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Prognostic role and clinicopathological features of *SMAD4* gene mutation in pancreatic cancer: a systematic review and meta-analysis

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ABSTRACT

Purpose: The prognostic value of *SMAD4* in pancreatic cancer has been evaluated in several studies. However, the conclusions remain controversial. Therefore, we aimed to evaluate the prognostic value of the *SMAD4* gene in pancreatic cancer to aid in the design of therapeutic strategies.
Methods: This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria and the proposed methodology was registered on PROSPERO. An electronic search was made in MEDLINE (via PubMed), Web of Science and Google Scholar to identify all relevant studies. Eligible studies were those written in English including patients with Pancreatic Ductal Adenocarcinoma (PDAC), who underwent pancreatic surgery with curative intent and provided data on *SMAD4* gene status or SMAD4 protein expression and its association to prognosis, measured by overall survival (OS). The effect measure of interest was the hazard ratio (HR) for OS between PDAC with or without SMDA4 mutations.
Results: A total of 24 studies on *SMAD4*, with 4340 samples, were included in this systematic review and meta-analysis. Our pooled results demonstrated that patients with tumours with *SMAD4* alteration had significantly worse prognosis for OS (HR= 1.43, 95% CI = 1.17–1.76, p-value = 0.002).
Conclusion: This systematic review and meta-analysis support the use of driver mutations in the *SMAD4* gene as a prognostic marker for pancreatic cancer.

Introduction

Pancreatic cancer (PDAC) is currently the six-leading cause of cancer death worldwide in 2022. It will become the third by 2030. PDAC is the gastrointestinal cancer with the worst prognosis, with a 5-year overall survival (OS) rate 12 %. Even for the small percentage (15 %) of people diagnosed with localised disease, the 5-year survival rate is only 44 % [1–3].

Despite advances in diagnostic and staging methods, most patients with PDAC have a late diagnosis, due to the lack of specific early clinical symptoms and biomarkers, or to difficulties in imaging at early stage. Most patients are diagnosed with metastatic or locally advanced disease. Only 20 % of patients have potentially curable tumours that are surgically resectable at diagnosis.

Upfront surgery, the standard-of-care for resectable PDAC, is the only therapeutic option that can achieve a cure. It is typically followed by adjuvant chemotherapy to reduce the likelihood of disease recurrence. Several criteria are used for the selection of adjuvant treatment, including patient́s performance status and comorbidities, prognostic factors such as American Joint Committee on Cancer (AJCC) tumour-node-metastasis (TNM) stage, tumour grade, presence of perineural invasion (PNI) and evaluation of resection margins for tumour cell involvement. Nevertheless adjuvant treatment results only in a small improvement in PDAC prognosis[4,5]. It is currently unclear the reasons

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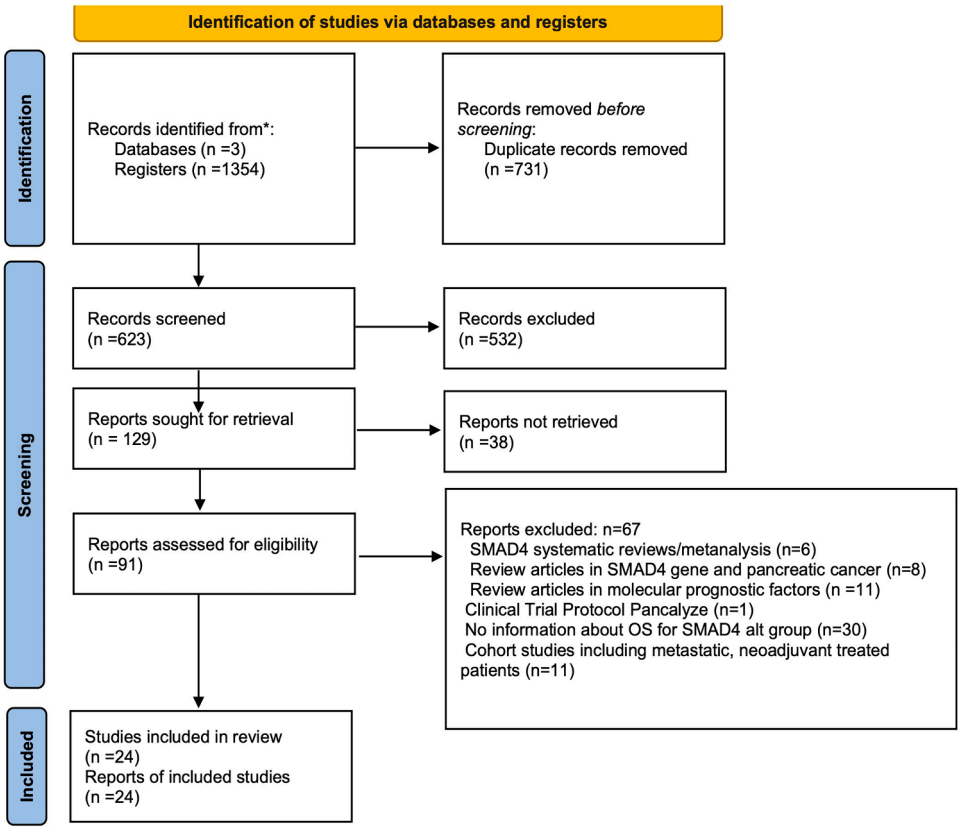

