the area where eight studies (blue circles) were included when the trim-and-fill method was applied. (B) Contour-enhanced funnel plot for meta-analysis of the association between the *PIK3CA* mutation status and progression-free survival. The left blank area represents the area where five studies (blue circles) were included when the trim-and-fill method was applied.

Tumor behavior may depend on multiple differing driver events as well as interaction of many molecular alterations in tumor, not solely on *PIK3CA* mutation status. Moreover, host factors such as immune response to tumor may influence prognosis. Thus, it is not surprising to find that any driver mutation (such as *PIK3CA* mutation) has no substantial prognostic role.

In the meta-analysis of OS, moderate inter-study heterogeneity was observed ($I^2 = 40.9\%$, $P_{\text{heterogeneity}} = 0.027$). We found that one study with a large sample size contributed to almost all of the observed heterogeneity [8]. Sensitivity analyses showed that exclusion of this study did not largely alter the pooled estimate (HR 1.02; 95% CI 0.92–1.13; $I^2 = 0$, $P_{\text{heterogeneity}} = 0.95$). The results using the trim-and-fill model, Copas model and subgroup analyses based on some main clinical variables were consistent with our primary analyses, indicating that our results were robust and not affected by publication bias. However, caution is warranted when interpreting the results, because publication bias is ubiquitous [52] and statistical tests for publication bias are imperfect.

Three previous systematic reviews have outlined the *PIK3CA* mutation status for predicting outcome in metastatic CRC [53–55]. The first meta-analysis by Mao et al. found that stage IV patients with *PIK3CA* exon 20 mutations had a shorter PFS and OS, which was observed only in one study with *KRAS* wild-type metastatic CRC patients treated with anti-EGFR monoclonal antibodies. The findings of the other two reviews by Wu et al. and Therkildsen et al. were consistent with the results of the first, which showed that *PIK3CA* mutations were significantly associated with worse PFS and shorter OS in metastatic CRC with anti-EGFR treatment. However, no previous systematic reviews or meta-analyses have assessed overall prognostic

significance of tumor *PIK3CA* mutation in patients with CRC in all stages.

Our meta-analysis is the first to provide robust statistical evidence against substantial prognostic role of *PIK3CA* mutation status in CRC patients. Although four previous studies [9, 11, 13, 14] showed a prognostic association of *PIK3CA* mutation in CRC, the statistical power was limited due to the small sample sizes of these studies (ranging from 67 to 616). Several factors might have contributed to the seemingly contradictory results of these studies. First, sample sizes enormously vary from study to study in the literature. It is well known that smaller studies generate unstable estimates of effect sizes for any association, and are prone to publication bias. The funnel plots (Figure 4) clearly demonstrated asymmetrical distribution of studies with low statistical power. Secondly, study designs greatly vary; many studies used a convenience sample from a single hospital (with unaccountable selection bias), while others used cases in clinical trials (with highly selected enrolment samples) either in a single institution or multiple institutions, or population-based CRC samples in epidemiologic settings. Thirdly, there are differences in mutation detection assays; two main detection systems used are pyrosequencing and Sanger sequencing. The previous validation study demonstrated higher analytic sensitivity of pyrosequencing over Sanger sequencing [56]. Fourthly, somewhat related to the second point, disease characteristics including disease stage and tumor location vary from study to study, which might cause heterogeneity in study findings. Studies have shown that *PIK3CA* mutations are more common in proximal colon cancer than in distal colon cancer and rectal cancer [10, 51, 57]. We conducted subgroup analyses according to various study characteristics, and found no substantial heterogeneity in the prognostic association of tumor *PIK3CA* mutation. Moreover, we applied the trim-and-fill model and Copas model for adjustment, and the results were consistent, indicating no significant association between *PIK3CA* mutation and CRC patient survival.

There are limitations in our systematic review. First, the statistical analysis of publication bias was not well powered because of the limited number of included studies ($n = 28$), although the results were adjusted by two models (trim-and-fill and Copas). Secondly, we could not perform sensitivity analyses related to the *KRAS, BRAF* mutation or microsatellite status, patient treatment regimen or detailed subgroup analyses according to the tumor site (such as left colon cancer, right colon cancer and rectal cancer) and disease stage (stages II, III and IV, separately) because of limited availability of subgroup analysis data in the included studies. Some of these factors have been associated with both *PIK3CA* mutation and prognosis in CRC patients. Thirdly, the different survival analysis methods could have affected the accuracy and precision of the pooled estimates. Although the majority of the studies used the multivariate Cox proportional hazards model, other studies did not report the statistical model [15, 26, 34], while another study applied univariate analysis only (without providing multivariate analysis data) [28]. In addition, adjustment variables varied considerably. Fourthly, we could not adequately assess the risk of bias for each of publication because details on the analysis were not available in many of the original reports. In addition, we were not able to contact the authors or sponsors of some studies to retrieve the data [58]. Although additional data such as Kaplan–Meier curves were provided for us to estimate the HRs and 95% CI in some studies [15, 18, 26, 33, 34], such estimations might have led to uncertain bias for pooled estimates. Fifthly, as reported [55, 59, 60], detection limits (analytical sensitivity) of the Sanger sequencing and pyrosequencing techniques were generally 5%–25% of mutant alleles among all alleles, and hence, false-negative laboratory results remain problems in our meta-analysis.

The present work has several important strengths. First, we conducted a systematic, comprehensive and reproducible search of the relevant studies in multiple online databases without language or publication status limitations, enabling us to select the appropriate studies for our meta-analysis. Secondly, the large sample size including over 12 000 patients enabled us to quantitatively assess the association of the *PIK3CA* mutation status with CRC prognosis, making it the most powerful and comprehensive synthesis of the evidence on this issue to date. Thirdly, appropriate subgroup analyses were carried out for certain key study characteristics, such as the study design, disease stage, mutation detection method, follow-up period and overall study quality, and we obtained generally consistent findings independent of most of the study characteristics. Fourthly, although there was subjectivity in our assessment, we formally rated the strength of evidence based on the quality scale for the existing prognostic studies. Fifthly, most studies provided null associations with *PIK3CA* mutation status, while others with negative or positive associations, which indicated the uncertainty of the survival outcomes for *PIK3CA* mutation in CRC. Besides, the potential for selection bias was acknowledged, we believe it was minimized by the strict pre-specification of screening process based on study eligibility criteria. Furthermore, we utilized the multiple modalities, to assess the extent of publication bias.

In summary, our current systematic review and meta-analysis provide evidence that do not support a substantial prognostic role of *PIK3CA* mutation status in CRC. Our data suggest a differential prognostic association of aspirin use after the diagnosis of CRC according to tumor *PIK3CA* mutation status, which needs to be confirmed by further studies. Large-scale or multi-center studies with patient-level data are warranted to establish the validity of the predictive role of *PIK3CA* mutation status in CRC for specific clinical or molecular profiles as well as treatment outcomes.

standardized official symbols for genes and gene products

We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including BRAF, EREG, KRAS, MGMT, PIK3CA and PTEN, all of which are described at www.genenames.org.

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disclosure

The authors have declared no conflicts of interest.

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Best practice guidelines in the psychosocial management of HPV-related head and neck cancer: recommendations from the European Head and Neck Cancer Society's Make Sense Campaign

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Over the past three decades, oral human papillomavirus (HPV) has been associated with an increase in the incidence of oropharyngeal squamous cell carcinoma (OPSCC) in several countries. Specialist oncologists in head and neck cancer are observing a wider range of demographics, sexual behaviours, and survival outcomes with their patients. Additionally, there are fewer smokers, consumers of alcohol, or people of lower socioeconomic status than in previous decades. In order to support patients, the European Head and Neck Society's Make Sense Campaign aims to promote best practice in the management of head and neck cancer through the delivery of counselling, psychological assessment, support with the patient experience following HPV-related cancer diagnosis, sexual impact (in terms of communication, behaviour and prevention), facilitating access to educational resources about HPV in head and neck squamous cell carcinoma and OPSCC, and early referral if necessary. New concerns about psychosocial distress and unmet psychosocial needs following diagnosis, therefore, exist throughout the disease and treatment periods. Oncologists treating patients with HPV-related head and neck cancer must

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