Fig. 1. PRISMA flow diagram of the literature review process for studies assessing the prognostic value of the *SMAD4* gene in pancreatic cancer patients.

why some patients face early recurrence, making PDAC a medical unmet need.

Identifying PDAC-specific prognostic biomarkers for local and distant recurrence could result in more efficient PDAC diagnostic strategies. It could also lead to better selection of personalised therapy and, hopefully, to improved survival.

SMAD4 is a well-known tumour suppressor gene located on chromosome 18q21.2. The majority of SMAD4 gene mutations in human cancer are missense, nonsense and frameshift mutations at the mad homology 2 region (MH2), which interfere with the homo-oligomer formation of SMAD4 protein and the hetero-oligomer formation between SMAD4 and SMAD2 proteins, resulting in disruption of TGFβ signalling[6].

Inactivating mutations affect around 27–60 % of PDAC cases, leading to loss of SMAD4 protein. SMAD4 is a co-factor that facilitates gene transcription and tumour suppression through the TGFβ- signalling pathway. TGFβ SMAD4 signalling pathway regulates tumour development by mediating growth arrest and inducing apoptosis[7–11].

Previous meta-analysis have looked into the effects of SMAD4 loss/gene inactivation in PDAC[12–14].

The most recent meta-analysis[15], examined 31 studies involving 3373 patients with PDAC. The aim was to estimate the prognostic effect of TP53 over-expression, SMAD4 loss and CDKN2A/p16 and KRAS mutations. The analysis showed that the loss of SMAD4 expression had a statistically significant adverse effect on survival. However, this conclusion relied solely on eight studies, which is a limitation[16–23]. The loss of SMAD4 protein, triggered by frequent mutations or deletions in the *SMAD4* gene, can promote PDAC. However, the potential prognostic value of *SMAD4* in PDAC is unclear due to inconsistent results. It is uncertain whether pathogenic mutations of *SMAD4* reduce OS in all PDAC patients and its implications in the recurrence pattern of PDAC are also uncertain.

To investigate the association of *SMAD4* status with clinicopathological characteristics and prognosis in patients with PDAC, we conducted a systematic review and meta-analysis.

Methods

This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. The proposed methodology for the systematic review was registered in the International Prospective Register of Systematic Reviews PROSPERO [CRD42023410090].

Search strategy and eligibility criteria

A thorough electronic search was conducted to find all relevant studies. We looked thorough MEDLINE (via PubMed), Web of Science and Google Scholar to identify relevant studies. The search date was last updated in November 2023. We examined the references list of included studies. The detailed search strategy is available in the PROSPERO register. To avoid translation-related biases, only studies written in English were included.

- They described one or more cohort(s).
- They included patients with PDAC who had undergone pancreatic surgery with curative intent.
- They provided information on *SMAD4* gene status or SMAD4 protein expression, obtained from tumour samples from these patients and its association with patients' prognosis, measured by OS.
- Studies concerning animals or cell lines, case series, letters, editorials, comments, reviews, and abstracts were excluded.

Exposure

The exposure of interest was *SMAD4* mutational status in patients

Table 1
Characteristics of the eligible studies.

First Author	Year	Country	Number of patients	Follow-up Median (months)	Recruitment Interval	SMAD4 alteration (%)	SMAD4 Method	Antibody SMAD4 (Dilution)	Survival Endpoint	Study Type (Cohort)	SMAD4 Prognostic Value	Study Quality (NOS)
Tascilar et al.	2001	USA	249		1990–2000	55	IHC/ PCR	Santa B8 1:100	OS	Retrospective	Proven	7
Biankin et al.	2002	Australia	348	9.5	1972–2000	78	IHC	Santa B8 1:100	OS	Retrospective	Not Proven	7
Toga et al.	2004	Japan	88	NA	2001–2002	85	IHC	Santa B8 1:100	OS	Prospective	Not Proven	5
Khorana et al.	2004	USA	138	16	1994–2002	52	IHC	Santa B8 1:400	OS	Retrospective	Not Proven	8
Blackford et al	2009	USA	91	12.9	1989–2007	44.9	NGS	NA	Mortality (30 days)	Retrospective	Proven	7
Ottenhof et al.	2011	Netherlands	78	NA	NA	43	IHC	Santa B8 1:300	OS	Retrospective	Proven	8
Bachet et al.	2012	France/ Belgium	471	54	1996–2009	60	IHC	Santa B8 1:50	OS	Retrospective	Not Proven	7
Jiang et al.	2012	China	162	NA	1995–2009	73	IHC	Abcam 1:15	OS	Retrospective	Proven	7
Shin et al.	2013	Khorea	272	NA	2004–2008	81.6	IHC	Abcam 1:100	OS	Retrospective	Proven	6
Oshima et al.	2013	Japan	106	17.3	2000–2011	60.4	IHC	Santa B8 1:100	OS	Retrospective	Proven	7
Voorneveld et al.	2013	Netherlands	41	NA	NA	53.7	IHC/ NGS	Santa B8 1:1600	OS	Retrospective	Proven	6
Yamada et al.	2015	Japan	174	16,7	2004–2010	59.8	IHC	Santa B8 1:100	DSS	Prospective	Proven	8
Wang et al.	2016	China	284	NA	2004–2014	52.5	IHC	Abcam 1 : 150	OS	Retrospective	Proven	6
Lee et al.	2017	korea	210	NA	NA	72.5	IHC	Abcam 1:100	OS	Retrospective	Proven	7
Herman et al.	2018	USA	145	NA	1994–2009	61	IHC	NA	OS	Retrospective	Proven	8
Hsieh et al.	2019	Taiwan	168	NA	NA	33	NGS	NA	OS	Retrospective	Not Proven	6
Mahadevan et al.	2019	USA	158	16	1999–2013	31.6	IHC	Santa B8 1:25	DSS	Retrospective	Not Proven	8
Xu et al.	2019	China	237	NA	2010–2013	70.9	IHC	Santa B8 1:200	OS	Retrospective	Proven	8
Kaissis et al.	2020	Germany	103	NA	2006–2019	60.8	IHC/ NGS	NA	OS	Retrospective	Not Proven	7
Sinn et al.	2020	Germany	107	NA	1998–2004	10	NGS	NA	OS	Prospective	Not Proven	7
Yokose et al.	2020	Japan	146	18.7	2013–2018	32	NGS	NA	OS	Retrospective	Proven	6
Hoyer et al.	2021	Germany	293	NA	2008–2013	27	NGS	NA	OS	Prospective	Proven	8
Park et al.	2021	USA	125	23.2	2014–2017	62	IHC	Abcam 1:100	OS	Prospective	Not Proven	7
Gits et al.	2021	USA	146	68.4	2000–2010	92	IHC	Santa B8 1:100	OS	Prospective	Not Proven	8

NA: not available, IHC: immunohistochemistry, PCR: protein chain reaction, NGS: next generation sequencing OS: overall survival, DSS: Disease specific survival, NOS: Newcastle-Ottawa scale.

with a resectable PDAC.

Outcome measure for meta-analysis

The Hazard Ratio (HR) was the measure used to determine the impact of *SMAD4* status on PDAC patients' OS. An HR value higher than 1 indicates a worse prognosis for individuals with tumours with SMAD4 alterations.

When studies report both unadjusted and adjusted HR, the adjusted figure was considered the less biased estimate and was used as the result. If estimated HR is not present, the p-value of the Wald test was used to derive the HR estimate. If the p-value was unavailable, the Kaplan-Meyer (KM) curves were used to reconstruct the patient-level data using Guyot's algorithm[24] with WebPlotDigitizer Software WebPlotDigitizer (utk.edu). After digitising the plots, the HR estimate along with its standard error and 95 % confidence interval was computed using the R Packages *survHE*[25] and *survival*[26]. When available, risk set data were used.

To analyse the variation across studies using shared characteristics concerning the patients such as recruitment time outcome, median follow-up time, ethnicity, type of analysis, antibody source, age, sex, AJCC stage, Tumour staging (T), Lymph node (LN) status, PNI and surgery resection margin type (R1 vs R0), subgroup analysis (for factors) and univariate meta-regressions (for covariates) were performed.

Data collection and analysis

Two of the authors of this review independently evaluated the

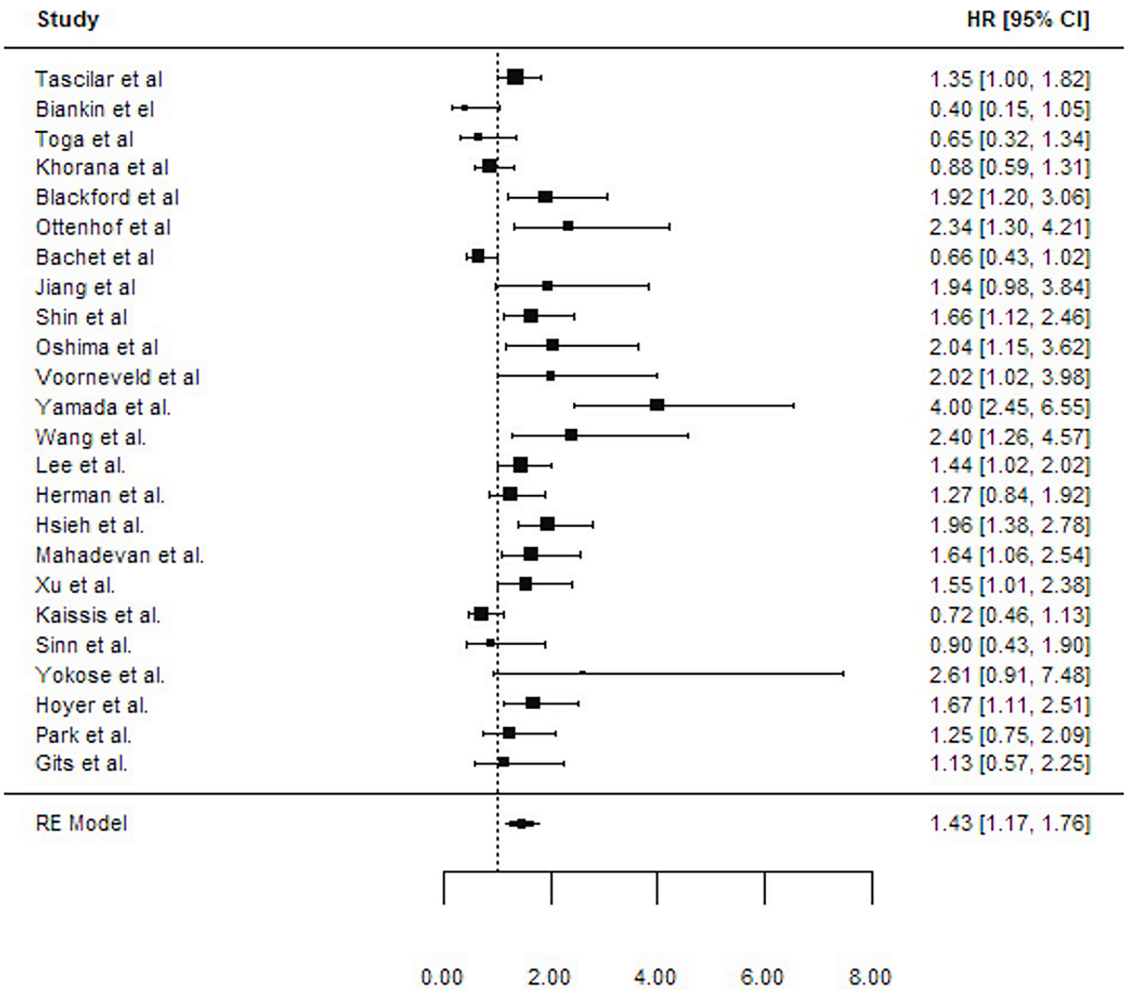

Fig. 2. Forest plot of included studies - Studies are displayed in ascending order (from top to bottom) according to publication year. The hazard ratio is represented by individual squares proportional to the precision of the estimates and the horizontal lines represent the 95 % CIs for each included study.

eligibility of each study by reviewing its title and abstract. Discrepancies were resolved by consensus.

All articles that passed the title/abstract screening were subject to inclusion assessment through a full-text review by two authors. The authors independently extracted information from all selected papers to a form, including study design, participants characteristics, sample size description of exposure, outcomes, and quality assessment indicators. Discrepancies in study selection were resolved through consensus. Following the systematic review, a meta-analysis was carried out using the R Package *metafor*[27]. The associated confidence intervals were based on logarithmic transformation. Forest plots were created to provide a graphical overview. The Cochran's Q test and the I^2 statistic were used to assess inter-studies heterogeneity. A value greater than 0.75 was considered high heterogeneity, and the pooled estimate was regarded as low-certainty evidence. If the I^2 statistic was <0.25, a fixed effects model was used. Otherwise, a random effects model was applied. Model estimation was performed using the restricted maximum likelihood approach with Knapp-Hartung modification. Adjusted estimates were obtained through logistic regression, and pooling was based on the log HR and standard deviation, with the exponential of the pooled result re-expressed as HR.

Funnel plots were used to assess the possibility of publication bias visually. The trim and fill method and Egger's test were applied to screen for potential publication bias[28].

Quality assessment

To determine the risk of bias, two authors examined the studies included in this meta-analysis independently using validated critical appraisal tools. A third reviewer resolved inconsistencies. The methodological quality of the included studies in meta-analysis was assess using the Newcastle-Ottawa Scale (NOS). The NOS considers the selection and the comparability of the cohorts, and the ascertainment of the exposure and outcome of interest. The scales ranges from 0 to 9 with a higher score indicating a higher quality study. Studies attaining a score of at least 8 points were considered high quality. The identification of *SMAD4* mutations after the date of diagnosis was not considered a source of bias. To control the effect of studies with an NOS score lower than 8 and other study characteristics, a sensitivity analysis was carried out using a multivariate meta-regression model. An omnibus test was applied to determine the overall significance of the model's coefficients, and a two-tailed p-value <0.05 was considered statistically significant.

Results

The search identified a total of 623 records after removing duplicates which were screened for eligibility. A total of 24 studies/papers were included in the meta-analysis, all cohort studies. The PRISMA flowchart (Fig. 1) outlines the selection process.

The main characteristics reported in the included studies are summarised in Table 1 and Table S1. Studies included were published in-

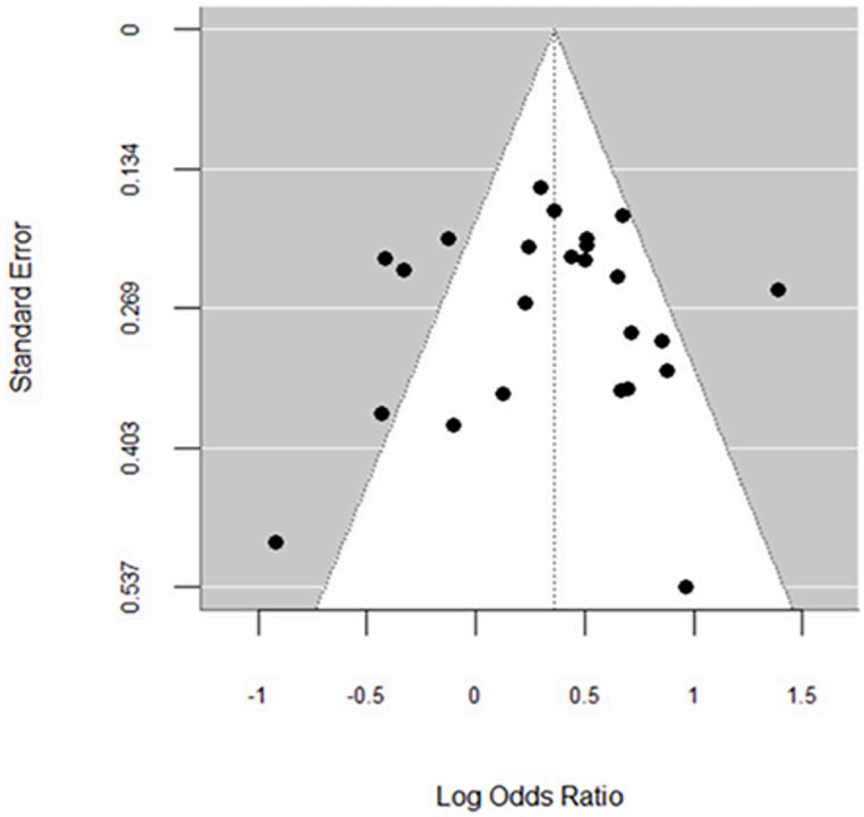

Fig. 3. Funnel plot for the random model-Egger's test p-value was 0.70.

Table 2
Subgroup analysis.

		Number of studies	HR (95 % CI)	I^2	*p*-value
Ethnicity	Asia	10	1.84 (1.35–2.52)	64.0	*0.032
	Other	14	1.21 (0.93–1.57)		
Age (years)	>65	9	1.13 (0.76–1.66	70.9	0.139
	≤65	12	1.57 (1.16–2.12)		
AJCC	≤ 6	6	1.11 (0.63–1.96)	67.8	0.299
lymph nodes (No.)	> 6	13	1.44 (1.10–1.87)		
***SMAD4* NGS**	Yes	7	1.51 (0.99–2.30)	72.3	0.747
	No	17	1.40 (1.08–1.83)		
SMADA4 antibody	Santa Cruz	12	1.34 (0.91–1.99)	75.3	0.478
	Other	5	1.58 (1.22–2.05)		
Median follow-up (months)	≤18	7	1.39 (0.86–2.34)	72.1	0.494
	>18	3	1.08 (0.32–3.59)		

p- value-significant at a 0.05 level

Table 3
Univariate meta-regression analysis.

Heterogeneity	Number of studies	Coefficient (95 % CI)	*p*-value
Age (median)	21	0.98 (0.91–1.06)	0.598
Gender (% female)	22	0.96 (0.92–1.01)	0.098
T1+T2(%)	15	1.01 (1.00–1.02)	0.213
LN Status Positive (%)	19	1.00 (0.98–1.02)	0.735
Perineural invasion (%)	9	1.00 (0.97–1.04)	0.832
Surgery R1 (%)	15	1.00 (0.98–1.02)	0.927
Follow up (median)	10	0.99 (0.96–1.02)	0.435
SMAD4 alt (%)	24	0.99 (0.98–1.00)	0.171

p- value-significant at a 0.05 level

between 2001 and 2021 and most were retrospective. Out of 4340 patients with PDAC who underwent pancreatic surgery, 3845 were eligible for survival analysis and were recruited from 1972 to 2019. Nineteen studies used immunohistochemistry (IHC) to identify SMAD4 protein loss of expression SMAD4 protein aberrant expression with varying cut-off values applied across studies. Loss of nuclear stain or weak cytoplasmic stain indicates SMAD4 loss and is a surrogate for *SMAD4* gene mutation/ deletion[29]. One study analysed *SMAD4* gene mutational status using Protein Chain Reaction (PCR) while seven used Next Generation Sequence (NGS) techniques[20,30–36].

The size of sample cohorts ranged from 41 to 471 in the 24 studies. The reported HR ranged from 0.4to 2.71. For 18 of the studies, the extracted HR was higher than one whereas the remaining six reported an HR lower than 1. Further details can be found in the forest plot (Fig. 2).

As pointed out by $I^2 = 71.1\%$ and Cochran's Q=73.3 ($p < 0.001$) results, the heterogeneity between studies is high indicating a larger variation across studies rather than within samples in a study.

SMAD4 gene mutations were significantly correlated with worse OS in PDAC patients, with an HR of 1.43 and a 95 % CI of 1.17–1.76, p-value=0.002.

Risk of bias assessment

To assess publication bias, a funnel plot was generated for the random model (Fig. 3). Egger's test p-value was 0.70. The trimm and fill method did not identify any missing study in both models, suggesting that publication bias was absent.

Out of the 24 studies, 8 were considered high quality (NOS score of 8). Ten studies attained a score of 7, five studies a score of 6 and only one study had a score of 5. The overall results are summarised in Table 1. A sensitivity analysis was also performed to determine the influence of the non-high-quality studies and of some study characteristics, such as prospective or/versus retrospective, design, estimation method (univariate or/versus multivariate analysis), year of publication, sample size and timing of last recruitment. A mixed-effects meta-regression was performed. The omnibus test for overall model significance yielded a p-value of 0.421.

Subgroup analysis and meta-regression

The source of heterogeneity was assessed through a subgroup analysis of demographic factors such as age, gender, ethnicity, tumour characteristics like AJCC stage, T1+T2, LN status, PNI, surgery resection margins (R1 vs R0), and presence and type of *SMAD4* alteration. AJCC lymph nodes (No.) were divided into ≤ 6 and >6 to achieve the most balanced number of studies in each group When IHC was the method used to determine *SMAD4* status, SMAD4 antibody source was analysed. Type of analysis (univariate vs multivariate), recruitment time endpoint, and median follow-up time were considered (Table 2). This analysis identified ethnicity as the only factor with a significant effect on HR. The covariates were assessed through a univariate meta-regression (Table 3).

Discussion

Despite extensive research, pancreatic cancer remains one of the most challenging malignancies due to its poor prognosis. Therefore, it is essential to better categorise and select patients based on prognostic factors for current therapies and to develop new therapeutic strategies. The tumour progression of PDAC involves various genomic alterations, including oncogene activation and loss of tumour suppressor genes [37–39]. Multiple studies have shown inconsistencies in the association between SMAD4 expression status and prognosis in PDAC patients due to the small sample size and varying methodologies[40–43,16]. The use of different techniques to evaluate *SMAD4* status, such as SMAD4 protein expression by IHC technique with distinct anti-SMAD4 antibodies, versus *SMAD4* gene mutational status with NGS, limits data interpretation[40–43,16]. Differences can occur when analysing the positive rate of SMAD4 gene alterations detected with IHC and NGS. While we have not detected significant differences between both techniques with our subgroup analysis, other cancers also present gene/protein analysis concordant/discordant results from IHC and NGS techniques.

One limitation of our analysis encompasses the limited or absent information presented in the included studies regarding LOH using NGS for SMAD4 genomic analysis, which, if not performed, can have a negative impact on the interpretation of SMAD4 results. In addition, due to the lack of available data, our univariate meta-regression analysis could not be performed on the effect of adjuvant chemotherapy on PDAC prognosis. Despite some limitations, to our knowledge, the current study is the largest of its kind to date. The analysed pooled data from twenty-five studies indicated that *SMAD4* alterations are significantly associated with prognosis in PDAC patients. The study included a subgroup analysis and several factors, including age, gender, and other medical-related factors, but no statistically significant effects were found. Contrary to other studies[12,14,15] that have identified factors such as positive LN, presence of PNI and positive surgical resection margin (R1) as conferring worse OS, no significant effect was observed in our study. The results from this meta-analysis can be considered robust, as the sensitivity analysis did not show a relation between the quality of the studies and the results. Moreover, evidence of publication bias was not found.

The combined findings of the present study show that SMAD4 gene alterations, detected in the resected tumour specimens from PDAC patients are associated with poor OS, irrespective of other prognostic factors. Ultimately, the incorporation of SMAD4 mutation status with additional prognostic factors, either in the format of clinical variables, blood (CA19–9, platelet count, IL-15)[44] or molecular biomarkers (BAX, Bcl-2, survivin, Ki-67, COX-2, E-cadherin)[45] can help tailor treatment strategies to improve patient outcomes.

Conclusion

The combined findings of the present study show that SMAD4 gene alterations, detected in the resected tumour specimens from pancreatic ductal carcinoma patients are associated with poor OS, irrespective of other prognostic factors. However, further large well-matched prospective studies are needed to validate these findings.

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CRediT authorship contribution statement

Tânia Rodrigues: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joana Albuquerque:** Writing – review & editing, Data curation. **Joana Cardoso:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Ivo Sousa-Ferreira:** Writing – review & editing, Software, Data curation. **João Paulo Martins:** Writing – review & editing, Validation, Supervision, Software, Formal analysis, Data curation. **José Luís Passos Coelho:** Writing – review & editing, Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2025.101091.

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